Chronic effects of isoflavones on cognition and aggression in a female population across the menstrual cycle

Doctor of Philosophy

Naomi Laura Perry

Centre for Human Psychopharmacology

Swinburne University

Melbourne

Australia

2014

Abstract

Premenstrual symptoms are common amongst women of reproductive age, and for many women these symptoms compromise quality of life. Aggression is a widely experienced symptom, yet potential non-pharmacological therapies for premenstrual aggression have remained largely unexplored in the current scientific research literature. Many women also complain of cognitive impairment during certain phases of the menstrual cycle. There is increasing scientific interest in the potential benefits of soy isoflavones on mood and cognitive function. Findings regarding the effects on mood and premenstrual symptoms in healthy young women have been mixed, and whilst there is some evidence that isoflavones may improve cognition, studies of the effects across the menstrual cycle are lacking, as are investigations into potential changes in brain activation underlying these effects. No human trials have specifically addressed the effects of isoflavones on aggression.

This thesis reports the findings of a randomised, double-blind, placebo-controlled trial which investigated the effects of supplementation with soy isoflavones across different cycle phases for two menstrual cycles. The outcomes examined included measures of aggression, mood and physical premenstrual symptoms, as well as several cognitive domains. To investigate potential changes in brain electrical activity, event related potentials (ERP) were also assessed during tasks of response inhibition, which is closely related to aggression. Participants were normally cycling women aged 18-35 years, 23 of whom received 200 mg soy isoflavones and 26 of whom received placebo for the duration of the intervention. In addition, a matched sample of 23 oral contraceptive users were included as a positive control. It was predicted that treatment with soy isoflavones would result in improvements in premenstrual symptoms including aggression and mood, as well as improvements in cognitive function, particularly executive functions which have been shown to be sensitive to isoflavone supplementation. It was further hypothesised that isoflavone supplementation would result in greater amplitude of ERP components associated with response inhibition.

The results of this trial indicated that supplementation with soy isoflavones was associated with increased aggression and poorer mood outcomes, as well as poorer

performance of some cognitive tasks, during the luteal phase of the menstrual cycle. However, during menses some symptoms were improved with soy isoflavones, suggesting that the effects of these compounds may depend on endogenous steroid hormone levels. Furthermore, some tasks with an increased cognitive load showed improvements with soy isoflavones, whereas less demanding tasks showed no effects. Isoflavone supplementation also increased latency of the later ERP component during response inhibition of face stimuli, suggesting a slowing of neural processes.

The findings from this study suggest that whilst soy isoflavones may be useful for the treatment of premenstrual symptoms during periods of low circulating steroid hormones, they may be detrimental to mood and other symptoms during periods where endogenous hormone levels are high.

Acknowledgements

There are many people who I wish to thank and without whom this would not have been possible. Firstly I must thank all my participants for their time and their interest in my study which gave me the motivation to keep going. Also thanks to Amy Gibbs, Karen Savage and Sarah Benson for giving up so much of their time to help with testing so that I could go home and visit my family and friends back in the UK, who I must also thank for their support and for not making me feel guilty about choosing to do this on the other side of the world.

I would also like to thank my supervisors for their much appreciated guidance. Thanks to Prof Andrew Scholey for his patience and feedback regarding all aspects of this study, as well as responding to my emails at all times of the day, Dr. Matthew Hughes for teaching me about EEG and being more helpful than I could have asked for, Prof Louise Dye for her expertise in this field and fantastic guidance with methodological issues, and Dr. Patrick Johnston for making it possible for me to study in Australia in the first place. I would also like to give special thanks to my unofficial supervisors Dr. David Camfield for so generously spending hours of his time teaching me about linear mixed modelling, and Dr. David White for helping me so much with EEG data preprocessing. Thanks also to Ass Prof Denny Meyer for explaining some of the statistical issues I came across, and Sam McIlraith for helping with the very long task of data entry.

Finally, I would like to thank all the friends I have made throughout this process, of whom there are too many to mention them all by name but they know who they are. Each of you has helped me in ways that I will always be grateful for. In particular, thanks to Chris Neale and James Kean for answering my stupid questions, proof reading and generally fixing all my problems. Also my housemates deserve special thanks for putting up with my mood swings and not getting angry when I wake them up in the middle of the night, and for always supplying me with wine and hugs when I need them. Last but certainly not least, the last year would have been unbearable were it not for the Burger Friday team. You guys gave me something to look forward to once a week and I will be eternally grateful.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of this thesis. This thesis contains no material previously published or written by another person except where due reference is made. Where the work is based on joint research or publications, discloses the relative contributions of the respective authors.

Signed 

Dated 23/12/2014

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Chapter 1

Introduction and Overview

Mood disturbances in Australia are common and widespread, and have been estimated to cost $20 billion annually (ABS, 2009). Almost half the population have experienced a mood disorder at some stage and they are more common in women than in men (Kuehner, 2003; Staley et al., 2006). For many women, whether they have been clinically diagnosed with a mood disorder or not, the menstrual cycle can play a large role in mood which can change according to fluctuating hormone levels (Halbreich & Kahn, 2001).

Premenstrual syndrome (PMS) is experienced by between 30% and 80% of Western premenopausal women (Singh, Berman, Simpson, & Annechild, 1998) with around 50% of women seeking medical treatment for the condition (Campbell, Peterkin, O'Grady, & Sanson-Fisher, 1997), thus placing a burden on health care. It is characterised by various behavioural, somatic and affective symptoms such as irritability, mood swings, fatigue, breast tenderness, headaches and food cravings. These typically begin during the 7-10 days prior to the onset of menstruation and are alleviated at, or shortly after, commencement of menses (Freeman & Halbreich, 1998). Symptom severity peaks either on the first day of menses or just before (Meaden, Hartlage, & Cook-Karr, 2005). Although there is no absolute consensus for the diagnosis of clinically significant PMS (see chapter 2), the National Institute of Mental Health (1983) recommend that PMS should be diagnosed if the participant demonstrates an increase in symptoms of at least 30% premenstrually (6 days before menstruation) compared to during days 5-10 of the menstrual cycle. A more severe form of PMS, premenstrual dysphoric disorder (PMDD) is experienced by approximately 3-8% of women of fertile age (Sveindóttir & Bäckström, 2000) and is classed in the DSM-5 (APA, 2013) as a depressive disorder. Although drugs such as antidepressants may alleviate some of these symptoms, they are not effective for all symptoms and may not be well tolerated by all women.

Aggression is a common symptom of premenstrual syndrome, and is of particular social concern in addition to causing functional impairment for many women (Yonkers, O'Brien, & Eriksson, 2008). In fact, it has been suggested that aggression may underlie other premenstrual symptoms (Yonkers, 1997). The fact that PMS and premenstrual psychosis have often been used as criminal defence in court (Brockington, 2011) further highlights the need to investigate female aggression, particularly during the

premenstrual phase. Some cognitive domains have also shown impairment during certain phases of the menstrual cycle, with some women reporting noticeable complaints during certain phases of the cycle (see Chapter 2). It is therefore important to investigate different therapies that may improve cognitive function as well as mood and aggression in young, healthy women to enable more stable cognitive functioning and mood across the cycle.

The use of oral contraceptives (OCs) in women of reproductive age is widespread, with over 100 million women worldwide being prescribed this form of contraception (Christin-Maitre, 2013). Whilst the majority of women using OCs use them for contraceptive purposes, they are also used by some women to alleviate the symptoms of PMS. However, whether OCs are effective in the treatment of mood, somatic symptoms or cognitive deficits is under debate, and some studies have found that OCs may in fact result in a worsening of symptoms in some women (see Chapter 3). Therefore many women seek alternative therapies to alleviate their symptoms.

The use of natural medicines has increased considerably in recent years, with two out of three Australians reporting having used complementary or alternative medicines during the past 12 months (NICM, 2011). Females are more likely to use nutraceuticals (foods or supplements which infer a greater benefit than nutrition alone) than males (Armstrong, Thiébaut, Brown, & Nepal, 2011; Eisenberg et al., 1998), and up to 91% of women suffering from PMS symptoms have been reported to self-medicate with nutraceuticals, preferring natural therapies over prescribed treatments (Domoney, Vashisht, & Studd, 2003). The nutraceutical of interest in this thesis is a subclass of phytoestrogens termed soy isoflavones (SIF). Phytoestrogens are non-steroidal compounds similar in structure to endogenous estrogen hormones (see Chapter 4.1.2) and isoflavones are the most widely studied subclass due to their availability in foods such as soy products. The majority of research to date has focused on postmenopausal women and alleviating the symptoms associated with the decrease in estrogen levels following menopause. There is evidence, discussed in Chapter 4, suggesting that isoflavones may also improve mood and cognitive function in younger females, although studies of the effects across the menstrual cycle are currently lacking.

Electroencephalography (EEG) is a commonly used non-invasive means of recording brain electrical activity from electrodes placed on the scalp. Examination of brain

activity may enable insight into the mechanisms underlying any observed effects of the menstrual cycle, OC use or isoflavone supplementation on mood, aggression and cognition. EEG has been used in the study of various nutraceuticals (Kim & Oh, 2012; Soares, Poitras, & Prouty, 2003), and in the current study the examination of event-related potentials (ERP) was used to assess the effects of isoflavone supplementation, in comparison to placebo and OC use, on brain activation responses to certain stimuli. Specifically, activation following inhibition of responses to abstract stimuli was examined due to the relationship between impulsiveness and aggression (Barratt, Stanford, Dowdy, Liebman, & Kent, 1999; Pawliczek et al., 2013). ERPs induced by inhibition of responses to faces displaying different emotions were also studied due to the evidence that aggressive and non-aggressive individuals respond differently to faces showing different emotional valence (Lee, Gill, Chen, McCloskey, & Coccaro, 2012), and that inhibition-related ERPs may depend on the emotionality of the stimulus (Johnston & Wang, 1991).

This thesis contains 11 chapters. Chapters 2, 3 and 4 review the literature on mood, aggression and cognition as affected by endogenous estrogen and the menstrual cycle (Chapter 2), OCs and synthetic estrogens (Chapter 3) and phytoestrogens (Chapter 4) and provide a rationale for the aims and hypotheses tested within the thesis. In Chapter 5, the literature on the neural underpinnings of inhibition and face emotion processing, as well as how these are affected by female sex hormones, is reviewed. Chapter 6 presents the aims and methods of the experiment focusing on behavioural measurements of the effects of OCs and SIF on mood, aggression and cognition across the menstrual cycle, as well as the neural correlates affected by OCs and SIF across the cycle as measured using electroencephalography (EEG). In Chapter 7, the baseline results are presented, describing the effects of oral contraceptives and changes across the menstrual cycle. The baseline findings are discussed with reference to the broader literature in Chapter 8. Post-treatment findings regarding the effects of soy isoflavones across the menstrual cycle are presented in Chapter 9, and these findings are discussed in Chapter 10. Finally, the general discussion of the thesis including limitations and future directions for this research are presented in Chapter 11.

Chapter 2

Endogenous estrogen fluctuations and the menstrual cycle

2.1. An Introduction to the Menstrual Cycle

The menstrual cycle has been extensively used as a non-invasive tool for the assessment of the effects of steroid hormones on behaviour. The menstrual cycle is controlled by changes in the concentrations of four hormones: the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as the steroid hormones estrogen (E) and progesterone (P). It is subdivided into different phases based on these hormonal changes and the reproductive effects that accompany them. It is common for the cycle to be divided into just 2 phases in the literature, the follicular and luteal phases, however for the purposes of this thesis two additional phases are also included, the menses and ovulatory phases.

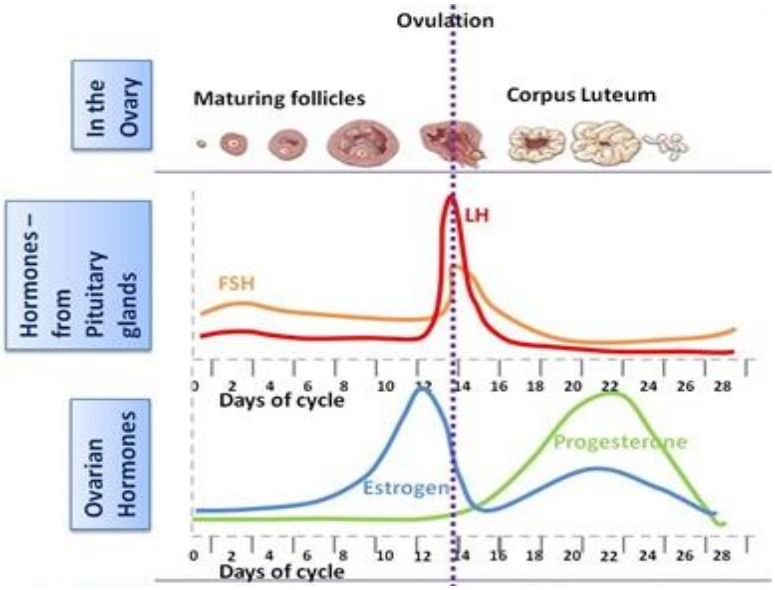

Figure 2.1 Fluctuations in gonadotropins and steroidal ovarian hormones across the menstrual cycle, as well as the progression from ovarian follicle to corpus luteum.

The early follicular phase (menses) begins with the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which stimulates the anterior pituitary to secrete FSH, whilst levels of LH, E and P are low. The high level of FSH stimulates the growth of ovarian follicles which secrete E, with one dominant follicle secreting large amounts of E. This results in high levels of E in the mid to late follicular phase, which

triggers LH secretion via positive feedback to the hypothalamus and pituitary gland and induces ovulation. During the luteal phase, the dominant ovarian follicle transforms into a corpus luteum, which produces E and P. Although E levels are lower than the preovulatory surge they remain high, whilst P levels are also high during the luteal phase, having increased 10- to 100-fold following ovulation. If fertilization does not occur, the corpus luteum regresses and E and P levels fall sharply at the end of the luteal phase, initiating the next menstrual period which begins the next menstrual cycle. Figure 2.1 illustrates these hormonal changes across the cycle.

2.2. Estrogen receptors in the brain

Although female sex steroids are best known for their effects on the reproductive organs, they are also known to cross the blood-brain barrier (Henderson, 1997; Wirth, 2010) and have effects on brain function (McEwen, 2002) , thereby potentially modulating mood and cognitive function. Since the focus of this thesis is on the effects of estrogen, a brief summary of estrogen receptors (ERs) and their distribution throughout the brain is provided here.

Nelson (2006) reported that there are two different types of ER, ER-alpha (ERα) and ER-beta (ERβ), which are distributed in different brain areas, for example ERα has been shown to be expressed in the lateral septum (LS), bed nucleus of the stria terminalis (BNST), anterior hypothalamus (AHA) and medial amygdala (MEA; Wood and Newman, 1995), whereas ERβ is highly concentrated in the BNST and the MEA, but not the LS or AHA. Importantly, the BNST and MEA where both forms of ER are expressed are regions known to be involved in the regulation of emotional behaviour (Nomura, Korach, Pfaff, & Ogawa, 2003). The most potent estrogen, estradiol, is reported to have similar binding affinities for both forms of ER (Kuiper et al., 1997), suggesting that endogenous estrogen may have effects on mood through actions on both ERα and ERβ in these brain regions. However, Walf and Frye (2006) reported in their review that ERβ is required for the effects of estrogen on mood, whereas ERα may be less important.

Whilst the majority of E is produced in the ovaries, research has shown that it is also produced in the hippocampus (Hojo et al., 2004). The hippocampus is known to be crucial for cognitive functioning, particularly memory functions (Olton, Becker, & Handelmann, 1979). In addition, the amygdala has been shown to be particularly sensitive to E_2 (Walf & Frye, 2006), and both the amygdala and hippocampus are known to regulate emotions such as anxiety and depression (LeDoux, 2000; Walker, Toufexis, & Davis, 2003). Taken together these findings suggest that actions of estrogen in the brain may result in alterations in mood and cognitive function.

2.3. Premenstrual Syndrome and mood changes throughout the menstrual cycle

Female sex hormones have long been acknowledged as potential moderators of mood, and one of the first studies investigating the mood enhancing effects of administering estrogen to women with various neuropsychological conditions was published over 100 years ago (Easterbrook, 1900; in Walf & Frye, 2006). The incidence of mood disorders such as anxiety and depression is higher in females than in males at all ages (Kessler et al., 2005; Kuehner, 2003; Staley et al., 2006; Steiner et al., 2005), which led to speculation regarding biological factors such as steroid hormones underlying the sex differences in the incidence of mood disturbance. Indeed, reductions in steroidal hormones following menopause and during the postpartum period have been associated with negative alterations in mood (Baines, Wittkowski, & Wieck, 2013; Weber, Maki, & McDermott, 2013). Furthermore, females' increased vulnerability to mood disorders occurs after puberty with the beginning of secretion of estrogen from the ovaries (Hayward & Sanborn, 2002). Menstrual cycle-related hormonal changes are widely believed to affect mood and other symptoms, however some women are more vulnerable to the negative effects of these hormonal fluctuations than others, as will be discussed in the following sections.

2.3.1. Definitions and characteristics of premenstrual syndrome and premenstrual dysphoric disorder

Premenstrual syndrome (PMS) is characterised by negative physical symptoms (e.g. bloating, headaches, breast tenderness) and psychological symptoms (e.g. irritability, depression, mood swings) that occur during the late luteal, or "premenstrual" phase of the menstrual cycle (six days before the onset of menstruation) and diminish at or shortly after the onset of menstruation (Haywood, Slade, & King, 2002; Ivey & Bardwick, 1968). Mild variations in mood as well as behavioural and physical symptoms are well known to be experienced by many women across the menstrual cycle, with estimates of up to 90% of women worldwide experiencing one or more symptoms in the premenstrual phase at some time of their life (Zaka & Mahmood, 2012), whilst some studies have reported that 100% of their participants experienced at least one premenstrual symptom of minimal severity (Bakhshani, Mousavi, & Khodabandeh, 2009; Tabassum, Afridi, Aman, Tabassum, & Durrani, 2005). The length of symptom expression can vary between a few days and two weeks, and symptoms generally peak around two days prior to the onset of menses (Pearlstein, Yonkers, Fayyad, & Gillespie, 2005) although it has been reported that symptoms peak as late as the first day of menses (Meaden et al., 2005). Although it is accepted that fluctuations in gonadal hormones trigger the symptoms during the premenstrual phase, the mechanisms underlying these effects are currently unclear and there is dispute over whether the high levels of steroid hormones during the midluteal phase or the sudden decline in these hormones just prior to menses cause the symptoms (see Section 2.3.4).

Whilst occasional and mild premenstrual symptoms are experienced by almost all women at some point, moderate to severe PMS is a less common complaint amongst women of reproductive age. Past research estimated that symptoms of significant severity were experienced by around 30% of fertile women, with 10% seeking treatment for PMS (Andersch, Wendestam, Hahn, & Ohman, 1986). More recent evidence has ranged from estimates of up to 20% of fertile women experiencing clinically relevant PMS (Yonkers et al., 2008) to 59% of women meeting the diagnostic criteria for PMS (Mahesh, Tirmizi, & Sanwer Ali, 2011). These varying reports of prevalence are likely due to the broad criteria used by different authors in defining what constitutes PMS, as well as the different instruments used in its measurement. According to the American Congress of Obstetricians and Gynecologists (ACOG, 2000), the diagnosis of PMS

must include at least one moderate to severe mood symptom and one physical symptom, and functional impairment must be attributed to the symptoms. Furthermore, the symptoms must be present for one to two weeks before menses and must remit by day 4 of menses, as measured by prospective documentation for at least two cycles. This point is of particular importance as retrospective recall correlates with prospective ratings in only 50% of cases (Rapkin, Li Chang, & Reading, 1988), and many past studies have used retrospective measurements. It has been estimated that affected women experience almost 3000 days of severe symptoms during the reproductive years (Rapkin & Winer, 2009). Symptoms do not occur in anovulatory cycles or following ovariectomy or treatment with ovulation inhibitors, therefore there is a general consensus that corpus luteum formation is necessary for the presence of symptoms (DeVane, 1991; Hammarback, Ekholm, & Backstrom, 1991; Yonkers et al., 2008).

In a small proportion of women, premenstrual symptoms are severe enough to warrant a diagnosis of premenstrual dysphoric disorder (PMDD), which is experienced by around 3-5% of women of reproductive age (Sveindóttir & Bäckström, 2000). Originally named Late Luteal Phase Dysphoric Disorder (LLPDD), PMDD is classified as a depressive disorder in the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5; APA, 2013) and, in order for a diagnosis to be made, certain conditions must be met. Firstly, the woman must demonstrate the presence of at least five symptoms during the luteal phase, at least one of which must be a mood symptom. Prospective daily rating of symptoms for two cycles must confirm the timings of symptoms during the luteal phase, and the woman must show evidence of functional impairment. Finally, the symptoms must not be an exacerbation of another psychiatric condition (Yonkers et al., 2008). Despite the fairly specific criteria for diagnoses of PMS and PMDD, the overlap between severe PMS and PMDD has been estimated to be up to 79% (Freeman, Rickels, Sondheimer, & Polansky, 1995).

2.3.2. Evidence of cyclical fluctuations in mood and other symptoms and the potential contribution of female sex hormones

Numerous studies have investigated various aspects of PMS/PMDD in order to characterise the prevalence, most common symptoms and potential causes of the disorder. Researchers have also investigated cyclical changes in symptoms in women not diagnosed with or seeking help for PMS/PMDD. An extensive historical review of the literature is beyond the scope of this thesis, therefore the following section is restricted to studies conducted since 2005 and those of historical importance. A search was conducted using various databases for studies of PMS as well as mood, depression and anxiety across the menstrual cycle. These studies are summarised in Table 2.1.

In women seeking treatment for PMS/PMDD, research has consistently shown that symptoms are rated as more severe during the luteal phase than the follicular phase (e.g. Baker & Colrain, 2010; Gingnell et al., 2012; Reed, Levin, & Evans, 2008; Seippel & Backstrom, 1998), with one study finding that symptom severity peaks for these women during the final five days before menses (Seippel & Backstrom, 1998). In particular, depression and anxiety are symptoms that are commonly experienced and are not only more severe during the luteal phase compared with the follicular phase, but are also more severe in women seeking help for PMS than asymptomatic women (Baker & Colrain, 2010; Reed et al., 2008).

Although cyclical changes in mood have also been displayed by non-help-seeking women in several studies, the timing of peak symptom severity is less clear. One study reported a similar pattern in non-help-seeking women as those diagnosed with PMS, such that severity was significantly greater during the luteal phase than the follicular phase (Reed et al., 2008). Conversely, it has been suggested that peak symptom severity occurs during menses in non-help-seeking women (Hampson, 1990a; Meaden, Hartlage, & Cook-Carr, 2005). Others have reported that peak symptom severity spans both the luteal phase and menses, with severity reducing during the follicular phase (Sakai & Ohashi, 2013). However, some studies have reported no cyclical variation at all in non-help-seeking women (Baker & Colrain, 2010; Hampson, 1990b; Phillips & Sherwin, 1992). It can be hypothesised that the lack of findings of these studies is due to their inclusion of only two phases, rather than prospectively measuring symptoms across the entire cycle. In a review of the available literature on cyclical changes in non-help-

seeking women, Romans, Clarkson, Einstein, Petrovic, and Stewart (2012) reported the majority of studies found either no association of mood with any cycle phase, or found an association between negative mood in the luteal phase combined with another phase. Very few found an association of negative mood with the premenstrual phase alone. These findings suggest that whilst women with and without PMS/PMDD all experience cyclical fluctuations in hormones, they may be affected differently by these biological changes. Theories of the mechanisms underlying the difference between women with and without PMS/PMDD are summarised in the following section.

Table 2.1 *Summary of studies of the timing of premenstrual symptoms in women suffering from PMS/PMDD and asymptomatic women, and their association with endogenous hormones*

Study	Sample/ Design/Duration	Measure(s)	Outcome	Comments
Baker & Colrain (2010)	9 PMS/PMDD 8 asymptomatic Age 18-40 yr At least two MC	BDI; POMS	PMS/PMDD sufferers had higher depression, fatigue and global mood disturbance in luteal compared with follicular phase and controls	No significant cyclical variation in depression in control women
Gingnell et al. (2012)	14 PMDD (34.9 yr) 15 asymptomatic (33.7 yr) One MC	STAI; MADRS-S; Hormonal assays	PMDD sufferers had higher anxiety and depression during luteal compared with follicular phase and controls	No significant differences between groups in hormonal levels, or between phases in E_2 levels
Hampson (1990a)	45 asymptomatic (23.7 yr) Individual testing sessions in 2 cycle phases	POMS	Enhanced vigour, reduced tension, depression and anger in preovulatory phase compared with menses	Measures performed once only in each phase, and in separate cycles
Hampson (1990b)	45 asymptomatic (23.7 yr) Individual testing sessions in 2 cycle phases	POMS	No difference between menses and midluteal phase in any affective domain	Measures performed once only in each phase, and in separate cycles
Kiesner & Poulin (2012)	47 asymptomatic Longitudinal study, tested annually from 12 to 21 yr	CDI; CES-D; Retrospective ratings of menstrual symptoms	Early adolescent increases in depression negatively associated with negative affect during menses, positively associated with mid-cycle negative affect, no association in premenstrual phase	Authors hypothesis underlying effect of reproductive steroids as early adolescence and mid-cycle are characterised by increases in female sex hormones; May also be result of physical symptoms
Meaden et al. (2005)	900 asymptomatic (13-53 yr) Two MC	DSMQ	Symptoms increase during premenstrual week; peak symptom severity on first day of menses	Findings suggest a lag between hormonal changes in luteal phase and peak symptom severity in asymptomatic women
Phillips & Sherwin (1992)	25 asymptomatic (24 yr) Individual testing sessions in 2 cycle phases	MAACL	No difference in mood between menses and luteal phase	Measures performed once only in each phase, and in separate cycles

Reed et al. (2008)	14 PMDD 15 asymptomatic Mean age 30 yr Two MC	Daily Ratings Form; BDI; STAI	PMDD sufferers had more severe dysphoria, depression and anxiety during luteal than follicular phase and controls	Control group also had significantly greater dysphoric mood in luteal phase than follicular phase; No difference between E_2 levels in luteal and follicular phases
Romans et al. (2012)	Review of 47 studies of asymptomatic women All included studies had a duration of at least one MC	Only prospective measures of menstrual symptoms included	38.3% of studies found no association of mood with any cycle phase, 38.3% found association between negative mood in premenstrual phase combined with another phase (in most cases menses), 14.9% found association of negative mood with premenstrual phase alone, 8.5% showed association between negative mood and a phase other than premenstrual	Concluded there is little evidence for premenstrual worsening of mood in non-help-seeking women
Sakai & Ohashi (2013)	29 asymptomatic (21.1 yr) Two MC	CES-D; MDQ	Higher depression and menstrual symptoms in menses and luteal phase than follicular	Study only included smokers
Schwartz et al. (2012)	19 asymptomatic (18-41 yr) Six weeks	DLQ; Hormone assays	Metabolite of estradiol measured several days prior to mood negatively associated with motivation and getting along with others, as well as anxiety; Metabolite of progesterone measured same day and day before mood associated positively with irritability, and when measured 5 days prior associated positively with difficulty coping	Small sample; Other factors such as stress and physical health also contributed to all mood outcomes as well as composite positive and negative mood measures
Seippel & Backstrom (1998)	30 PMS Age 27-44 yr Two MC	Prospective daily rating scale; hormone assays	Significant cyclical mood changes; peak severity during final five premenstrual days	High E_2 associated with mood and physical symptoms, high LH associated with mood symptoms, high P associated with less severe mood symptoms. No control group

PMS, Premenstrual syndrome; PMDD, Premenstrual Dysphoric Disorder; MC, Menstrual Cycle; BDI, Beck Depression Inventory; POMS, Profile of Mood States; STAI, State-Trait Anxiety Inventory; MADRS-S, Montgomery-Asberg Depression Rating Scale; CDI, Children's Depression Inventory; CES-D, Centre for Epidemiological Studies Depression Scale; DSMQ, Daily Symptom and Mood Questionnaire; MAACL, Multiple Affect Adjective Checklist; MDQ, Menstrual Distress Questionnaire; DLQ, Daily Life Questionnaire; E_2, Estradiol; P, Progesterone; LH, Luteinizing Hormone

2.3.3. Potential Mechanisms of Action of Endogenous Estrogens on Mood and Theories of Underlying Causes of PMS/PMDD

Some theories on the causes of PMS/PMDD centre on the idea that total steroidal hormone levels differ between women who do and do not experience symptoms. Findings from an early study suggested that there may be a small difference in the pattern of estradiol and progesterone secretion between women with PMS and those without (Halbreich, Endicott, Goldstein, & Nee, 1986). In a study comparing PMS sufferers with asymptomatic women, both plasma progesterone and estradiol levels were found to be within the normal range and did not significantly differ between groups, however when women with PMS were subdivided into groups according to symptom severity, estradiol levels were found to be significantly higher amongst women with more severe symptoms compared to those with less severe or no symptoms from the follicular to the luteal phase (Redei, 1995). Furthermore, progesterone levels were found to correlate positively with PMS symptoms, preceding the symptoms by 5-7 days (Redei, 1995), and administration of progesterone for one month resulted in a provocation of symptoms with some delay (Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998).

In contrast, Rapkin et al. (1997) reported that progesterone has anxiolytic properties due to the action of its metabolites at $GABA_A$ receptors, and suggested that rather than higher levels of progesterone causing premenstrual symptoms, it may be the decline in this hormone shortly prior to menstruation that induces the symptoms. Other researchers have supported this theory that the drop in progesterone in the late luteal phase is responsible for premenstrual complaints through changes in neurotransmitters such as GABA (Sundström Poromaa, Smith, & Gulinello, 2003). However, Yonkers et al. (2008) highlight that for many women the onset of symptoms is in the ovulatory or early luteal phase, which refutes this theory. Furthermore, RCTs of progesterone supplementation have not demonstrated efficacy for the treatment of PMS/PMDD (Freeman et al., 1995; Rapkin et al., 1987; Wyatt, Dimmock, Jones, Obhrai, & O'Brien, 2001). It has thus been suggested that either the preovulatory peak in estradiol, or the postovulatory peak in progesterone, or both, may trigger premenstrual symptoms (Schmidt et al., 1998). Despite the evidence that lowered endogenous estradiol is associated with mood complaints, especially depression (Young, Midgley, Carlson, & Brown, 2000), treatment with estradiol has been found to be ineffective as a treatment

for mood depression in several studies. Other studies have shown that GnRH agonists, which reduce steroid hormone levels, increase depressive mood symptoms (Warnock, Bundren, & Morris, 2000). Watson, Alyea, Cunningham and Jeng (2010) have suggested that these discrepancies may be due to the involvement of other estrogen metabolites such as estrone (E_1) and estriol (E_3) which have been less extensively studied than E_2. Dose may also play an important role, since animal studies have shown that E_2 administered in doses producing typical physiological levels reduce anxiety and depression, however lower or higher doses had no effect (Walf & Frye, 2005).

Findings from animal studies regarding the contributions of steroid hormones to mood are also unclear. During the late proestrous phase of the estrous cycle, when levels of E_2 are high, rodents show decreased levels of behaviour associated with anxiety and depression. During this phase, rats spend more time in the open arms of an elevated plus maze which indicates reduced anxiety, compared to rats during the diestrous phase when E_2 levels are lower (Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001). In that study, E_2 levels were found to differ between phases whereas P levels remained constant, suggesting that E_2 rather than P has anxiolytic effects. However, Frye and Walf (2002) reported the same behavioural observations during the proestrous phase, but that a metabolite of progesterone in the hippocampus mitigated these effects. Even further evidence has found that lifelong low levels of E_2 are associated with increased anxiety behaviour (Danilovich, Harada, Sairam, & Maysinger, 2003). These findings suggest that both E_2 and P may have anxiolytic effects, and that periods when these hormones are low may be characterised by more anxious behaviour.

Since several studies have found no difference in gonadal hormone levels between women with PMS/PMDD and controls (Gingnell, Morell, et al., 2012; Rubinow et al., 1988; Rubinow & Schmidt, 1995), more recent theories have centred around the idea that rather than physiological differences in hormone levels being responsible for PMS, women with PMS/PMDD may have an intolerance of or increased sensitivity to fluctuations in gonadal steroids (Yonkers et al., 2008). In line with this theory, administration of gonadal steroids following pre-treatment with an ovulation inhibitor provoked PMS-like symptoms in women with PMS but not in controls (Schmidt et al., 1998).

Symptoms associated with PMS/PMDD are similar to those seen with reduced serotonergic function (Rapkin, 1992), therefore it has been proposed that serotonergic dysfunction may be implicated in PMS/PMDD. Female sex steroids have been shown to modulate serotonin transmission in rodents (Hiroi, McDevitt, & Neumaier, 2006; Rubinow, Schmidt, & Roca, 1998) and in non-human primates (Bethea, Lu, Gundlah, & Streicher, 2002). Central Nervous System (CNS) serotonergic function is indeed altered during the luteal phase in women with PMS or PMDD compared with controls, with lowered whole blood serotonin (Rapkin et al., 1987) , decreased platelet uptake of serotonin (Ashby, Carr, Cook, Steptoe, & Franks, 1988) and decreased platelet monoamine oxidase (MAO) activity (Ashby et al, 1988) all being reported. In women who have not been diagnosed with PMS/PMDD, a negative correlation has also been observed between whole blood 5-HT concentrations and negative mood scores in the premenstrual phase (Kikuchi et al., 2010). In addition, several treatments which increase serotonin levels have been found to be effective in the treatment of PMS/PMDD (Rapkin, 2003), whereas impairment of serotonergic function through a tryptophan-free diet (Menkes, Coates, & Fawcett, 1994) or administration of a serotonin-receptor antagonist (Roca et al., 2002) provokes symptoms. A positron emission tomography (PET) study further revealed that women with PMDD demonstrated a significantly smaller change in 5-HT_{1A} receptor binding potential in the raphe nuclei from the follicular phase to the luteal phase compared with asymptomatic women, lending support to the idea that serotonergic function is disrupted in women with PMDD (Jovanovic et al., 2006).

It has also been suggested that altered amygdala reactivity in women with PMDD may account for their increased experience of negative mood during the luteal phase. However, a study utilising functional magnetic resonance imaging (fMRI) found no difference in amygdala activity between PMDD sufferers and controls in the luteal phase, whereas in the follicular phase women with PMDD displayed enhanced activity in response to emotional stimuli. This was accompanied by a correlation between progesterone levels and amygdala activity. Furthermore, when women with PMDD were divided into those with high and those with low levels of trait anxiety, women with PMDD and high trait anxiety did display enhanced amygdala activity during the luteal phase, both compared with controls and with their own follicular phase (Gingnell, Morell, et al., 2012). These findings suggest that increased activity in the follicular

phase may characterise women with PMDD, and for a subgroup of more anxious women with PMDD increased activity is seen in the luteal phase.

Estrogen may also affect mood outcomes via non-direct pathways, such as by altering production and secretion of the hormone prolactin (PRL). Estrogen is known to facilitate PRL production, and behavioural changes associated with high levels of PRL include increased depression (Watson et al., 2010). In support of this theory, thyroid activity has been found to be more variable in women with PMS compared to controls (Girdler, 1995), and some studies have shown that although absolute levels of PRL are not altered in women with PMS, the timing of their excretion may differ from controls (Parry & Newton, 2001).

In addition to female sex hormones, testosterone has also been implicated in the aetiology of PMS/PMDD. Eriksson, Sundblad, Lisjo, Modigh, and Andersch (1992) reported that women with PMS had higher serum levels of free testosterone (FT) and suggested that androgens may be associated with premenstrual irritability and dysphoria. Similarly, Dougherty, Bjork, Moeller, and Swann (1997) postulated that increased levels of testosterone (T) are related to irritability and impulsive symptoms, although in their study the relationship did not differ between women with and without premenstrual complaints, and a significant relationship was only found during the midfollicular phase.

Overall, there is compelling evidence for cyclical changes in mood as well as physical symptoms in women with PMS/PMDD, and perhaps also to a lesser extent in non-help-seeking women. In particular, mood appears to be impaired during the late luteal (premenstrual) and menses phases of the cycle, when steroidal hormone levels are low. Whilst the reduction in these hormones just prior to menses is probably the trigger for these symptoms, the exact mechanisms underlying the observed changes are as yet unclear. The effects of estrogen on the serotonergic system provide the most comprehensive theory to date, although complex interactions with other hormones and neurotransmitters are likely also involved.

2.4. Aggression and the Menstrual Cycle

2.4.1. Definition and Different Forms of Aggression

The study of aggression has been hampered by the lack of consensus on a definition of what aggression is and what constitutes aggressive behaviour. According to Archer (2009) a generally accepted definition of aggression is that it is a behaviour intended to harm another individual. However, different types of aggression have also been described. Sex differences in aggression originally began being studied in the 1920s and focussed on explicit forms of aggression such as physical and verbal forms. Later studies also explored other "relational" forms of aggression that were reported to be more typical of girls than boys (Lagerspetz, Bjorkqvist, & Peltonen, 1988). Björkqvist (1994) used the terms "direct" and "indirect" aggression to refer to overt (e.g. physical) and covert (e.g. malicious gossip) acts of aggression, respectively, and suggested that examination of both forms of aggression is necessary when studying human aggression.

Hormones have long been known to be involved in the aetiology of aggression and the relationship between endocrinology and dominance behaviour has been of interest since it was observed that the removal of a rooster's testes resulted in a reduction in dominance behaviour, whilst replacement of the testes led to resurgence of this behaviour (Berthold, 1849; in Cooke, 2006). Testosterone and other androgens are widely believed to have the most influence over this behaviour since males are typically more aggressive than females (Baillargeon et al., 2007; Eagly & Steffen, 1986; Wilkowski, Hartung, Crowe, & Chai, 2012). Indeed, a relationship between testosterone and aggression has also been found in women, with free testosterone being correlated with both physical and verbal aggression (Von Der Pahlen, Lindman, Sarkola, Mäkisalo, & Eriksson, 2002). Whether the relationship between testosterone and aggression in women changes across the menstrual cycle is unclear, as it has been suggested that this relationship may only be evident during the midfollicular phase (Dougherty, Bjork, Moeller, et al., 1997).

However, there is some evidence that female sex hormones may also play a role in aggression, and emphasis has more recently been placed on investigating these relationships. In the early 1970s, the aromatisation of testosterone to estrogen was found to be responsible for some sexual behaviour (Whalen & Hardy, 1970), leading to the

idea that this process may also be involved in aggression. In support of this theory, not only has administration of estrogen been shown to increase aggression in several species (as discussed below), but aromatase activity in the brain was also found to correlate with aggression (Schlinger & Callard, 1989; Soma et al., 2000).

One way in which the effects of female sex hormones on aggression have been studied is by looking at changes in aggression across the menstrual cycle as levels of female sex hormones fluctuate. As discussed above, many females experience mood changes during the late luteal phase of the cycle, and it has been suggested that aggression is one of the most commonly experienced premenstrual symptoms and may even underlie some of the other mood related symptoms (Yonkers, 1997), yet aggression as a specific symptom has been largely overlooked in the literature. This is surprising given the reports of an increased likelihood of women committing violent crime during the premenstrual phase compared to other phases (D'Orban & Dalton, 1980), as well as an increase in psychotic episodes during this phase (O'Dwyer, Friedman, & Clifford, 1997).

2.4.2. Aggression across the menstrual cycle

Although few studies have focussed on cyclical changes in aggression as a primary endpoint, several studies included this outcome in investigations of premenstrual symptoms more broadly. These studies as well as studies investigating direct hormonal relationships with aggression are summarised in Table 2.2. Several studies have reported more severe anger and aggression during the luteal phase, particularly in women with PMS (Bond, Critchlow, & Wingrove, 2003; Van Goozen et al., 1996). Others have suggested that aggression is increased in women with PMS regardless of cycle phase (Dougherty, Bjork, Huang, & Moeller, 1997; Dougherty, Bjork, Cherek, Moeller, & Huang, 1998). On the other hand, it has been reported that physical aggression is increased during menses in asymptomatic women (Ritter, 2003). Some studies have also reported no cyclical variations in aggression in women with and without PMS (Brambilla, Speca, Pacchiarotti, & Biondi, 2010; Dougherty, Bjork, Moeller, et al., 1997). It has been suggested that the increase in aggression in the luteal phase in symptomatic women may only be apparent in response to provocation (Van

Goozen et al., 1996), which could provide an explanation for the lack of findings in some of these studies.

2.4.3. Theories of mechanisms underlying menstrual cycle effects on aggression

It is well established that T is positively correlated with aggression, and it should be taken into consideration that levels of plasma T are higher during the ovulatory and luteal phases (Eriksson, Fukunaga, & Lindman, 1994). However, Dougherty et al. (1997) reported that the relationship between T and aggression in women was only significant during the midfollicular phase and not during other phases, and that although plasma T fluctuated across the cycle aggressive behaviour did not. Furthermore, they found a trend for women whose plasma T levels remained fairly constant to be more aggressive overall.

The direct effects of female sex hormones may therefore explain fluctuations in aggressive behaviour. In particular the effects of E will be discussed here since E is the focus of this thesis, however the contribution of P will also be considered where appropriate. As mentioned in section 2.2, ERs are highly concentrated in the BNST and MEA, and these regions have been shown to be involved in the regulation of emotional behaviours, including aggressive behaviour (Nomura et al., 2003). Interestingly, research by Ogawa, Lubahn, Korach, and Pfaff (1997) using "Knockout mice" showed that the two types of ER had opposite functions. In studies of knockout mice, genes are disrupted so that the proteins controlled for by the specific gene will not operate. When the ERα gene was disrupted, these mice showed decreased levels of aggressive behaviour during resident-intruder tests, suggesting that ERα may be aggression-promoting. However, when ERβ was disrupted, mice showed increased levels of aggression during these tests, indicating that ERβ may inhibit aggression. Reduced ERα activity in brain regions associated with aggressive behaviour has been found following treatment with an aromatase inhibitor, as well as a reduction in aggressive behaviour (Trainor, Greiwe, & Nelson, 2006), lending support to the theory that ERα is aggression-promoting.

Table 2.2 *Summary of studies of aggression across the menstrual cycle and its association with endogenous hormones*

Study	Sample/ Design/Duration	Measure(s)	Outcome	Comments
Bond et al. (2003)	24 PMDD sufferers 18 asymptomatic controls 24-45y Follicular and late luteal phases Mixed between-within subjects design	Conflict Tactics Scale	More aggressive problem-solving tactics during luteal compared with follicular phase in women with PMDD only. Women with PMDD also had higher lifetime history of aggression.	Testing conducted during different menstrual cycles introducing issues of inter-cycle variability
Brambilla et al. (2010)	15f asymptomatic (27.3y) Early follicular, midluteal and late luteal phases Within-subjects	Buss-Durkee Rating Scale	Non-significant cyclical variations in aggression. Positive correlation between estrogen and verbal aggression during follicular phase, and between estrogen and resentment during late luteal phase.	Findings suggest some specific aspects of aggression may be related to hormonal changes across the cycle. No correlation between testosterone and any outcome.
Dougherty, Bjork, Huang, & Moeller (1997)	40f High PMS symptoms (n=20; 27.1y) or Low PMS symptoms (n=20; 26.6y) Menses, late luteal phase and rest of cycle Between-subjects	PSAP	Participants with high premenstrual symptom scores showed higher rates of aggressive responding than those with lower symptom scores regardless of phase.	Aggressive responding correlated with negative affect and behaviour change during menses and luteal phase only, suggesting increased aggressive behaviour in these phases in women with more severe PMS symptoms
Dougherty, Bjork, Moeller, & Swann (1997)	12f (24.8y) 18-39y High PMS symptoms (n=5) or Low PMS symptoms (n=7) Menses, midfollicular, ovulatory and late luteal phases Mixed between-within subjects design	PSAP	More aggressive responding in high symptom group. No cyclical changes in aggression in either group.	Little relationship between testosterone and aggression during menses and late luteal phase
Dougherty et al. (1998)	22f High PMS symptoms (n=11; 28.1y) or	PSAP; POMS	More aggression in high symptom group than low symptom group regardless of cycle phase.	

	Low PMS symptoms (n=11; 24.1y) Menses, midfollicular, ovulatory and late luteal phases Mixed between-within subjects design		Aggression correlated with behavioural and psychological, but not somatic, symptoms.	
Ritter (2003)	29f (21.6y); 22m (22.3y) Menses and midluteal phase Mixed between-within subjects design	Buss-Perry Aggression Questionnaire	Physical aggression higher during menses than midluteal phase. Trend effect for verbal aggression in the same direction.	No significant sex difference when women were in menses, but increased physical aggression in men compared with women in luteal phase
Stanton & Schultheiss (2007)	53f (19.96y) Acute	Picture story exercise; Questionnaire containing dominance and aggression scales	Positive correlation between basal estradiol levels and power motivation. Relationship stronger in single women and those not using hormonal contraceptives. Contest winners high in implicit power motivation showed post-contest increases in estradiol, whereas losers showed post-contest decreases.	No relationship between basal testosterone and dominance. Supports the theory that estrogen rather than testosterone may mediate aggression in women.
Stanton & Edelstein (2009)	44f (18.58y) Acute	Picture story exercise	Positive correlation between estradiol and implicit power motivation.	Effect only seen in single women and those not using hormonal contraceptives.
Van Goozen et al. (1996)	58f Premenstrual complaints tested in luteal phase(n=20; 29.4y); no complaints tested in luteal phase (n=20; 28.1y) intermediate tested in follicular phase (n=18; 29.2y) Between-subjects	POMS; STAS; ASQ; Angry Responding	Anger more prominent in luteal phase in response to provocation, particularly in women with premenstrual complaints. Objective measures and self-reports both showed increased anger in women with premenstrual complaints in luteal phase compared with both other groups	No baseline differences in self-reports

PMDD, Premenstrual Dysphoric Disorder; y, Years; f, Female; PMS, Premenstrual Syndrome; PSAP, Point Subtraction Aggression Paradigm; POMS, Profile of Mood States; m, Male; STAS, State-Trait Anger Scale; ASQ, Anger Situation Questionnaire

The serotonergic system has been suggested to be the most likely mediator between female sex hormones and aggression. ERβ messenger ribonucleic acid (mRNA) and protein are abundant in the midbrain raphe nuclei, including the dorsal and median raphe, where the largest population of serotonin (5-HT)-synthesising neurons are found (Mitra et al., 2003), and as described in section 2.3.4, female sex steroids have been shown to modulate serotonergic transmission. Reduced serotonergic function is known to be related to impulsive aggression, and tryptophan depletion which results in lowered levels of serotonin has been shown to increase aggression in hostile men (Cleare & Bond, 1995). Moreover, the reduced rate of serotonin synthesis following tryptophan depletion is more pronounced in women than in men (Nishizawa et al., 1997), and tryptophan depletion during the premenstrual phase has been shown to result in increased levels of aggression in response to provocation compared to when a control drink was administered (Bond, Wingrove, & Critchlow, 2001). Conversely, however, one study has actually demonstrated a positive association between plasma tryptophan and trait hostility, anger, anger expression and an antagonistic interpersonal style in women (Suarez & Krishnan, 2006). In that study tryptophan was not related to measures of impulsivity. These findings may suggest that whilst serotonin may be associated with increased levels of anger and hostility, the lack of increase in impulsivity could prevent the outward, or physical, expression of these tendencies, which has been measured in the majority of other studies. The authors also speculated that the down-regulation of 5-HT receptors associated with aggression may be a compensatory response to higher levels of 5-HT.

The increase in levels of oxytocin in the presence of estrogen has also been suggested as an explanation for the effects of estrogen on aggression. Oxytocinergic projections to the amygdala are thought to be important for social recognition in mice, which helps to prevent aggression. Since only ERβ are expressed in oxytocinergic cells, this may explain the inhibitory effects of ERβ on aggression discussed above (Mong et al., 2003).

2.5. Cognition and the Menstrual Cycle

Cognitive function refers to human information processing and includes functions such as attention, memory, learning, language processing, problem solving and abstract reasoning (Kolb & Whishaw, 2009). Although cognitive impairment is not usually included in measures of premenstrual symptoms, women who report experiencing somatic and affective symptoms often also report impairment in cognitive functioning during the luteal phase of the cycle (Rubinow & Byrne, 1984). This has led researchers to examine whether performance on various cognitive tasks is actually impaired during the luteal phase as subjective reports suggest. Whilst academic and work performance on the whole has been shown not to be affected (Omu, Al-Marzouk, Delles, Oranye, & Omu, 2011), the examination of specific cognitive domains has revealed some interesting findings. Particular attention has been paid to tasks which show differences in performance between males and females, such as verbal fluency and fine motor skills which favour females, and spatial ability which favours males, and how performance on these tasks varies across the menstrual cycle. Sex differences in performance on these tasks has long been hypothesised to be due to hormonal differences between the sexes (Halpern, 1992), and studies from the past two decades have served to verify this assumption. Cyclical changes in cognitive functions in both PMS/PMDD sufferers and non-help-seeking women will be reviewed in this section, with specific focus on the contribution of estrogen to these effects where appropriate.

2.5.1. Cognitive function across the menstrual cycle in women with PMS/PMDD

In one study, women meeting the criteria for PMS/PMDD were compared with control women, and women with more severe symptoms were found to exhibit poorer performance on a digit-span task during the premenstrual phase, whereas women with low symptom severity performed best during this phase. Women with high symptom severity also responded significantly slower on a letter detection task in both the premenstrual and postmenstrual phases (Diener, Greenstein, & Turnbough, 1992). However, the authors concluded that the differences in cognitive function were minimal and that the difference in symptom severity had a greater effect than cycle phase. It

should be noted that this study employed a small sample of only 16 women aged 27 to 43 years, and that symptoms were rated retrospectively, therefore the validity of the findings may be questionable.

In another study comparing symptomatic women with women displaying no cyclical symptoms, several domains of cognitive function were assessed both pre-menstrually and post-menstrually. Women with PMDD were found to perform significantly worse in the luteal phase than control women on tasks of delayed digit recognition, immediate and delayed word recall, delayed word recognition and a digit symbol substitution task. Both groups showed a trend for poorer performance on a divided attention task during the luteal phase compared to the follicular phase (Reed et al., 2008). These findings suggest that certain women may experience more pronounced changes in cognitive function across the menstrual cycle than others, but even women not meeting the criteria for PMS or PMDD may also experience impairment in some tasks during the luteal phase. The study was limited by its small sample size and the fact that some, but not all, participants received a caffeinated beverage with breakfast, which may have strongly influenced the cognitive outcomes.

2.5.2. Cognitive function across the menstrual cycle in non-help-seeking women

An early study of cognitive changes across the menstrual cycle found that reaction times were faster at times of high progesterone levels, but were actually fastest during the premenstrual phase when levels of both estrogen and progesterone decline. This led the authors to suggest a time lag between the period of highest progesterone levels and optimal performance on reaction time tasks (Wuttke et al., 1975). A similar pattern was seen with a complex calculation task in the same study, where reaction times as well as accuracy were improved during the luteal phase.

Several studies have reported improved performance on tasks showing a female bias and impairment on tasks showing a male bias during the luteal phase when estrogen and progesterone levels are high, whereas the opposite is found during the menses and follicular phases when levels of these hormones are lower. In a study designed to examine the effects of estrogen independently of progesterone on tasks specifically

chosen to reflect sexually dimorphic abilities, women were tested during menses, when estrogen is low, and during the preovulatory phase when estrogen peaks. Progesterone during both these phases is low and therefore should not contribute to any observed differences in performance. Spatial ability, which is usually favoured by males, was significantly enhanced in menses compared to the preovulatory phase, a pattern which was also found for the male-positive task of deductive reasoning although this was not significant. Tasks of articulatory and manual speed are generally performed better by females than males and were significantly enhanced during the preovulatory phase, as was expected. Contrary to expectations, however, perceptual speed and verbal fluency showed no significant differences between phases (Hampson, 1990a). Cyclical changes in spatial memory were also reduced to a trend effect when mood subscales were included as covariates, although these covariates were not themselves significant. Taken together these findings suggest that performance on certain cognitive tasks may be influenced by cyclical changes in estrogen, however as the author noted, these fluctuations in performance are relatively small and other factors may contribute more to cognitive functioning.

In a similar study by the same author, women were tested during the menstrual and midluteal phases of the cycle in a counterbalanced order. When only the first test session was analysed, women tested during the menstrual phase outperformed those tested during the midluteal phase on tasks measuring spatial ability and deductive reasoning, which are usually favoured by males. Women tested during the midluteal phase performed significantly better on tasks of articulation, which are usually favoured by females. However, as in the previous study the magnitude of these phase differences were reported to be relatively small on the whole (Hampson, 1990b). When within-subject comparisons were conducted between the two phases, performance during the luteal phase was found to be enhanced on speeded articulation, manual speed/coordination, verbal fluency and two subtests of perceptual speed, confirming the enhancement of tasks generally performed better by females during the luteal phase. However, for male-specific tasks the enhancement of performance during the menstrual phase was only evident for deductive reasoning, whereas for spatial abilities the expected variation was not observed. The author suggested that this was due to an asymmetric carryover effect, in that subjects who initially completed the task under favourable hormonal conditions developed better skills for performing the task a second

time. The enhanced performance on tasks of verbal fluency and perceptual speed during the luteal phase in the second study, but not in the preovulatory phase in the first study, suggest that progesterone may play more of a role in these abilities than estrogen.

In normally cycling healthy women, delayed recall of visual stimuli was shown to be impaired during the menstrual phase compared to the luteal phase (Phillips & Sherwin, 1992). In that study, no other measures of memory including digit span, paired-associate learning, immediate recall of visual stimuli or immediate or delayed paragraph recall showed differences in performance between the phases. However, paired-associate learning was positively correlated with plasma estradiol levels in the luteal phase, and delayed visual recall was positively correlated with plasma progesterone levels in the luteal phase. The authors also reported that only approximately one half of the sample experienced impairments in visual memory in the menstrual phase, whereas others showed no cyclical variation. In the subgroup that did experience detriments, the correlations between hormones and performance were stronger. This was not due to differences in hormone levels between groups, suggesting an increased sensitivity of a subgroup of women to physiological variations in sex-steroid levels across the menstrual cycle.

In contrast to the findings of Hampson (1990a; 1990b), Gordon and Lee (1993) reported no significant differences across the menstrual cycle on either a "visuospatial" or a "verbosequential" composite of cognitive function. In that study, women were tested during the menses, follicular and luteal (days 20-24) phases. Analysis of the relationship between the gonadotropins and the steroidal hormones with the cognitive outcomes suggested that gonadotropins may play a larger role in predicting cognitive performance than steroid hormones. Possible explanations for these divergent findings include the higher than average performance of women in the sample, potentially giving rise to ceiling effects, as well as the different tests used in this study. Furthermore, analysis of individual tasks may have revealed significant effects on certain tasks where the combination of tasks into a composite may have masked the results.

In a study that focussed solely on the mental rotation task, which is generally performed better by males than females, performance was enhanced in menses compared to the luteal phase as expected, and testosterone was reported to have a positive influence on performance whilst estrogen had a negative influence (Hausmann et al., 2000). Further

research found significant cyclical changes in verbal working memory, but no changes in spatial ability. In a small study of eight young normally cycling (NC) women, verbal span was increased in the middle of the cycle compared with the beginning and end of the cycle, whereas no differences were seen in a mental rotation task (Rosenberg & Park, 2002). The pattern seen in performance on the verbal memory task parallels the body's estrogen levels. However, the lack of hormone assays to measure circulating estrogen means that no associations could be explored in this study. Furthermore, the small sample size including only college students makes generalisation to the broader population impossible.

In a small study of performance on several different tasks during the early follicular and mid luteal phases, no difference between phases was found on a task measuring explicit memory, however performance on a task of implicit memory was improved in the mid luteal phase compared with the follicular phase. Interestingly, women who were first tested during the follicular phase also showed more priming than those first tested during the luteal phase on a fragmented object identification task, a measure of perceptual implicit memory, although the overall magnitude of priming did not vary across the cycle. The authors suggested that high levels of ovarian hormones may inhibit perceptual priming, and that women who first perform the task when these hormones are low maintain a high level of performance when tested again (Maki, Rich, & Rosenbaum, 2002). In accordance with other studies, verbal fluency and fine motor skills were also found to be improved during the luteal phase, whereas mental rotation was worse during this phase compared with the follicular phase. Since estradiol was found to correlate positively with verbal fluency and negatively with mental rotation and perceptual priming, it was concluded that estrogen, and not progesterone, was responsible for the observed effects.

In a small study assessing performance on tasks measuring sustained attention, executive function, manual coordination, visuo-spatial memory, verbal fluency and spatial ability, nine 19-34 year old women with regular menstrual cycles were tested during the ovulatory, early luteal, late luteal and menstrual phases. Sustained attention was improved during the early luteal phase, when progesterone levels are high. During the ovulatory phase, when estrogen levels are high, visuo-spatial memory was improved

whereas verbal fluency was impaired. No other cognitive components showed significant cyclical variations (Solís-Ortiz & Corsi-Cabrera, 2008).

Reaction time to both visual and auditory stimuli has recently been shown to be affected by menstrual cycle phase. A study of 19 women during the menses, early follicular, late follicular and midluteal phases found slower reaction times during menses and the early follicular phase, when levels of steroid hormones are low, compared to the other phases where levels of either estrogen alone or estrogen and progesterone are high (Šimic & Ravlic, 2013). Although the authors did not measure circulating hormone levels, basal body temperature was measured and is thought to be a reliable method of estimating cycle phase.

To investigate the effects of steroidal hormones on cerebral asymmetry, Can, Hahn, Ocklenburg, Ball, and Güntürkün (2012) tested women during the menses, follicular and midluteal phases of the menstrual cycle on a verbal dichotic listening task. Although no effect of cycle phase was seen in the total sample of women, when only those who had available estradiol measurements were analysed there was a difference in the number of correct responses between the menses and follicular phases, such that scores were higher during menses. The degree of language asymmetry did not change across phases, suggesting that estradiol affects auditory recognition without altering language asymmetry as expected.

In a brief review of the more extant literature in this area, Sherwin (2012) concluded that there is strong evidence to suggest that changes in endogenous hormones across the menstrual cycle influence performance on several cognitive tasks, however the magnitude of changes may not be clinically significant. Furthermore, when considering the studies reviewed above it appears that women who experience changes in physical and emotional symptoms across the menstrual cycle may show more pronounced changes in performance of different cognitive tasks across the menstrual cycle.

In addition to fluctuations in steroidal hormones across the menstrual cycle as a means of examining the effects of hormones on cognition, the decline in endogenous estrogen levels following menopause has also been used to investigate the effects of estrogen (or lack of) on cognitive function. Dumas, Hancur-Bucci, Naylor, Sites, and Newhouse (2006) reported no significant differences in cognition following three months treatment

with 1 mg 17β-estradiol compared with placebo, suggesting no positive impact of estrogen on any cognitive domains. Estrogen did, however, protect against cognitive impairment induced by anticholinergic drugs on some tasks (see section 2.5.3). The authors suggested that the lack of significant findings of some studies in this area may be due to ceiling effects and an inability to detect changes in cognitively normal subjects. This may particularly apply to the studies reviewed above since younger populations are less likely to display cognitive deficits.

2.5.3. Potential mechanisms of action of endogenous estrogen on cognition

17-β-estradiol is known to have neuroprotective effects. Preincubation of mouse hippocampal cells with 17-β-estradiol prior to insult with the neurotoxins amyloid β protein, hydrogen peroxide and glutamate resulted in a prevention of oxidative stress-induced cell damage and cell death, and cell survival was increased almost to the level of untreated control cells (Behl, Widmann, Trapp, & Holsboer, 1995). In the same study, DNA degradation caused by glutamate was also blocked by estradiol. Other steroid hormones did not protect the cells, suggesting that estradiol alone may underlie cognitive fluctuations across the menstrual cycle rather than other female sex hormones. Furthermore, the effects were found to be estrogen receptor-independent since protection was only evident at higher doses, thus suggesting that the antioxidant activity of the hormone may underlie the effects rather than receptor-mediated effects.

Dendritic spine density in hippocampal CA1 pyramidal cells has also been shown to be increased during times of increased estradiol in the rodent estrous cycle as well as following administration of estradiol (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley, Gould, Frankfurt, & McEwen, 1990; Woolley & McEwen, 1993). The increase in dendritic spines is paralleled by increased density of synapses, indicating that new spines form synaptic contacts (Woolley & McEwen, 1992). Dendritic spine function has been implicated in synaptic integration and neuroprotection (Segal, 1995; Yuste & Denk, 1995), and increased spine density results in increased excitatory input to CA1 pyramidal cells, which therefore increases hippocampal excitability and plasticity (Woolley, 1998). Long term potentiation (LTP), which is a model for cellular changes that might be involved in learning and memory, has also been shown to be affected by E_2, with administration of E_2 reducing the threshold for LTP induction

(Woolley, 1998). The increase in spine density in the hippocampus suggests a potential mechanism by which E_2 may affect cognitive function, and although there is a lack of consistent evidence for the effect of ovarian hormones on hippocampus-dependent spatial abilities there is a suggestion that sustained elevation of E_2 positively influences hippocampus-dependent behaviour (Woolley, 1998). In addition, intrahippocampal administration of estradiol following training in a water maze resulted in improved retention 24 hours later (Packard & Teather, 1997).

It has been suggested that the effect of estradiol on neuroprotection is dependent on the activation of *N*-Methyl-D-Aspartate (NMDA) by estradiol, since the effects of estradiol administration were blocked by treatment with both a competitive and a non-competitive NMDA receptor antagonist (Woolley & McEwen, 1994). Woolley, Weiland, McEwen, and Schwartzkroin (1997) also demonstrated that NMDA receptor binding was 30% greater in the stratum radiatum of the CA1 region in estradiol-treated rats compared with OVX controls, and 46% greater in the stratum oriens. Interestingly, rats treated with estradiol followed by progesterone had NMDA receptor binding levels intermediate between those treated with estradiol only and OVX rats. This suggests that the dendritic spines and synapses induced by estradiol might be specifically enriched in NMDA receptors. Furthermore, the increase in dendritic spine density was correlated with sensitivity to NMDA receptor-mediated synaptic input (Woolley et al, 1997).

It is thought that the effects of E on cognition may be due to increases in brain activity only during times of heavy cognitive load. Administration of 1mg 17-β-estradiol to postmenopausal women resulted in increased frontal activation during conditions of difficult working memory load compared to women treated with placebo, as measured using functional magnetic resonance imaging (fMRI) (Dumas, Kutz, Naylor, Johnson, & Newhouse, 2010). This would explain why cyclical variation in cognitive performance is only seen in some, usually more demanding, tasks involving frontal functions.

It has been demonstrated that estrogen affects the extracellular regulated kinase/mitogen activated protein (ERK/MAP) kinase pathway, which may explain the association between estrogen and increased synaptic plasticity and therefore the effects on cognition. In ovariectomised (OVX) rats not treated with estradiol, levels of active ERK2 were lowered by 95%, but this effect was almost completely reversed by

estradiol implantation. In addition, levels of tyrosine phosphorylation of NR2 subunits of NMDA receptors in OVX females with E_2 implants were double the levels of those without implants. Furthermore, in intact females there was a 3-fold increase in the state of activation of ERK2 kinase during proestrous (when endogenous estrogen levels are high) compared to diestrous (when estrogen levels are low), as well as a 50% increase in levels of tyrosine phosphorylation of NR2 subunits at proestrous compared to diestrous. This was accompanied by greater long term potentiation in hippocampal CA1 tissue from females in proestrous compared to tissue from females in diestrous (Bi, Foy, Vouimba, Thompson, & Baudry, 2001). The authors suggested that circulating levels of estrogen regulate the activation state of ERK2, which in turn results in the tyrosine phosphorylation of NR2 subunits of NMDA receptors and therefore an increase in NMDA receptor function. This would account for the changes in magnitude of long term potentiation observed across the estrous cycle. This pathway is known to be critical for learning and memory and may explain cognitive fluctuations across the menstrual cycle in humans.

Estrogen has also been shown to affect the cholinergic system (Kaufman, Vadasz, & Lajtha, 1988) which is thought to be particularly vulnerable to cognitive decline (Gibbs, Wu, Hersh, & Pfaff, 1994) and is important for attention and memory encoding (Gais & Born, 2004). OVX rats administered 17-β-estradiol demonstrated increased choline acetyltransferase (ChAT) activity in the caudate nucleus, cortex, hippocampus and hypothalamus (Kaufman, Vadasz, & Lajtha, 1988). ChAT catalyses acetylcholine synthesis and is a marker of cholinergic neurons, therefore these findings suggest that E_2 increases cholinergic activity.

In a small scale crossover trial of postmenopausal women, 15 women were administered either 1 mg 17β-estradiol or placebo for three months, after which they performed a series of cognitive tasks following anti-cholinergic challenges. Estrogen pre-treatment was found to attenuate the anti-cholinergic drug-induced impairments in tasks of attention as well as tasks with speed components (Dumas et al., 2006). Further studies by the same group revealed that E_2 reduced impairment on a verbal memory task specifically following administration of a muscarinic receptor antagonist in younger postmenopausal women, whereas only a trend in the same direction was found for a nicotinic antagonist. Furthermore, in older postmenopausal women E_2 impaired

performance on the same task (Dumas, Hancur-Bucci, Naylor, Sites, & Newhouse, 2008). A later study using fMRI revealed that estradiol significantly reduced activation in the left medial frontal gyrus during anti-muscarinic challenge, whereas it increased activation in the precuneus during anti-nicotinic challenge, demonstrating that estradiol modulated cholinergic system regulation of brain activation related to cognitive function (Dumas, Kutz, Naylor, Johnson, & Newhouse, 2012).

2.6. Chapter Summary

The literature regarding the effects of fluctuations in endogenous estrogens across the menstrual cycle as well as across the life span is extremely broad, and only the more recent and more historically important findings have been reported here. These findings suggest that cyclical fluctuations in mood, physical symptoms, aggression and cognitive function may be evident in many women, but are particularly pronounced in a subgroup of women who meet the criteria for PMS/PMDD. The divergent findings observed in some studies may be due to differences in the measures used to assess outcomes as well as differences in the way cycle phases are defined. It is difficult to ascertain the specific influence of estrogen through observations of behaviour across the menstrual cycle due to the fact that other hormones also fluctuate across the cycle. However, the evidence seems to suggest that estrogen may affect general mood and aggression through actions on the serotonergic system, whereas cognitive effects are more likely due to actions on the cholinergic system. The next chapter will review the literature on the effects of oral contraceptives and synthetic estrogens which may help to shed more light on this topic.

Chapter 3

Oral Contraceptives and Synthetic Estrogens

3.1. An Introduction to Oral Contraceptives

Oral contraceptives (OCs) are the most widely prescribed form of reversible contraception, and are used by over 100 million women worldwide (Christin-Maitre, 2013). Over one quarter of Australian women of child bearing age use OCs, with women aged under 24 years being most likely to use this form of contraception (Mazza et al., 2012). The most common type is the combined oral contraceptive pill (COC), which contains varying amounts of ethinyl estradiol and progestins depending on the brand. The COC was originally formulated by accident in 1959 when the progestin-containing contraceptive being tested was contaminated with the ethinyl estrogen mestranol, and was found to result in less breakthrough bleeding than progestin alone. Since their introduction in the 1960s, the gonadotropin-inhibiting properties of ethinyl estrogens have been recognized and as mestranol is rapidly converted to ethinyl estradiol (EE), EE is now the estrogen used in almost all COCs (Amy & Tripathi, 2009).

Besides the obvious utilisation of OCs for the prevention of pregnancy, OCs have also been described as having several non-contraceptive related benefits. OC use is associated with multiple health benefits, such as lower risk of endometriosis as well as endometrial and ovarian carcinoma (Maia & Casoy, 2008) and reducing the risk of benign breast disease such as chronic cystic disease (Vessey & Yeates, 2007). On the other hand, OC use is also associated with increased risk of cardiovascular and gall bladder diseases (Farmer, Lawrenson, Thompson, Kennedy, & Hambleton, 1997; Petitti et al., 1996) and has been shown to have deleterious effects on immune system function (Scanlan, 1995). Previous research has shown that OC users may be less aggressive than non-users (Perry, Canning, Scholey, & Dye, under review), therefore a group of OC users were included as a positive control in the current study. The effect of OC use on mood and premenstrual symptoms is controversial, and the debate over whether COCs may be effective in improving mood, premenstrual symptoms or cognition is ongoing and contributes to the worldwide discussion of whether OC use is beneficial or detrimental to overall health. Some of the key literature regarding these effects will be discussed in this chapter.

3.1.1. Chemistry of Oral Contraceptives

The exact chemical composition of COCs depends on the brand, but they generally contain between 20 and 35 µg EE and varying amounts of different progestins. They can, however, be broadly divided into different generations. First generation OCs are no longer prescribed as they contained higher doses of EE which resulted in more serious side effects (Stanczyk, Archer, & Bhavnani, 2013). Second generation OCs (e.g. Levonorgestrel) contain progestins that are derived from testosterone, therefore they are more androgenic than the other OCs. Third generation OCs (e.g. gestodene, norgestimate, desogestrel) were developed using progestins with less androgenic activity. Newer generation OCs, such as Yasmin and Yaz, contain the progestin drospirenone (DRSP) which is derived from spironolactone rather than progesterone or an androgen, and has both anti-mineralocorticoid and anti-androgenic properties.

3.1.2. Pharmacokinetics of Oral Contraceptives

There is great variability in the pharmacokinetics of EE due to the fact that they undergo enterohepatic recirculation. Oral EE is very rapidly absorbed, commonly 90% during the first hour, however in some individuals absorption takes up to two hours. The peak blood level of EE is commonly reached within two hours, but again there is great individual variability, taking up to six hours in some women (Goldzieher, 1989). In addition to inter-individual variability in the pharmacokinetics of EE, there is also day-to-day intra-individual variability, such that the same dose of EE taken on the same cycle day during consecutive menstrual cycles can result in up to a four-fold variation in the amount of EE absorbed (Goldzieher, 2008). It has been reported that the peak plasma concentrations of ethinylestradiol (100pg/ml) and the progestin levonorgestrel (6ng/ml) are similar to those of 17β-estradiol and progesterone in women during the mid-luteal phase of the menstrual cycle (Diliberti, O'Leary, Hendy, Waters, & Margolis, 2011). The bioavailability of EE ranges from 25% to 65% of the amount ingested, and the half-life ranges from 6 to 27 hours (Goldzieher & Stanczyk, 2008). The variability in pharmacokinetics may underlie discrepancies between studies in this field.

The binding affinity of EE to the estrogen receptor (ER) has been shown to be 1-2 times higher than estradiol (E_2) in humans (Tilton, Foran, & Benson, 2005). Because oral estrogens are metabolised through the hepatic portal system, this results in higher conversion to estrone and lower bioavailability of E_2.

3.2. Oral Contraceptives, Mood and Premenstrual Symptoms

Although numerous studies have investigated the effects of OC on mood and premenstrual symptoms, the findings reported in the literature are somewhat contradictory and a consensus has not yet been reached on whether the effects, if there are any, are positive or negative. These studies are summarised in Table 3.1. It has been estimated that although most women using COCs are satisfied with the effects of their OC use, between four and ten per cent of users experience mood side effects including depressive symptoms, irritability and mood swings (Kelly et al., 2010) and mood-related side effects are one of the major reasons for discontinuing OC use (Kay, 1984; Lindh, Blohm, Andersson-Ellström, & Milsom, 2009; Sanders, Graham, Bass, & Bancroft, 2001). Newer generation pills containing anti-androgenic progestins have been shown to have a better mood side effect profile than older more androgenic progestins such as levonorgestrel and are the primary focus of most current research, as discussed below (Poromaa & Segebladh, 2012).

Early research generally focussed on the prevalence of depression in OC users versus non-users, and observational and cross-sectional studies are still common in this field. Several authors reported increased rates of depression in OC users (Cullberg, 1972; Herzberg, Johnson, & Brown, 1970; Kulkarni, Liew, & Garland, 2005; Nilsson & Almgren, 1968; Oddens, 1999). However, lower rates of depression and reduced symptom severity in OC users were also reported (Berenson, Odom, Breitkopf, & Rahman, 2008; Deijen, Duyn, Jansen, and Klitsie, 1992; Herzberg, Draper, Johnson, and Nicol, 1971; Mordecai, Rubin, & Maki, 2008; Nyberg, 2013; Ott, Shew, Ofner, Tu, & Fortenberry, 2008; Toffol, Heikinheimo, Koponen, Luoto, & Partonen, 2011; Toffol et al., 2012), as well as no positive or negative effects of OC use (Duke, Sibbritt, & Young, 2007; Graham, Bancroft, Doll, Greco, & Tanner, 2007; Griksiene & Ruksenas, 2011; Rosenthal, Cotton, Ready, Potter, & Succop, 2002; Sveindottir & Backstrom,

2000; Vessey, McPherson, Lawless, and Yeates, 1985). Much of the variability in findings of these studies is likely due to the different OC formulations used, in particular those studies that found negative effects of OC use on mood were mostly older studies and included women using older generation OCs. However, other confounding factors may also impact on these findings, and causality cannot be inferred from observational and cross-sectional studies. It should be noted that a large amount of bias is evident in these studies as most women who experience negative side effects of OC discontinue use, therefore the women included in the above studies were most likely those who did not experience negative side effects (Robinson, Dowell, Pedulla, & McCauley, 2004).

Although there are ethical considerations regarding randomising women to contraceptive treatment, some randomised controlled trials (RCTs) have been conducted. Several researchers have reported positive effects of OC use on mood (Graham & Sherwin, 1993; O'Connell et al., 2007; Walker & Bancroft, 1990) however these positive effects were also often observed following placebo treatment. In other studies, no significant differences between OC and placebo were found (Grinspoon et al., 2003). However, in the majority of studies, attrition rate was higher in those treated with OC than placebo (Graham & Sherwin, 1993; Grinspoon et al., 2003). In one study, mood was found to be worsened following OC treatment compared with placebo (Gingnell, Engman, et al., 2012). It should be noted that only women with a history of negative side effects of OC use were included in that study, and therefore they may be more prone to the negative effects. Furthermore, the progestin used in that study was levonorgestrel, which has androgenic properties and is more likely to have negative effects on mood than newer generation OCs.

Table 3.1 *Summary of studies of the effects of oral contraceptive use on mood and other premenstrual symptoms.*

Study	Sample size (n) Age Design	Dosing/ Treatment	Duration	Measure(s)	Outcome
Akerlund et al. (1993)	1000f 18-40y Randomised, double blind, no placebo control	30µg EE/150µg DSG or 20µg EE/150µg DSG	12 months	Not specified	Lower dose of EE associated with more side effects
Bancroft et al. (1987)	37f Randomised, no placebo control	Combined pill (Microgynon; n=18) or Triphasic pill (Logynon; n=19)	2 cycles	Daily ratings of mood, physical symptoms and sexual feelings	Women with history of premenstrual negative mood more likely to experience negative mood effects with combined OC, opposite for women without such a history.
Berenson et al. (2008)	608f 16-33y Longitudinal prospective study No placebo control	20µg EE/ 0.15mg DSG taken for 21 days, then 2 days placebo and 5 days 10µg EE	24 months	Symptom checklist	Less breast tenderness, cramping, hair loss, acne, nervousness and mood swings with OC. Increased inter-menstrual bleeding. DMPA did not show same beneficial effects on acne and breast tenderness, suggesting estrogenic mechanism on these symptoms
Deijen et al. (1992)	200 OC starters; 370 OC switchers; 140 NC 16-45y Observational	Minulet (30µg EE/75µg GSD)	3 months	AMQ; SIP	Starting OC use had no negative effect on depression, moodiness, anxiety or anger. Those who switched from a different OC showed improvements
Duke et al. (2007)	8636f 18-23y Cross-sectional	Various OCs	Retrospective reports of past 12 months	CESD-10	No difference between groups in likelihood of being depressed
Endrikat et al.	649f	20µg EE/75µg GSD	12 cycles	Self-reported	Lower dose of EE associated with more side

(1997)	18-39y Double blind, no placebo control	(n=428) or 30µg EE/75µg GSD (n=221)		adverse events	effects
Freeman et al. (2001)	82 PMDD sufferers 18-40y Double blind, placebo controlled RCT	30µg EE/ 3mg DRSP or placebo	3 cycles	COPE; BDI; POMS	Greater improvement in total COPE score with OC but not significant. Items "increased appetite", "desire to be alone" and "hot flushes" significantly improved with OC. Also greater improvement in BDI and POMS scores with OC
Freeman et al. (2012)	Review of 3 RCTs and one open-label study	20µg EE/90µg LNG continuous	Between 2 and 4 cycles	Daily Record of Severity of Problems or DSR	Concluded although findings are inconsistent there is an overall improvement in symptoms with continuous OC use. Studies had low statistical power and large placebo effect evident in RCTs
Gingnell et al. (2013)	34f with history of negative mood with OC 18-45y Double blind, placebo controlled RCT	30µg EE/0.15mg LNG or placebo	1 cycle	fMRI; MADRS-S; STAI-S; Cyclicity Diagnoser Scale	Increased depressed mood, mood swings and fatigue in the last week of treatment cycle with OC use compared with placebo. Also significant increase from baseline. Lower activity in left insula, left middle frontal gyrus and bilateral inferior frontal gyri with OC use
Graham & Sherwin (1993)	45 PMS sufferers 18-35y (29.5y) Double blind, placebo controlled RCT	Triphasic OC (n=20) or placebo (n=25)	3 months	Daily ratings of mood and sexual interest	Improved mood in luteal phase after 3 months OC use but also placebo. Reduced sexual interest with OC use. Side effects of OC use such as breakthrough bleeding and breast tenderness
Graham et al. (2007)	61f 18-31y (20.1y) Pre-post design, no placebo control	Triphasic OC with either 35µg EE or 25µg EE and NGM	3 months	BDI; Side Effects Questionnaire	No changes from baseline after 3 months, but not measured across the cycle
Greco et al.	48f	Triphasic OC with	3 months	BDI;	Slight improvement in mood. Lower dose gave

(2007)	18-30y (19.7y) Pre-post design, no placebo control	either 35µg EE or 25µg EE and NGM		Self-reported side effects	more improvement in mood, higher dose more improvement in physical symptoms
Griksiene & Ruksenas (2011)	20 NC (21.1y); 23 OC (21.8y) Cross-sectional	Various OCs	Testing during 3 phases of one cycle	PANAS	No difference between groups in positive or negative affect
Grinspoon et al. (2003)	45f 18-40y (26.5y) Double blind, placebo controlled RCT	35µg EE/180-250µ NGM or placebo	3 cycles	POMS	No group differences in global mood. More adverse events with OC, including anxiety and depression
Joffe et al. (2003)	658f 36-45y Cross-sectional	Various OCs	Retrospective reports of 3 months or more	Structured clinical interview	Worsening of mood with OC in women with history of depression only. Improved in women with early onset premenstrual mood disturbance or dysmenorrhea
Kelly et al. (2010)	424f 16-40y Randomised, single blind, parallel groups with no placebo control	30µg EE/3mg DRSP (n=282) or 30µg EE/150µg LNG (n=142)	7 cycles	MDQ	Similar beneficial effects on water retention and impaired concentration with both OCs. DRSP superior for improving physical well-being and negative affect during menses only. Most common reasons for withdrawal were related to mood
Kulkarni et al. (2005)	26 OC; 32 NC 18-50y Cross-sectional	Various OCs	Acute	BDI; HAMD; MADRS	Significantly higher depression symptoms with OC use. Mean scores of 2 measures suggested mild depression for OC users, normal scores for NC
Mordecai et al. (2008)	36f 18-40y Cross-sectional	Various OCs	Testing twice during one cycle	PANAS; CES-D; MDQ	Greater positive affect in OC users than non-users
Nyberg (2013)	24f 23-45y (31.1y) Pre-post design, no	35µg EE/ 250µg NGM	3 months	Cyclicity Diagnoser Scale	Significant improvements in mood, swelling, and effects on daily life with OC in those with more severe symptoms only

	placebo control				
O'Connell et al. (2007)	76f 16.8y Double blind, parallel groups RCT	20µg EE/100mg LNG (n=38) or placebo (n=38)	3 months	CES-D	No difference in depressive symptoms. Both groups showed improvements.
Oddens (1999)	1466f 20-49y Cross-sectional	Various OCs	Retrospective	Population survey	Irritability and depressed feelings worsened by OC use, but improvements in feeling relaxed. Past users reported more negative effects than current users
Oinonen & Mazmanian (2002)	Review of available literature	Various OCs	Various durations	Various measures	No overall positive or negative effect of OC on mood, but stabilising effect. Reductions in mood negative affect may reflect improved physical symptoms rather than pharmacological effect
Ott et al. (2008)	328f 14-22y (16.7y) Longitudinal study	Various OCs	Two 12-week periods per year for up to 41 months	Positive mood, negative mood and sexual interest scales	Positive mood higher and negative mood lower in stable OC users. Stable OC use associated with less cyclical mood variation
Pearlstein et al. (2005)	64f 18-40y (31.8y) Double blind RCT crossover	20µg EE/3mg DRSP or placebo 24/4 regimen	3 cycles	Daily Record of Severity of Problems	In first treatment period, mood improvement experienced by 51% OC and 31% placebo. In second treatment period, improvement experienced by 34% OC, deterioration experienced by 17% placebo
Poromaa & Segebladh (2012)	Review of placebo controlled RCTs	Various OCs	Various durations	Various measures	OCs may reduce mood swings and cycle variability. No mood deterioration evident as long as OC use initiated due to contraceptive need. Subgroup of women including those with PMDD most likely to benefit
Rosenthal et al. (2002)	43f 13-19y	20µg EE/100µg LNG	6 cycles	Not specified	Majority did not report worsening in any symptoms, despite over 50% expecting

	Pre-post design, no placebo control				increased weight gain and mood changes. All but one satisfied with OC
Sanders et al. (2001)	79f 22.5y Pre-post design, no placebo control	Randomly assigned to monophasic (35µg EE/250µg NGM) or triphasic (35µg EE/180-250µg NGM)	12 months	Sexual experience scale; Menstrual health questionnaire; Side effects questionnaire	Only 38% continued on same OC for 12 months. Physical and emotional side effects most common reasons for discontinuation, however more women perceived improvements than worsening of symptoms with OC use
Sangthawan & Taneepanichskul (2005)	99f 18-35y Open-label randomised trial	30µg EE/3mg DRSP or 30µg EE/150µg LNG	6 cycles	WHAQ	Significant decrease from baseline in negative affect category, anxiety, irritability, feeling sad or blue and weight gain with DRSP in luteal phase compared with LNG
Sulak et al. (2007)	111 current OC users 18-48y Pre-post design	30µg EE/3mg DRSP continuous	24 weeks	Daily headache scale; DSR	Reduction in severity of headaches with continuous use compared with 21/7 regimen in women with severe headaches at baseline only
Sveindottir & Backstrom (2000)	83f 20-40y (30.8y) Cross sectional	Various OCs	6 cycles	Women's Daily Health Diary	No difference between groups in cyclicity of symptom severity. Only one of the five women meeting criteria for PMDD was using OC
Toffol et al. (2011)	1987f 30-54y Cross-sectional	Various OCs	Retrospective report of past 2 weeks	BDI	No significant detrimental effects of OC use on mood. Scores on some BDI items and risk of major depressive disorder lower in OC users. OC use associated with worries about health and higher risk of alcohol dependence. Not representative of younger women who are the most common users of OCs
Toffol et al. (2012)	8586f 25-54y (39.8y) Cross-sectional	Various OCs	Retrospective report of past month	BDI; Self-report of 13 symptoms	OC use associated with lower depression. Duration of OC negatively correlated with "feeling tense and nervous" and "frightening

					thoughts"
Vessey et al. (1985)	16746f Cross-sectional	Various OCs	Unknown	Referrals for psychiatric disorders	No difference in prevalence of psychotic and non-psychotic disorders between OC users and non-users
Winkler et al. (2004)	998f 17-45y (28.2y) Open-label randomised trial, parallel groups	20µg EE/150µ DSG (n=500) or 20µg EE/100µg LNG (498)	6 cycles	POMS; Diary cards	Both OCs reduced dysmenorrhea and premenstrual symptoms, more problems with irregular bleeding and spotting with LNG. Greater improvement in mood with DSG
Yonkers et al. (2005)	450 PMDD sufferers Double blind RCT	20µgEE/3mg DRSP or placebo 24/4 regimen	3 cycles	Daily Record of Severity of Problems	Significant improvement in physical, mood and behavioural symptoms with OC. 15% attrition rate in OC group not included in analysis

F, Female; y, Years; EE, Ethinyl Estradiol; DSG, Desogestrel; OC, Oral Contraceptive; DMPA, Depot Medroxyprogesterone Acetate; NC, Naturally Cycling; GSD, Gestodene; AMQ, Amsterdam Mood Questionnaire; SIP, Sickness Impact Profile; CESD-10, Centre for Epidemiologic Studies Short Depression Scale; PMDD, Premenstrual Dysphoric Disorder; RCT, Randomised Controlled Trial; DRSP, Drospirenone; COPE, Calendar of Premenstrual Experiences; BDI, Beck Depression Inventory; POMS, Profile of Mood States; LNG, Levonorgestrel; DSR, Penn Daily Symptom Report; fMRI, Functional Magnetic Resonance Imaging; MADRS-S; Montgomery-Asberg Depression Rating Scale; STAI-S, State-Trait Anxiety Inventory State Scale; PMS, Premenstrual Syndrome; NGM, Norgestimate; PANAS, Positive and Negative Affect Scale; MDQ, Menstrual Distress Questionnaire; HAMD, Hamilton Rating Scale for Depression; WHAQ, Women's Health Assessment Questionnaire

Different doses of OC as well as different formulations may also have different effects on premenstrual symptoms. Greco et al. (2007) reported that a lower dose of EE resulted in improvements in mood, whereas a higher dose of EE resulted in improvements in physical symptoms. Others have reported more side effects with lower doses of EE (Akerlund, Rode, & Westergaard, 1993; Endrikat, Muller, & Dusterberg, 1997). In terms of the different progestins contained in OCs, desogestrel has been shown to have greater positive effects on mood than levonorgestrel, as well as fewer side effects (Winkler, Ferguson, & Mulders, 2004). The most recently developed progestin used in OCs is drospirenone, which is an analogue of the diuretic drug spironolactone that has been shown to alleviate premenstrual symptoms. Drospirenone also has anti-mineralocorticoid and anti-androgenic properties. More recent studies have therefore focussed on formulations containing this progestin as a possible treatment for PMS.

Findings regarding the effects of OCs containing drospirenone appear promising, with improvements in mood compared to placebo (Freeman et al., 2001; Pearlstein, Bachmann, Zacur, & Yonkers, 2005; Yonkers et al., 2005). In fact, in one study the overall effect size was similar to that found for selective serotonin reuptake inhibitors which are currently approved as effective treatments for PMDD (Yonkers et al., 2005). However, it should be noted that in all these studies, although drospirenone-containing OCs were superior over placebo, there was still a large placebo response. Drospirenone-containing OCs have also shown reductions in negative affect compared with levonorgestrel (Kelly et al., 2010; Sangthawan & Taneepanichskul, 2005). Again, there are issues with these studies in that women experiencing adverse effects of these OCs withdrew, resulting in a "healthy survivor" effect, however these findings so far appear promising for the use of OCs containing drospirenone in the treatment of PMS.

In addition to the effects of OC use on severity of mood and other premenstrual symptoms, the effects on the timing of these symptoms have also been studied. Sveindottir and Backstrom (2000) reported that the pattern of symptom severity did not differ between OC users and non-users, and that symptoms were more severe during the luteal phase than the follicular phase. However, others have reported a stabilising effect of OCs on mood throughout the cycle (Oinonen & Mazmanian, 2002; Ott et al., 2008; Poromaa & Segebladh, 2012). On the other hand, it has been suggested that symptoms

may be more severe during the inactive pill phase rather than the premenstrual phase (Coffee, Sulak, & Kuehl, Kelly et al., 2010; Sulak, Scow, Preece, Riggs, & Kuehl, 2000; Walker & Bancroft, 1990). For that reason, some researchers have investigated the effectiveness of including shorter inactive pill phases or continuous OC use. When a four day inactive pill phase was used, improvements were seen with OC compared with placebo (Pearlstein et al., 2005). However, this may have been due to the administration of a drospirenone-containing OC formulation, which has consistently shown beneficial effects on mood regardless of treatment regimen. In a study with no inactive pill phase, continuous OC use resulted in a reduction in the severity of headaches in women reporting severe headaches at baseline (Sulak, Willis, Kuehl, Coffee, & Clark, 2007). Freeman et al. (2012) also concluded from their review of four studies of continuous OC use that there does seem to be an overall improvement in menstrual symptoms.

From the available literature, it appears that OCs may be beneficial for mood and premenstrual symptoms in some women. This may depend on the generation of OC, dose, and individual characteristics of the woman. Furthermore, monophasic pills are more likely to stabilise mood throughout the cycle than triphasic regimens, although they have a higher probability of inducing mood change during the pill withdrawal phase (Walker & Bancroft, 1990). The dearth of placebo controlled trials in this area, as well as the fact that some side effects of OC use (e.g. breast tenderness, bloating and depression) overlap symptoms of PMS/PMDD, make firm conclusions regarding the effects of OC use difficult to draw. Future studies in this area should aim to use prospective tools to measure mood and premenstrual symptoms, as well as including a placebo control, although this latter recommendation is difficult to implement due to ethical considerations.

3.3. Oral Contraceptives and Aggression

Whilst aggression is often described as being one of the most common and troublesome of the premenstrual symptoms (Canning, Waterman, Simpson, & Dye, 2012), research on the effects of the OC have tended to primarily investigate PMS as a whole, or divided effects into mood, behavioural and physical symptoms, for example. The

literature examining the effects of OC use specifically on aggression is therefore rather scant.

Findings from animal studies have been somewhat conflicting, with different species showing different effects of OC or the active ingredient ethinylestradiol (EE). Exposure to EE resulted in reduced aggression in males of several fish species (Colman, Baldwin, Johnson, & Scholz, 2009; Dzieweczynski, 2011; Filby, Paull, Searle, Ortiz-Zarragoitia, & Tyler, 2012; Saaristo, Craft, Lehtonen, & Lindström, 2010; Salierno & Kane, 2009). Conversely, EE has been shown to increase aggression in rodents. Female rats developmentally exposed to 0.4 μg/kg/day 17α-ethinylestradiol, a dose chosen to be equivalent to that received by women using OCs, displayed enhanced aggression towards males compared to those receiving a lower dose of 0.004 μg/kg/day or vehicle, despite no effect on plasma estradiol levels (Della Seta, Farabollini, Dessì-Fulgheri, & Fusani, 2008).

Triphasic oral contraceptive administration was found to increase the amount of contact aggression received by female monkeys during the year following cessation of OC treatment, as well as reducing vigilance and increasing social stress (Shively, 1998). However, the amount of contact aggression displayed by OC treated monkeys was not affected. This finding was replicated when cynomolgus monkeys administered a triphasic OC for two years in the diet received significantly more contact aggression than controls, and also tended to display more contact aggression although this effect was not significant (Henderson & Shively, 2004).

In a study of a very small sample of gorillas administered a COC, aggression was found to occur evenly throughout the cycle, which differs from behaviour usually seen in naturally cycling gorillas where aggression is more evident around ovulation (Sarfaty, Margulis, & Atsalis, 2011). Although this study did not explicitly compare OC treated animals with naturally cycling animals, the findings suggest a similar stabilising effect on aggression as that seen on mood in humans.

Whilst animal research can provide some insight into the effects of OC use, one important difference between animals and humans in this area is that in animals, aggression is necessary for survival and dominance. However, in humans aggression is seen as an unfavourable trait, and therefore the effects of OC use on aggression may be

different. Less research has focussed specifically on the effects of OCs on aggression in humans. Grant (1998) briefly reported on "aggressive mood changes" with OC use, particularly in women using a middle dose of norethisterone with EE, however it is unknown how the aggressive mood was measured and no statistical analyses are described. In a study of the relationship between estradiol and implicit power motivation in women, a positive correlation was found in naturally cycling women whereas in OC users the relationship was not significant (Stanton & Schultheiss, 2007). Implicit power motivation may encompass several behaviours including direct and indirect aggression. This finding was replicated by Stanton and Edelstein (2009) who also found that despite the lack of relationship between circulating estradiol and power motivation in OC users, there was no difference between NC women and OC users in their overall levels of power motivation. However, these studies did not directly assess aggression, and in fact it has been suggested that implicit power motivation may not include direct aggression as this is not seen as socially acceptable in humans and more successful outlets would be chosen by women with higher power motivation (Winter, 1988).

In a cross-sectional study, Perry, Canning, Scholey, and Dye (under review) found that OC users rated themselves as less aggressive than non-users as assessed by scores on the Buss-Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1972) as well as the items "anger" and "aggression" added to the Penn Daily Symptom Report (DSR; Freeman et al., 1996). Non-users also responded slower to both direct and indirect aggression related stimuli on an emotional stroop task during the premenstrual phase, suggesting an increased salience of these stimuli to non-users. However, these findings must be interpreted with caution as increased salience may not indicate a higher level of aggression. Victims of aggressive attacks may also respond slower on this task (Riemann & McNally, 1995), therefore it cannot be concluded that these individuals are more aggressive, nor can causality be inferred due to the cross-sectional nature of the study and lack of placebo control. Further human research is needed to confirm these findings and further explore the relationship between OC use and aggression.

3.4. Oral Contraceptives and Cognition

Synthetic estrogens are commonly used to alleviate symptoms associated with menopause, which include a decline in cognitive function. Findings regarding the cognitive effects of hormone therapy (HT) have been mixed, with some authors reporting impairments in domains such as verbal learning and immediate digit recall (Grady et al., 2002; Maki, Gast, Vieweg, Burriss, & Yaffe, 2007; Maki et al., 2009; Resnick et al., 2006), but other domains such as verbal fluency being improved (Maki et al., 2009). These findings suggest that synthetic estrogens and progestins may also have effects on cognitive function in younger women. The majority of research on the cognitive effects of OCs has centred on sexually dimorphic tasks such as verbal memory, in which females usually outperform males (Hausmann, Schoofs, Rosenthal, & Jordan, 2009; Weiss, Kemmler, Deisenhammer, Fleischhacker, & Delazer, 2003), and mental rotation, in which males typically outperform females (Halpern, 1992; Maccoby & Jacklin, 1974). Since hormonal differences are thought to underlie these gender effects, it is logical that hormonal effects of OCs would be most apparent in these tasks.

Indeed, significant differences between OC users and NC women have been reported. Whilst NC women perform better on verbal working memory tasks during periods of the cycle when estrogen is higher, this cyclical variation is not seen in OC users (Rosenberg & Park, 2002), although performance is better during the active pill phase than the inactive pill phase (Mordecai et al., 2008). The effects on verbal memory may depend on the generation of OC used, since Griksiene and Ruksenas (2011) reported that although NC women outperformed OC users overall, when generation of OC was considered, third generation OCs were shown to have detrimental effects whilst new generation OC users did not differ from NC women. It has also been suggested that qualitative rather than quantitative differences in verbal memory may exist, with OC users demonstrating enhanced recall of the gist of a story, and NC women more likely to recall details (Nielsen, Ertman, Lakhani, & Cahill, 2011). Whilst improvements in verbal memory *per se* with OC use have not been shown, it has also been suggested that OCs may protect against impairment induced by cortisol exposure (Kuhlmann & Wolf, 2005).

Table 3.2 *Summary of studies of the effects of oral contraceptives and synthetic estrogens on cognitive function.*

Study	Sample size (n) Age Design	Dosing/ Treatment	Duration	Cognitive domains/Tasks	Outcome
Bartholomeusz et al. (2008)	30f 18-38y (22.59y) Double blind RCT	100µg/day transdermal estradiol (n=16) or placebo (n=14)	31 days	Multiple cognitive domains tested	Trend towards improvement on long-delay free recall task with estradiol, and significant improvement in spatial working memory. Estradiol also protected against scopolamine-induced cholinergic insult on long-delay recall task. No effect on any other domain
Gogos (2013)	16 OC; 29 NC; 15m 20-43y Cross-sectional	Various OCs	One testing session, NC women tested in either follicular or luteal phase (parallel groups)	Global Congitive Function measured using RBANS	OC users similar total score to NC women in mid-luteal phase, and slightly better than NC in follicular phase, significantly better than males. OC users significantly better than males on immediate memory and attention, better than NC in early follicular phase and males on delayed memory
Griksiene & Ruksenas (2011)	20 NC (21.1y); 23 OC (21.8y) Cross-sectional	Various OCs	Testing during 3 phases of one cycle	Verbal Fluency; Mental Rotation	NC women generated more words than OC users overall, although non-significant. Significant reduction in number of words generated by third generation OC users compared to NC women and new generation OC users. Third generation OC users had slower reaction times to rotated stimuli, other OC users did not differ from NC women
Holloway et al. (2011)	15OC; 37 NC; 20m 18-23y Cross-sectional	Various OCs	One testing session, NC women tested in either follicular or luteal	Attention; Inhibition	OC users learned conditioned stimulus/unconditioned stimulus association faster than NC and males. Prepulse inhibition reduced with OC suggesting impaired

			phase (parallel groups)		information processing
Kuhlmann & Wolf (2005)	20 OC; 27 NC 20-34y (24.81y) RCT crossover (cortisol administration) Cross-sectional (OC use)	Various OCs; 30mg hydrocortison or placebo	Testing once during the same phase of two cycles	Verbal Memory	Significant detriment to memory when cortisol administered to NC women, no effect in OC users. Suggests a possible protective effect of OCs against impairments
Mordecai et al. (2008)	36f 18-40y Cross-sectional	Various OCs	Testing twice during one cycle	Verbal Memory (CVLT)	Improved verbal memory in active pill phase. No differences between phases in NC women
Nielsen et al. (2011)	34 OC; 32 NC 18-35y Cross-sectional	Various OCs	Two testing sessions one week apart	Verbal Memory	Improved recall of "gist" of a story with OC use but poorer recall of details
Nielsen et al. (2013)	36 OC; 42 NC 18-35y (20.37y) Cross-sectional	Various OCs	Two testing sessions one week apart	Verbal Memory	In OC users, enhanced memory for negative information if noradrenergic activation to negative stimuli, enhanced memory for positive images if cortisol response to a stressor. No effect in NC women
Rosenberg & Park (2002)	10 OC (19.4y); 8 NC (19.4y) Cross-sectional	Various monophasic OCs	One cycle	Verbal Working Memory; Mental Rotation	No cyclical variation in OC users on verbal working memory, NC women performed better in middle of cycle when estrogen high. On mental rotation, no difference between OC and NC
Wharton et al. (2008)	56 OC; 90 NC 19.23y Cross-sectional	Second generation (n=19), third generation (n=28) or Yasmin (n=9)	One testing session, NC women tested in either follicular or luteal phase (parallel groups)	Mental Rotation	No difference between OC and NC when all OC users grouped. New generation OC users significantly worse, second generation non-significantly better

Wuttke et al. (1975)	16 OC; 16 NC 18-25y Cross-sectional	Various OCs	One cycle	Reaction Time; Visual Orientation; Arithmetic; Memory	Detriments to reaction time and arithmetic with OC. Detriments to other tasks with OC when compared with NC women in luteal phase only

OC, Oral Contraceptive; NC, Naturally Cycling; y, Years; m, Male; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; CVLT, California Verbal Learning Task

The generation of OC used also appears to be of importance in the effects on mental rotation. Whilst no effects were found when all OCs were grouped (Rosenberg & Park, 2002; Wharton et al., 2008), new generation users have been shown to perform significantly worse than other users and NC women, whilst second generation users outperformed other groups (Wharton et al., 2008). Third generation users were also shown to have slower reaction times to rotated stimuli, whilst other users did not show effects on reaction time (Griksiene & Ruksenas, 2011).

Other researchers have taken a broader approach to studying the effects of OCs on cognition. An early study reported detrimental effects of OC use on reaction time and mental arithmetic, whilst poorer performance in other cognitive domains was also observed only when compared to NC women in their luteal phase (Wuttke et al., 1975). However, OC formulations have changed dramatically since that study was conducted, therefore the effects of newer OCs may be less deleterious. Indeed more recent studies have shown improvements in attention and inhibition (Holloway, Beck, & Servatius, 2011), as well as superior performance on a global cognitive function battery, in particular a delayed memory task, compared with NC women in their follicular phase and men (Gogos, 2013).

Unfortunately, due to the ethical issues with randomising women to OC or placebo, there is a lack of randomised controlled trials in this area. One study investigated the effects of transdermal estradiol treatment on cognitive function and found improvements in free recall and spatial memory, as well as protection against scopolamine-induced cholinergic insult (Bartholomeusz et al., 2008). These findings suggest that synthetic estrogens may indeed improve some areas of cognitive function. However, since the route of administration was different than that of OCs, and estradiol was administered alone rather than combined with a progestin, these results cannot be generalised. Further randomised controlled trials will be needed to investigate the specific effects of OCs.

The available literature suggests that OCs may have beneficial effects on some cognitive domains, and that fluctuations in cognitive abilities that are evident across the menstrual cycle in NC women are reduced in OC users. Estrogen alone appears to be more effective than combined OCs, however this may be due to the route of administration (transdermal rather than oral) and not due to the formulation itself.

Different generations of OCs may also differentially affect cognitive function, and this should be taken into consideration when interpreting findings.

3.5. Putative Mechanisms of Action of Oral Contraceptives and Synthetic Estrogens

3.5.1. Effects on Endogenous Hormones

Oral contraceptives suppress levels of endogenous estrogens by inhibiting gonadotropin-releasing hormone (GnRH) via negative feedback. This also results in prevention of the mid-cycle surge in LH, which in turn suppresses ovulation and is one of the ways in which OCs prevent pregnancy. The reduction of 17β-estradiol and progesterone by OCs has been demonstrated in several studies (Griksiene & Ruksenas, 2011; Liening, Stanton, Saini, & Schultheiss, 2010; Likis, 2002), and is known to occur both peripherally (Rapkin, Morgan, Sogliano, Biggio, & Concas, 2006) and centrally (Follesa et al., 2002). This reduction in female sex hormones may explain the negative effects of OCs on verbal fluency tasks, which are modulated by estrogen and progesterone. However, no correlation was found between salivary 17β-estradiol and number of words generated (Griksiene & Ruksenas, 2011).

Alterations in androgen biosynthesis may also play a crucial role in the effects of OCs. In male fish, plasma androgens were reduced following EE exposure, as well as suppressive effects on testosterone-producing enzymes and an enzyme that aromatises testosterone (Filby et al., 2012). In humans, reduced testosterone in OC users has also been shown compared to non-users in their ovulatory phase (Griksiene & Ruksenas, 2011) although this reduction may not be stable across time (Liening et al., 2010), and different OC formulations vary in the extent to which they lower FT (Coenen, Thomas, Borm, Hollanders, & Rolland, 1996; Greco, Graham, Bancroft, Tanner, & Doll, 2007; Schultheiss, Dargel, & Rohde, 2003). It is thought that OCs lower free testosterone (FT) levels through suppressing ovulation, blocking production of total testosterone (T) and increasing levels of sex hormone binding globulin (SHBG; Boyd, Zegarac, Posvar & Flack, 2001). Women with PMS have been reported to have elevated levels of testosterone in the luteal phase (Eriksson et al., 1992), therefore the ability of OCs to reduce FT may explain the positive effects of OCs on some premenstrual symptoms.

However, it is unlikely that reductions in testosterone are the mechanism by which OCs may improve mood since reduced androgen levels were found not to be associated with depressed mood (Graham et al., 2007; Greco et al., 2007). In fact, several studies have shown a positive correlation between androgen levels and well-being in women (Bell, Donath, Davison, & Davis, 2006; Cawood & Bancroft, 1996), and although endogenous androgens are lowered in OC users, androgenic progestins can be much higher depending on the generation of OC (McFadden, 2000), which would suggest that other mechanisms must underlie the effects of OCs on mood and premenstrual symptoms.

3.5.2. Effects on Neurotransmitters

Synthetic estrogens are known to affect serotonergic (5-HT) activity by increasing the number of 5-HT receptors, increasing 5-HT synthesis and decreasing monoamine oxidase (MAO) activity (Halbreich & Kahn, 2001). In an early study, urinary 5-HT and its metabolites were higher after a dietary tryptophan load in women using OCs than in naturally cycling women (Toseland & Price, 1969). Increased serotonergic activity is known to have positive effects on mood (e.g. Flory, Manuck, Matthews, & Muldoon, 2004; Hamon & Blier, 2013), which may explain the improvements in mood found with OC use in some women. However, other studies have shown reduced serotonergic activity with OC use. Henderson and Shively (2004) found that OC administration increased the cortisol response and decreased the prolactin (PRL) response to fenfluramine in monkeys, both of which suggest decreased serotonergic function. This is in line with earlier work which described reduced synthesis of 5-HT due to altered tryptophan metabolism (Daabees, Mohy El-Din, Zeitoun, & Makar, 1981) Oinonen and Mazmanian (2002) suggest that this may actually be due to a progesterone-mediated increase in MAO activity, although it is unclear under what circumstances these opposing effects occur.

The dopaminergic system may also be affected by OC use. Levels of plasma tyrosine, the precursor of dopamine (DA), were significantly lower in women using OCs than in naturally cycling women (Moeller, 1981). An increase in dopaminergic inhibitory control of prolactin release in response to a challenge was also found in women taking OCs (Chalmers, Fulli-Lemaire, & Cowen, 1985), which may be due to estrogen decreasing the sensitivity of the presynaptic dopamine autoreceptor (Shively, 1998).

Dopamine interacts with the serotonergic system in the promotion of positive mood (Sasaki-Adams & Kelley, 2001; Willner, Hale, & Argyropoulos, 2005), therefore reductions in DA activity may suggest that mood should be worsened in women using OCs. Since studies have not concurrently investigated changes in DA activity and mood it is difficult to determine whether DA underlies any negative effects of OC use on mood.

Studies have demonstrated reduced γ-aminobutyric acid-A (GABA) receptor sensitivity and reduced plasma GABA in the luteal phase in women suffering from PMS (Poromaa & Segebladh, 2012; Sundström Poromaa et al., 2003). Administration of EE induces upregulation of γ2 subunit gene expression in $GABA_a$ receptors in the cerebral cortex (Follesa et al., 2002), providing a possible mechanism by which OCs may alleviate premenstrual symptoms. Furthermore, the metabolites of progestins used in OCs have a similar chemical structure to allopregnanolone and therefore also have $GABA_A$ receptor effects (Andréen et al., 2009). On the other hand, increases in aggression may also be explained by actions at the $GABA_A$ receptor as several $GABA_A$ agonists have been shown to induce aggression in both animals and humans (Andréen et al., 2009).

The cholinergic system has been suggested by several authors to mediate the positive effects of exogenous estrogens on cognition (Acosta et al., 2009; Miller & Franklin, 1999). Cholinergic activity is known to be crucial in cognition, particularly in learning and memory formation and attention (Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Newhouse, Potter, Kelton, & Corwin, 2001). Both ERα and ERβ have been identified in the basal forebrain, which is the primary cholinergic innervation to the cerebral cortex, hippocampus and hypothalamus (Toran-Allerand et al., 1992). In animals, estrogen replacement has been shown to modulate acetylcholine (ACh) release (Gibbs, Hashash, & Johnson, 1997) as well as ameliorate a memory deficit induced by the muscarinic receptor antagonist scopolamine (Acosta et al., 2009; Gibbs, Burke, & Johnson, 1998).

The protection against scopolamine injury has also been demonstrated in postmenopausal women following three months estrogen treatment. In that study, estrogen also protected against cognitive impairments following administration of the anti-nicotinic drug mecamylamine, although for both challenges the only cognitive abilities protected were those involving attention, psychomotor function and tasks

involving speed (Dumas et al., 2008). Furthermore, postmenopausal women using estrogen therapy had significantly higher muscarinic receptor density than never-users in the left striatum, left hippocampus, lateral frontal cortex and thalamus (Norbury et al., 2007). However, in a study in young healthy women, estradiol was found to minimally protect against cognitive impairment following scopolamine insult, suggesting only a small role of the cholinergic system in the improvements in cognition seen following estradiol treatment (Bartholomeusz et al., 2008).

3.5.3. Other Potential Mechanisms

Hormonal contraceptives have been shown to reduce cortisol responses to physical and psychosocial stressors (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). This may be a mechanism by which OCs affect cognition, as cortisol is known to impair delayed recall and was found to impair verbal retrieval in NC women but not in those using hormonal contraception (Kuhlmann & Wolf, 2005). It is unknown at this stage whether the low endogenous estradiol or the synthetic EE in the OC leads to the reduced cortisol response, however it is assumed that estrogen rather than progesterone mediates this relationship since there was no difference in response between women in the luteal phase and those in menses (Kuhlmann & Wolf, 2005). Furthermore, it appears that OCs may not reduce cortisol levels in the absence of a stressor, but may stabilise cortisol over time (Liening et al., 2010).

It has been suggested that OCs may improve cognitive function by reducing functional cerebral asymmetries (FCAs) through improved interhemispheric interaction. Women showed larger leftward bias in a visual line-bisection task during OC intake than during the OC withdrawal phase, suggesting improved interplay between the two hemispheres (Cicinelli et al., 2011).

Gender differences in brain structure have been well described in the literature, however more recent studies have also shown differences in brain structure between OC users and non-users. Pletzer et al. (2010) found significantly larger prefrontal cortices, pre- and post-central gyri, parahippocampal and fusiform gyri and temporal regions in OC users compared to non-users. The hippocampus, fusiform and parahippocampal gyri have been implicated in spatial abilities (Epstein, Parker, & Feiler, 2007; Meulenbroek

et al., 2010) and differences in the structure of these regions may help to explain effects of OC use on spatial abilities.

3.6. Chapter Summary

The available literature suggests that OC use may be beneficial for mood and premenstrual symptoms in some women, although this is not a robust finding. Newer generation OCs in particular show promise for the treatment of PMS, and monophasic preparations may stabilise mood during the active pill phase although symptoms often worsen during the pill withdrawal phase. The lack of human research on the effects of OCs on aggression make drawing conclusions about these effects difficult, however from the one study that has been conducted to date it appears that long term use of OCs may be associated with reduced premenstrual aggression. Some cognitive outcomes may also improve with OC use although again the lack of placebo-controlled trials makes it difficult to draw firm conclusions. The mechanisms underlying these effects are at present unclear, however alterations in the activity of neurotransmitters such as serotonin and dopamine likely play a key role.

Chapter 4

Soy Isoflavones and Other Phytoestrogens

4.1. Introduction to phytoestrogens

Phytoestrogens (PE) are non-steroidal plant-derived phenolic compounds similar in chemical structure to mammalian estrogens, which are most commonly found in soy products. They are also found in lower concentrations in other leguminous plants such as fruits, vegetables and whole grains (Kurzer & Xu, 1997). The three main classes of phytoestrogens are isoflavones, lignans and coumestans, with isoflavones being the most commercially consumed due to their availability in a variety of food products (Lephart, Setchell, & Lund, 2005). Isoflavones are also the most extensively studied class of phytoestrogens, with a search for the term "isoflavone" generating over 9,000 results in the Scopus database at the time of writing. The amount of isoflavones found in soy products can differ greatly depending on the soy variety and processing methods. For example, soy flour has a very high concentration of PE (150-170 mg/100 g) as does soy protein isolate (91 mg/100 g), whereas levels are much lower in alcohol extracted soy protein concentrate (11 mg/100 g), tofu (25-30 mg/100 g) and soy milk (1-3 mg-100 g). Even within certain soy products the exact concentration varies between brands (Dwyer et al., 1994; Umphress, Murphy, Franke, Custer, & Blitz, 2005). Since the focus of this thesis is on soy isoflavones (SIF) they will be extensively reviewed here, with other phytoestrogens being discussed as appropriate.

4.1.1. Why Study Phytoestrogens?

Asian populations are known to have lower incidences of many diseases and health problems, such as cardiovascular disease, obesity, diabetes, breast cancer (and other hormone-dependent cancers), osteoporosis and menopausal symptoms, as compared with Western cultures (Henderson & Bernstein, 1991; Maskarinec, Pagano, Yamashiro, & Issell, 2003; Wu et al., 1996; Ziegler, 2004). Since soy is an integral part of the Asian diet, and Asian diets contain approximately 10-fold higher concentrations of PE than Western diets, this observation of better health outcomes in a population consuming more soy products has led to the theory that soy and its constituents may have health benefits. In particular, research has primarily focused on postmenopausal women and climacteric symptoms due to the theory that lowered estrogen following menopause is

responsible for these symptoms, and that the estrogenic effects of PE (see section 4.1.3) may alleviate them. Conventional hormone therapy (HT) containing estrogen and progestogens have been found to increase the incidence of breast cancer and the risk of cardiovascular diseases (Rossouw et al., 2002). Therefore postmenopausal women are increasingly turning to natural alternatives such as PE supplementation, which have been shown to have a relatively low risk of side effects (Tempfer et al., 2009).

Following a plethora of clinical trials, PE are now recognised as compounds that reduce menopausal symptoms, particularly hot flushes, as well as protecting against breast cancer and coronary heart disease, although for the latter a greater effect is observed in postmenopausal women than men (Albertazzi & Purdie, 2002). In addition, PE have been shown to lower plasma low-density lipoprotein (LDL) cholesterol (Chilibeck et al., 2013) and increase high-density lipoprotein (HDL) cholesterol in a dose-related manner (Terzic, Dotlic, Maricic, Mihailovic, & Tosic-Race, 2009).

Despite the widely held belief that phytoestrogens are beneficial for health, some concerns have been raised. Phytoestrogens are now recognised endocrine disruptors (Patisaul & Jefferson, 2010) and some evidence has emerged suggesting that these compounds may pose a risk to infants and the unborn (Rozman et al., 2006). However, in 1999 the US Food and Drug Administration (FDA) approved the use of the health claim that soy is effective in reducing the risk of coronary artery disease which led to a rapid increase in sales of food products containing soy. Further research is clearly needed to address the issue of whether the widely held belief that phytoestrogens are beneficial to health is warranted.

4.1.2. Phytochemistry of isoflavones

Isoflavones are most commonly sourced from the soybean (*Glycine Max*) which contains between 18 to 562 mg/100g total isoflavones (Mortensen et al., 2009). Soy beans contain three primary isoflavones in their biologically inactive conjugated glycoside form: genistin, daidzin and glycitin. These glycosides are high in molecular weight and hydrophilicity and therefore are poorly absorbed in the small intestine. The conjugates contain glucose or carbohydrate moieties and are metabolised by bacterial β-

glucosidases in the intestinal wall to form the bioactive aglycones genistein, daidzein and glycitein respectively, which are more easily absorbed. Intestinal glucosidases can also metabolise biochanin A and formononetin to genistein and daidzein respectively (Axelson, Sjovall, Gustaffson, & Setchell, 1984). Daidzein can be further metabolised to the more metabolically active equol in the intestine, although a specific type of intestinal microbe is needed for this bioconversion and only 30-50% of individuals possess this microbe (Atkinson, Frankenfeld, & Lampe, 2005), with vegetarians and people of Asian origin being most likely (Lampe, Karr, Hutchins, & Slavin, 1998). The alternative metabolite is O-desmethylangiolensin (O-DMA), which is less estrogenic than equol.

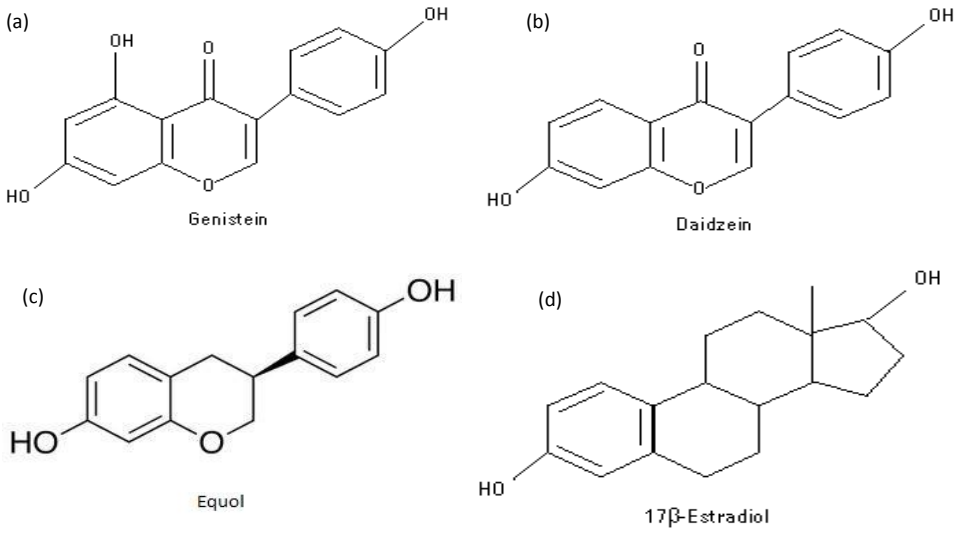

Figure 4.1. Chemical structure of the isoflavones genistein (a) and daidzein (b) and the metabolite equol (c) in comparison with 17-β-estradiol (d).

The average time taken to reach peak plasma concentrations after ingestion of aglycones has been reported to be 4-7 h, and for β-glycosides the time is delayed to 8-11 h. The half-lives of daidzein and genistein were also reported to be 9.3 and 7.1 h respectively, indicating that isoflavones and/or their metabolites are rapidly excreted (Setchell et al., 2001). In fact the majority of genistein and daidzein are eliminated within 24 hours. However, a diet high in soy (50 mg/day isoflavones) results in a steady state of high

plasma concentrations (50-800 ng/mol) far greater than concentrations of plasma estradiol (40-80 pg/mol; Setchell, Boriello & Hulme, 1984).

4.1.3. Pharmacokinetics of isoflavones

The phenolic ring structures of isoflavones (see Figure 4.1) are similar to those of steroidal estrogens, allowing them to cross the blood-brain barrier and bind to estrogen receptors (ER; Barnes et al., 2000) exerting both weak estrogenic and anti-estrogenic effects (Kuiper et al., 1998). When levels of endogenous estrogens are high, isoflavones may block full estrogen activity by occupying ER, whereas when endogenous estrogens are low the estrogenic activity of isoflavones may become apparent (Kuiper et al., 1998). These effects may also depend on the target tissue being investigated.

Isoflavones have a greater affinity for the more recently discovered ERβ than the classic ERα (Kuiper et al., 1997). The affinity of genistein for ERβ is thought to be 20-30 times higher than for ERα, and is comparable to the affinity of native 17-β-estradiol (Kuiper et al., 1998). The affinity of other isoflavones may be up to 500 times lower than that of 17-β-estradiol. In the brain, ERα are located primarily in the hypothalamus and regions associated with reproductive behaviour (Gibbs & Gabor, 2003), whereas ERβ are predominantly found in the frontal and prefrontal cortex, hippocampus and basal forebrain, areas of importance for cognitive function (Sherwin & McGill, 2003). Once they have crossed the blood brain barrier, isoflavones concentrate in tissue sites that have a high expression of ERβ, such as the prefrontal cortex (PFC) which contains far more ERβ than ERα (Lephart, Rhees, Setchell, Bu, & Lund, 2003). Due to their actions on ER, it can be stated that isoflavones act as natural selective estrogen receptor modulators (SERMs) in various tissues throughout the body (Setchell, 2001).

Phytoestrogens have been shown to increase levels of sex hormone binding globulin (SHBG) in humans (Adlercreutz, 1998), yet only around 50% of isoflavones are bound by SHBG. This therefore potentially lowers levels of unbound endogenous estrogen, while there are high levels of plasma PE that can bind to ER and modulate estrogen hormone action. It has been reported that PE can be present in the blood at levels up to 10,000 times higher than those of steroidal estrogens (Adlercreutz et al., 1991; Wise,

2003). Research in this area is complicated by the variable individual metabolic response to PE, with Murkies, Wilcox and Davis (1998) reporting up to a 1000-fold variation in isoflavone excretion following ingestion of a controlled quantity of soy. This variability in human metabolism of isoflavones is thought to be explained by differences including the composition of intestinal flora, redox potential of the colon and intestinal transit time (Setchell et al., 1984).

4.2. Phytoestrogens, mood and premenstrual symptoms

4.2.1. Animal Studies

It has been suggested that due to the effects of PE on serotonin (5-HT) and other neurotransmitters (see section 4.5.3), as well as the effects of endogenous estrogen on mood and premenstrual syndrome (PMS; see chapter 2), that PE consumption may similarly alter mood and symptoms of PMS. Administration of these compounds to animals may help to provide insight into not only the behavioural effects of PE, but also their potential mechanisms of action.

In one of the earliest studies investigating the effects of PE on anxiety, Lund and Lephart (2001a) fed male and female rats a diet containing either no PE (PE-free) or 600 µg of PE/g (PE-600) for 75 days and measured anxiety using the elevated plus maze (EPM). Animals fed the PE-600 diet made significantly more entries into the open arms of the maze, and spent more time in the open arms than those fed the PE-free diet, indicating reduced anxiety with PE treatment. This was supported more recently in a study where a 200 mg/kg daidzein diet fed to male mice for 30 days resulted in more entries to the open arms compared to controls (Zeng et al., 2010).

The anxiolytic effects of PE have also been demonstrated in ovariectomised female rats, where doses of 0.5 and 1.0 mg/kg genistein were found to reduce anxiety related behaviour comparable with the effects of diazepam in a black and white model and an open field test. Increased grooming and rearing following treatment were also reported, which are indicators of low stress, and the anxiolytic effects could not be explained by increased locomotor activity as this outcome was not affected (Rodríguez-Landa, Hernández-Figueroa, Hernández-Calderón, & Saavedra, 2009).

In contrast, Hartley et al. (2003) reported anxiogenic effects of a diet containing 150 µg/g of genistein and daidzein for 18 days in male rats assessed using the elevated plus maze and social interaction. This lower dose and shorter duration of treatment may explain the disparity between studies, as it has been suggested that at low doses PE inhibit estrogenic effects by inhibiting aromatase activity, whereas at higher doses they produce estrogenic effects (Almstrup et al., 2002). This theory is supported by a study in which 10 mg/kg body weight of equol injected for four days beginning on the day of birth resulted in increased anxiety in adult male rats (Patisaul & Bateman, 2008).

Gender may also play a role in whether PE have anxiolytic or anxiogenic effects. A diet containing 95 mg isoflavones/kg fed to rats for between 7-14 days produced anxiolytic effects in proestrus females (when endogenous estrogens are high) but anxiogenic effects in males (Patisaul, Blum, Luskin, & Wilson, 2005). It was suggested by the authors that timing of intervention may be an important factor in whether the effects in males are anxiolytic or anxiogenic. In the study by Lund and Lephart (2001) rats were exposed to the same diet from conception through to testing, including the perinatal period. It is thought that early exposure may be crucial for positive effects in males. However, since neonatal exposure to equol resulted in increased anxiety in adulthood in male rats more research is needed to determine whether this is the case (Patisaul & Bateman, 2008).

In a series of experiments by Blake, Fabick, Setchell, Lund and Lephart (2011) the effects of different doses and durations of treatment with isoflavones, as well as differing ages of the animals on depression-related behaviours in female rats were investigated. In females with natural ovarian failure, a low PE diet resulted in greater immobility in the forced swim test indicating increased depression, as well as lower levels of serum serotonin, than those on a high PE diet. The effect of the low-PE diet was reversed following injection with equol, suggesting that equol may be an effective antidepressant. This effect was only observed in animals following natural ovarian failure and was not seen in intact females or those who were ovariectomised shortly after puberty. This suggests that ovarian status may play a role in the antidepressant effects of PE.

4.2.2. Human Studies

As previously stated, consumption of isoflavones is greater in Asian populations than Western populations. The prevalence of moderate to severe PMS symptoms have also been found to be lower in Asian than Western populations, suggesting a possible beneficial effect of isoflavone intake on PMS symptoms (Takeda, Tasaka, Sakata, & Muraka, 2006) although this finding has not been replicated. The effects of isoflavones on premenstrual symptoms are beginning to be explored, as well as effects on mood more generally. These studies are summarised in Table 4.1.

Several studies have shown improvements in mood with isoflavone supplementation in post-menopausal women (Casini et al., 2006; de Sousa-Munoz & Filizola, 2009; Ishiwata, Melby, Mizuno, & Watanabe, 2009; Lipovac et al., 2010). Whilst some studies have found no effects on anxiety in this population (Casini et al., 2006), others have reported significant reductions in self-reported anxiety (Ishiwata et al., 2009; Lipovac et al., 2010). Beneficial effects on other parameters of mood such as depression have been more consistently found (Casini et al., 2006; de Sousa-Munoz & Filizola, 2009; Ishiwata et al., 2009; Lipovac et al., 2010). However, several researchers also reported reductions in anxiety and depression with placebo treatment, highlighting the large placebo effect that is often observed in studies of climacteric symptoms and the need to include placebo controls in these studies.

Few studies have investigated the effects of isoflavones on mood in younger humans, or the effects on premenstrual syndrome (PMS). From the literature that does exist, it appears that physical symptoms of PMS may be reduced by treatment with isoflavones (Bryant et al., 2005; Ishiwata, Uesugi, & Uehara, 2003), and that women suffering from menstrual migraines may benefit from their consumption (Burke, Olson, & Cusack, 2002; Ferrante, Fusco, Calabresi, & Cupini, 2004). Psychological symptoms have proved to be less affected by isoflavone treatment, although reductions may still be observed to a lesser extent than physical symptoms (Ishiwata et al., 2003). As with research on menopausal symptoms, a large placebo effect is evident in studies of PMS, as highlighted by Bryant et al. (2005) who reported that the majority of symptoms in their study were significantly reduced in severity following placebo treatment as well as isoflavone treatment. This again underlines the importance of including placebo controls in these trials.

Some researchers have not used placebo controls and rather have conducted cross-sectional studies investigating the correlation between estimated isoflavone intake and retrospective ratings of severity of PMS symptoms. Negative correlations between isoflavone intake and symptom severity have been reported during menses only (Kim, Kwon, Kim, & Reame, 2006) although others have reported no association in any phase (Gold et al., 2007; Nagata, Hirokawa, Shimizu, & Shimizu, 2004). Findings from these studies must be interpreted with caution, as no causal effect can be concluded from cross-sectional studies, and retrospective measures of PMS symptoms are far less reliable than prospective measures due to the issue of recall bias. In addition, Gold et al. (2007) included only middle aged women in their study and the possibility of the symptoms reported being menopausal rather than premenstrual symptoms cannot be excluded. More randomised, double-blind, placebo-controlled trials are needed to further examine whether PE may improve mood or alleviate PMS symptoms, although the findings at this stage appear to suggest that at least some symptoms, in particular physical symptoms, may benefit from PE supplementation.

Table 4.1 *Summary of studies investigating the effects of isoflavones on mood and premenstrual symptoms.*

Study	Sample size (n) Age Design	Treatment	Duration	Outcome(s)	Effect of soy isoflavones	Comment
Bryant et al. (2005)	23f PMS sufferers 18-35y RCT crossover	68mg/d IF from ISP (aglycone equivalent) or milk protein placebo	7 menstrual cycles	DSR	Reduction in total PMS symptom scores following IF treatment. For most symptoms improvements also seen following treatment with placebo.	Only headache and breast tenderness significantly reduced by IF but not placebo. Equol production did not enhance symptom reduction
Burke et al. (2002)	49f MM sufferers 18-48y Parallel groups RCT	Extract of soy (30mg), dong quai (50mg) and black cohosh (25mg) 2 x daily, or placebo	24 weeks	Daily diary for recording details of MM	Significant reduction in average frequency of MM	
Casini et al. (2006)	76f Post-menopausal 50y (4y) RCT crossover	60mg/d aglycone IF (tablet) or placebo	6 months	STAI BDI POMS	Reduced depression, improved global mood scores on POMS, no effect on anxiety	Statistical analysis (t-tests) with no correction for number of tests performed or order of treatment
De Sousa-Munoz & Filizola (2009)	84f Post-menopausal 45-60y Parallel groups RCT	120mg/d IF or placebo	16 weeks	CES-D	Reduction in depressive symptoms, but no significant difference between IF and placebo	Lack of significant effect likely due to large placebo response in treatment of climacteric symptoms
Ferrante	10f MM	56mg genistein	3 months	Daily diary for	Average number of days with	Very small sample size. No

et al. (2004)	sufferers Pre-post design	and 20mg daidzein consumed during perimenstrual period		recording details of MM	migraine decreased significantly with IF treatment	control group.
Gold et al. (2007)	3302f 42-52y Cross-sectional	Dietary intake of isoflavones assessed with FFQ	Acute	Retrospective rating of premenstrual symptoms including abdominal cramps, breast pain, weight gain, mood changes, increase in appetite, anxious, muscle pain and severe headaches	No association between genistein intake and any premenstrual symptoms	Retrospective measures subject to recall bias. Many premenstrual symptoms not included e.g. depression. Only included middle aged women, some of whom were in perimenopause therefore may have been experiencing menopausal rather than premenstrual symptoms.
Ishiwata et al. (2003)	56f PMS sufferers 18-21y RCT crossover	40mg/d IF or placebo	8 menstrual cycles	PMS symptom questionnaire	Significant reduction in physical symptoms. Psychological symptoms also reduced but not significant	Headache showed the biggest reduction in severity following IF treatment
Ishiwata et al. (2009)	127f Menopausal 40-59y RCT	10mg equol 1 x daily or 10mg equol 3 x daily or placebo	12 weeks	POMS Menopausal Symptom Questionnaire	Equol 3 x daily resulted in reduced depression, Tension-Anxiety, Depression-Dejection, and Fatigue, as well as increased Vigour, compared with placebo	Perimenopausal/postmenopausal equol nonproducers had significant improvements from baseline following treatment with equol 3 x daily on all outcomes except depression
Kim et al. (2006)	84f 28-40y Cross-sectional	Dietary intake of isoflavones assessed with FFQ	Acute	MDQ	IF intake negatively correlated with scores on autonomic reactions, behavioural changes and total MDQ scores during menses	No significant correlations in any other phase. Issues with recall bias due to retrospective measure of PMS, as well as issues with

						use of FFQ to estimate IF intake
Lipovac et al. (2010)	109f Post-menopausal >40y RCT crossover	80mg IF from red clover 2 x daily or placebo	90 days	HADS SDS	Significant reduction in scores on both scales	Improvement also seen with placebo, but only 21.7% with placebo compared with 76.9% improvement in HADS and 80.6% improvement in SDS with IF.
Nagata et al. (2004)	189f 19-34y Cross-sectional	Dietary intake of isoflavones assessed with FFQ	Acute	MDQ	No correlation between IF intake and PMS symptoms	Methodological issues regarding use of a retrospective measure of symptoms as well as FFQ to estimate IF intake

F, Female; PMS, Premenstrual Syndrome; y, Years; RCT, Randomised Controlled Trial; IF, Isoflavone(s); ISP, Isolated Soy Protein; DSR, Penn Daily Symptom Report; MM, Menstrual Migraines; STAI, State-Trait Anxiety Inventory; BDI, Beck Depression Inventory; POMS, Profile of Mood States; CES-D, Centre for Epidemiologic Studies Depression Scale; FFQ, Food Frequency Questionnaire; MDQ, Moos Menstrual Distress Questionnaire; HADS, Hospital Anxiety and Depression Scale; SDS, Zung's Self-Rating Depression Scale

4.3. Phytoestrogens and aggression

Given the compelling evidence for a role of estrogens in aggression and aggressive behaviour (see chapter 2.4) an examination of the effects of phytoestrogens is warranted. However, this area of research remains largely unexplored. In animals, two studies have investigated the effects of phytoestrogens on aggressive behaviour in fish. The first found reduced territorial aggression following exposure to paper mill effluent containing phytoestrogens (Johnsson, Parkkonen, & Förlin, 2003). However in a study where controlled amounts of genistein and equol were administered in water without other substances found in effluent, intensity of aggressive behaviour as measured by response to a perceived intruder was reduced (Clotfelter & Rodriguez, 2006).

In non-human mammals, findings have also been mixed. In male mice, both genistein and daidzein have been shown to reduce aggressive behaviour (Wisniewski, Cernetich, Gearhart, & Klein, 2005; Zeng et al., 2010). In the first study (Wisniewski et al., 2005) it was found that a low dose of genistein (5mg/kg) fed to female mice during pregnancy reduced aggression in male offspring compared to those not exposed to genistein, whereas there was no effect in male offspring of females fed a high dose (300mg/kg). In contrast, Zeng et al (2010) showed that consumption of a high dose (200mg/kg) of daidzein for 30 days resulted in lowered aggression compared to controls. These findings suggest a complex relationship between the timing of administration and dose.

In contrast, other species displayed increased aggression following phytoestrogen treatment. Male cynomolgus macaques fed a high-PE diet of 1.88 mg total isoflavones/g protein displayed 67% more aggressive acts than controls following a 15 month intervention (Simon, Kaplan, Hu, Register, & Adams, 2004). Shorter durations of treatment had similar effects, with adult male hamsters fed a diet containing 810 µg/g total PE for 4 weeks displaying more aggression towards a non-aggressive opponent than those fed a PE-free diet. In younger hamsters fed the same diet for 7 weeks, no significant difference between groups was found (Moore, Karom, & O'Farrell, 2004). Exposure to 10 mg/kg equol daily for four days during the neonatal period resulted in increased aggression compared to controls in the resident-intruder test in male rats (Patisaul & Bateman, 2008). It should be noted that in that study, the control group displayed very low levels of aggression and none made any attacks, which could

explain the difference between groups. It has also been reported that there is a dose-dependent U-curve in the effects of PE (Almstrup et al., 2002), therefore it is possible that with other doses either no effect or reductions in aggression would be seen.

There could be several factors contributing to the conflicting findings in animal studies, including the doses administered, different tests used and particular extracts used. Environmental factors could also play a role, even in well controlled laboratory studies. For example, it has been shown that estrogenic effects on aggression may differ depending on whether the bedding used in the animal facilities is made from corncob or not (Landeros et al., 2012). More standardised studies will need to be conducted before a consensus can be reached. The effects of phytoestrogens on aggression in humans have not yet been investigated, therefore although animal studies provide some insight into the possible effects in humans, as well as the potential mechanisms of action of PE, research is needed to investigate whether PE have aggression-promoting or aggression-inhibiting effects in humans.

4.4. Phytoestrogens and cognition

It has been observed that in addition to cultural differences in various health outcomes (see section 4.1.1), Asian populations, particularly Japanese, have a lower incidence of dementia than Western populations (Graves et al., 1996). This has unsurprisingly led to the question of whether PE play any role in protecting against dementia or improving cognitive function. Although findings regarding the effects of PE on dementia, and in particular Alzheimers disease (AD) are inconclusive at this stage, studies have emerged suggesting possible improvements in some cognitive domains. These studies will be reviewed below.

4.4.1. Animal studies

Chronic phytoestrogen supplementation has consistently shown improvements in spatial memory in female rats as assessed by maze tasks which rely on intact hippocampal function. Ovariectomised (OVX) rats fed an isolated soy protein (ISP) containing up to 144 mg PE demonstrated a dose-dependent improvement in spatial memory,

comparable to that of estrogen. (Pan, Anthony, Watson, & Clarkson, 2000). These effects were also observed in intact female rats exposed to a lifelong diet of 0.6 mg PE/g (Lund et al., 2001). Those who were later switched from the PE to the PE-free diet also showed poorer performance than those who remained on the PE diet. Luine, Attalla, Mohan, Costa and Frankfurt (2006) found similar results over the shorter time frame of 9 weeks in OVX rats fed a diet containing approximately 0.8 mg PE/g.

More recently, OVX rats receiving 0.4 g/kg or 1.6 g/kg of PE for 12 weeks were tested on a Morris Water Maze (MWM), and rats supplemented with the higher dose of phytoestrogens demonstrated improved performance comparable with that of treatment with 17-β-estradiol. Rats given the lower dose did not differ from controls (Pan, Li, Yeung, & Xu, 2010). Conversely, acute administration of both a low and a high dose of genistein to young OVX rats significantly improved performance on the MWM. No improvement was found in aged OVX rats in the same study (Alonso et al., 2010). It should be noted that the highest dose in this study was lower than the lowest dose in the study by Pan et al. (2010), suggesting a complex relationship between duration of treatment and dose.

In male rats the effects are less clear. Administration of a PE-rich diet resulted in poorer visual spatial memory in male rats compared to those fed a PE-free diet (Lund et al., 2001). These findings were supported in a companion paper by the same group (Lund & Lephart, 2001b) where males switched from a PE-rich diet to a PE-free diet demonstrated improved performance on a radial arm maze. Males treated with the antiandrogen drug flutamide prenatally showed the opposite effect, suggesting that PE improve spatial memory in "feminised" brains.

In a different study, 15 weeks of a soy isoflavone diet improved spatial memory. This effect was only observed with the low dose diet (0.3 g/kg) and not the higher dose (1.2 g/kg; Lee et al., 2004). This was partially supported in a more recent study where three different doses of aglycones were found to significantly improve spatial learning and memory (Yang, Jin, Ren, Luo, & Zhou, 2011). Although the authors concluded that even the high dose resulted in improved performance, it should be noted that the highest dose used in this study was 200 mg, which is lower than the lowest dose used by Lee et al. (2004).

Administration of genistein was shown to protect against ischemia-induced impairments in long-term memory as well as spatial memory in male gerbils (Donzelli et al., 2010). More recently, a dose of 200 mg/kg daidzein resulted in no significant differences from controls in performance on the MWM in male mice, suggesting no effect of isoflavones (Zeng et al., 2010). Acute administration of daidzein did, however, reverse impairments in spatial memory that were induced by cholinergic dysfunction (Kim et al., 2010). The discrepancy in findings may be due to differences in the age of the animals used, the cognitive tests and the treatment duration and dosage.

In a study of the effects of genistein on cognitive functions other than spatial memory, Neese et al. (2010) found impairments in performance on a working memory task in ovariectomised aged rats following chronic treatment with a high dose of genistein (323 µg/kg/d). This effect was not seen in younger rats, however there was a trend towards impairment in all age groups on a task of inhibitory control and timing. The tasks used in that study were specifically chosen to tap prefrontal cortex (PFC) function, and the authors have suggested that PE may impair performance on tasks that are not mediated by the hippocampus. However, the doses used in the study were moderate and the 4-hour half-life of genistein in rats means that the once daily dosing would not have produced constantly elevated concentrations of genistein. Furthermore, since genistein alone was administered it is not known whether the effects would have been the same if other isoflavones were administered concurrently. Since the products available for human use contain multiple isoflavones it would be beneficial to investigate the synergistic effects of these compounds together.

4.4.2. Human studies

The primary focus of research in this area in humans has been on postmenopausal women due to the theory that cognitive decline observed following menopause may be due to lowered levels of estrogen and other sex hormones, and in fact that women who use hormonal therapy show a 30-45% lower risk of dementia than those who do not (Yesufu, Bandelow, & Hogervorst, 2007). The potentially estrogenic effects of PE have highlighted the possibility of their positive influence on cognitive function when endogenous estrogens become lower following menopause. The effects in other populations have more recently begun to be explored more thoroughly. Studies

investigating the effects of isoflavones on cognitive function in different populations are summarised in Tables 4.2 and 4.3, and an overview of the cognitive domains that have been shown to be influenced by isoflavones is given in Table 4.4.

Several researchers have reported findings from cross-sectional studies, where intake or estimated intake of isoflavones is correlated with performance of cognitive tasks. In one such study, isoflavone intake was not correlated with cognitive function in elderly women (Kreijkamp-Kaspers et al., 2007), however tofu intake has been associated with superior memory in older men and women (Hogervorst et al., 2011). On the other hand, White et al. (2000) reported poorer cognitive performance in elderly men who had consumed high levels of tofu during midlife. There are several issues with these correlational studies which use food frequency questionnaires to estimate isoflavone intake, such as the variation in isoflavone content between different foods and different brands of foods, as well as the fact that foods such as tofu are highly processed and contain toxins. Placebo controlled experimental studies are far more informative regarding the effects of standardised doses of isoflavones.

Findings from randomised controlled trials have been somewhat conflicting. Whilst several studies have shown improvements in tasks of verbal fluency (Gleason et al., 2009; Kritz-Silverstein, Von Muhlen, Barrett-Connor, & Bressel, 2003) and verbal memory (Kritz-Silverstein et al., 2003) in postmenopausal women following isoflavone supplementation, impairments in this domain have also been reported (Fournier et al., 2007; Howes, Bray, Lorenz, Smerdely, & Howes, 2004). Other studies have shown no effect of isoflavones on verbal memory (Maki et al., 2009), although in that study isoflavones from red clover rather than soy were administered, which may explain the lack of findings since red clover contains much lower concentrations of genistein and daidzein. Tasks measuring executive function and thought to tap frontal lobe functions have also shown improvement in this population (Casini et al., 2006; Duffy, Wiseman, & File, 2003; Santos-Galduroz, Galduroz, Facco, Hacul, & Tufik, 2010), however some researchers have reported poorer performance on tasks of executive function with isoflavone consumption compared with placebo (Gleason et al., 2009). Furthermore, other researchers have reported no effect of isoflavones on executive function (Fournier et al., 2007). Similarly, some studies have shown positive effects of isoflavones on non-verbal memory (Casini et al., 2006; Duffy et al., 2003; Gleason et al., 2009; Henderson

et al., 2012; Howes et al., 2004) whilst others have found no effects on this domain (Fournier et al., 2007; Santos-Galduroz et al., 2010).

A number of randomised controlled trials have resulted in no differences in performance between isoflavones and placebo in any cognitive domain in postmenopausal women (Fournier et al., 2007; Ho et al., 2007; Kreijkamp-Kaspers et al., 2004). Several explanations have been proposed to explain these disparate findings. Firstly, the severity of menopausal symptoms at baseline may be a predictor of the effects of isoflavones, such that individuals with severe symptoms may be more likely to benefit from isoflavone supplementation. In the study by Fournier et al. (2007), the participants suffered only mild to moderate symptoms, which may explain the lack of positive findings in that study. The age of the participants may also be crucial, since both Ho et al. (2007) and Kreijkamp-Kaspers et al. (2004) included older women who had been post-menopausal for several years. This idea is supported by Hogervorst et al. (2011) who reported superior memory with increased tofu intake in participants below the age of 73, with an average age of 67, but no significant association in participants above the age of 73, with an average age of 80. A "critical window" hypothesis has been proposed, which states that as time from menopause increases, effects of phytoestrogens are less apparent due to the down-regulation of estrogen receptors (Hogervorst et al., 2011). It has also been suggested that the cognition-enhancing effects of isoflavones may only be apparent when task difficulty is high, since more complex tasks of verbal memory and executive function have shown positive effects (Duffy et al., 2003; Kritz-Silverstein et al., 2003).

Table 4.2 *Summary of studies of the cognitive effects of isoflavones on different cognitive domains in postmenopausal women and older men.*

Study	Sample size (n) Age Design	Treatment	Duration	Cognitive Domains	Effect of soy isoflavones	Comment
Casini et al. (2006)	78f 50y (4y) Placebo controlled crossover	60mg/d aglycone IF (tablet)	6 months	Digit symbol Digit span Visual scanning	More correct digit symbols Backward digit recall improved	Statistical analysis (t-tests) with no correction for number of tests performed or order of treatment
Duffy et al. (2003)	33f 57y (1y) 50-65y 2 parallel groups	60mg/d aglycone IF (tablet; n=18) or placebo (n=15)	12 weeks	Attention Memory - Episodic Memory -Long term Memory -Semantic Frontal/executive function	Improved picture recall, sustained attention, rule reversal & planning after IF	
File et al. (2005)	50f 51-66y 2 parallel groups	60mg/d aglycone IF (tablet; n=18) or placebo (n=15)	6 weeks	Attention Memory - Episodic Memory - Long term Memory - Semantic Frontal/executive function	Improved frontal lobe function (mental flexibility, rule reversal, planning), nonverbal short term memory	
Fournier et al. (2007)	79f 56y (0.9y) 48-65y 3 parallel groups RCT	Cows' milk & placebo or soy milk & placebo (72mg/d IF) or cows' milk & 70mg/d IF	16 weeks; after 4 weeks diet adjustment to low IF	Selective Attention (Stroop) Memory - digit ordering Spatial memory - short term (colour matching; Benton Visual Retention test) & long term (visual pattern recognition)	Decline in verbal working memory (digit ordering) in soymilk group No effects of soymilk or supplements on any other measured parameters	IF excretion similar in soy milk & supplement groups – greater than in control (cows milk).

				Forward digit span Corsi block tapping		
Gleason et al. (2009)	30 (15m; 15f) 62-89y Parallel groups RCT	100mg/d IF glycosides (n=15) or placebo (n=15)	6 months	Visuospatial Memory Verbal Fluency Speeded Dexterity Executive Function Verbal Memory	Improved VSM, verbal fluency and speeded dexterity Slower responses on 2 tests of executive function	Plasma genistein and daidzein levels elevated No equol producers in sample Similar side effect profile to placebo
Henderson et al. (2012)	313f 45-92y Parallel groups RCT	25g ISP/d (91mg aglycones weight; n=154) or placebo (n=159)	2.5 years	Cognitive composite score Executive/Expressive/Visuo spatial Factor Verbal Episodic Memory Factor Visual Episodic Memory Factor	No difference in global cognitive function Improved visual memory	
Hill et al. (2005)	21f 52.5y (4.3y) RCT crossover	100mg/d soy IF (aglycone; tablet)	8 weeks per treatment	Memory - Episodic Memory -Long term Memory -Semantic Frontal/executive function Spatial (mental rotation) Attention	Trends for improved Verbal learning/memory; planning; impaired spatial memory	Effect sizes reported (>10%); Effects apparent at 4 & 8 weeks dosing; Increased estrogen at 4 & 8 weeks, no effects on other hormones
Ho et al. (2007)	168f 55-76y Parallel groups RCT	80mg/d soy derived IF (n=80) or placebo (n=88)	6 months	Memory (list learning) Visual Perception (Rey Osterreith Figure) Executive function (trail making/verbal fluency) Attention (digit span/digit vigilance)	No differences for intervention & placebo after treatment; No change from baseline in groups	Ceiling effects on MMSE; low levels of vasomotor symptoms; diet not controlled – mean dietary IF intake 20mg/day in both groups so could reduce size of any effect

				Motor control (tapping) Naming MMSE		
Hogervorst et al. (2011)	142 (55m ; 87f) 56-97y divided into young (<73y with mean age of 67y) and old (>73y with mean age of 80y) age groups Cross-sectional	Dietary intake of isoflavones assessed with food frequency questionnaire	Acute	Hopkins Verbal Learning Test	Positive correlation between tofu intake and immediate recall in younger group, negative correlation in older group	
Howes et al. (2004)	28f >60y Parallel groups RCT	IF extract from red clover (25mg formononetin; 2.5mg biochanin; < 1mg daidzein and genistein; n=14) or placebo (n=14)	6 months	Comprehensive cognitive battery assessing many cognitive domains	Improved visual-spatial intelligence with IF Improved verbal memory with placebo Deterioration in digit recall with IF	All effects non-significant when adjusted for multiple comparisons
Kreijkamp-Kaspers et al. (2004)	175f 66.6y (4.8y) 60-75y Parallel groups RCT	25.6g soy protein = 99mg/d aglycone IF (powder; n=88) or placebo (n=87)	12 months	Memory Attention Verbal fluency Naming MMSE	No effect of soy IF on any aspect of cognitive function	
Kreijkamp-Kaspers et al. (2007)	301f 60-75y Cross-sectional	Dietary intake of isoflavones and lignans assessed with food frequency	Acute	Memory Processing capacity and speed Executive function	No association between isoflavone intake and cognitive function, but higher lignan intake	Correlational data only No tests of memory which have shown effects of IF

		questionnaire			associated with better processing capacity and speed, and executive function	
Kritz-Silverstein et al. (2003)	53f 55-74y Parallel groups RCT	55mg soy extract IF =110mg/d total IF (tablet; n=26) or placebo (n=27)	6 months	Trail making Category fluency Logical memory Verbal memory	Improved category fluency Trend for improved verbal memory & trail making	
Maki et al. (2009)	66f 53y Parallel Groups	0.625mg/2.5mg CEE/MPA; 128mg/d Black cohosh extract = 7.27mg triterpine glycosides; 398mg/d red clover extract = 120mg IF aglycones; Or placebo	12m	Verbal Memory Logical Memory Visuospatial Ability Verbal Fluency Attention Working Memory Visuoperceptual speed	No effects of either botanical treatment on any cognitive domain. Trend towards greater decline in verbal learning in CEE/MPA group	No measures of planning and mental flexibility – shown in other studies to be sensitive to phytoestrogen treatment
Santos-Galduroz et al. (2010)	38f 50-65y Parallel groups RCT	80 mg/d IF (n=19) or placebo (n=19)	4 months	Visual-Spatial test Digit Span (Central executive) Digit Symbol (Agility and attention) Similarity (Capacity to integrate information) Verbal Paired Associates (Declarative episodic memory)	Improved recall of semantically related words No improvement in episodic declarative memory or visual-spatial abilities	

White et al. (2000)	4236 (3734m; 502f) 71-93y Observational study	No intervention; participants classified as low-low, high-high or intermediate tofu consumers based on interviews and questionnaires	28 years	Cognitive Abilities Screening Instrument (CASI) Attention; concentration, orientation, short- and long-term memory; language ability; visual construction; verbal fluency; abstraction and judgment	Poorer performance on the CASI (score <74) associated with higher mid-life tofu consumption	Not a controlled trial, dietary intake not assessed at time of cognitive assessment. No actual measurement of urinary or serum phytoestrogens or their metabolites

IF, Isoflavone(s); RCT, Randomized Controlled Trial; m, Male; f, Female; y, Years; VSM, Visuospatial Memory; ISP, Isolated Soy Protein; MMSE, Mini-Mental State Examination; CEE, Conjugated Equine Estrogens; MPA, Medroxyprogesterone Acetate

In younger samples, both males and females have shown improvements in non-verbal memory following isoflavone consumption (Celec, Ostatnikova, Putz, & Hampl, 2004; File et al., 2001; Ostatnikova et al., 2007; Thorp et al., 2009), as well as improvement in performance of tasks of executive function (File et al., 2001), although these effects may be restricted to females depending on the task, since males showed no improvement in a planning task. In addition only young females showed improvement in verbal fluency, whilst males were impaired in this domain (File et al., 2001). Interestingly, Islam, Sparks, Roodenrys, and Astheimer (2008) reported that isoflavone consumption improved verbal learning and memory during menses, when performance is usually poorer. This finding highlights the need to investigate the effects of isoflavones in young women across the menstrual cycle. In contrast, Vanata and Metzger (2007) reported no difference between participants administered isoflavones and those given placebo in tasks of verbal memory. Pilsakova, Riecansky, Ostatnikova, and Jagla (2009) also reported no effects of isoflavone consumption on a mental rotation task, although this trial was not placebo controlled and therefore results should be interpreted with caution.

The discrepancies between findings suggest that cognitive enhancing effects of isoflavones in younger populations may be observed only when a higher dose is used over a longer duration, as was used in the study by File et al. (2001). However, since significant effects were also observed shorter interventions (Islam et al., 2008; Ostatnikova et al., 2007) it has been suggested that task difficulty may rather be a factor in whether effects are observed or not, and that when task difficulty is high, high doses of isoflavones may significantly improve performance.

Table 4.3 *Summary of studies of the cognitive effects of isoflavones on different cognitive domains in younger males and females.*

Study	Sample size (n) Age Design	Dosing/ Treatment	Duration	Cognitive Domains	Effect of soy isoflavones	Comment
Celec et al. (2004)	86 (54f; 32m) 18-25y Within-groups dietary intervention	2g soybeans/kg bodyweight per day	1 week	Spatial visualisation Mental Rotation	Improvements in both tasks in both sexes. Gender difference in performance reduced	Plasma estrogen and salivary testosterone decreased in females; no change in males
Celec et al. (2005)	16 females 23.4y (4.5y) Within-groups dietary intervention	900g soy/week (129g/d soy protein 154mg/d IF)	1 week tests on day 1 & 7	Spatial visualisation Mental rotation	Improvements in both tasks	No change in estrogen. Increased testosterone. No control group Limited cognitive domains tested
File et al. (2001)	27 (15m; 12f) Parallel groups dietary intervention	100mg/d soy protein or 0.5mg/d total IF	10 weeks	Attention Memory - Episodic Memory - Long term Memory - Semantic Frontal/executive function	Improved short & long-term memory, mental flexibility; Letter fluency & executive function improved in f only	No effects on attention or category generation
Hill et al. (2004)	22f 33.6y (6.1y) Randomised crossover	30.5g soy protein (68mg/d aglycone IF) or 116mg conjugated IF	7 menstrual cycles; 2 screening; 2 IF; 2 placebo	Memory - Episodic Memory -Long term Memory -Semantic Frontal/executive function Spatial (mental rotation) Attention	Improved long-term verbal memory; Short effect on verbal fluency; No effects on spatial, executive function or attention	Verbal fluency effect in 1 cycle; No effects of soy on urinary hormones
Islam et al.	28f	120mg/day IF (for	3 days		Improved working	Performance on verbal

(2008)	21y (4.15y) Within-groups dietary intervention	70kg person) from 2 daily servings of soy germ flour		Verbal Learning Paragraph Recall Working Memory Mental Rotation Verbal Fluency	memory; Improved verbal learning; Trend towards improved letter fluency	learning task following soy supplement equivalent to that in luteal phase without intervention; no difference between menses and luteal phases without intervention in working memory yet soy improved performance, suggests mechanism via non-estrogenic pathways. No control group
Ostatnikova et al. (2007)	86 (32m; 54f) 18-25y Within-groups dietary intervention	2g/kg per day soybeans (~170mg/kg IF)	1 week	Mental Rotation Spatial Visualisation	Significant improvement of both tasks in both sexes. Decline in salivary testosterone and estrogen in females only. Hormonal effects in males dependent on basal testosterone levels	No placebo control
Pilsakova et al. (2009)	36 (20m; 16f) Between-groups dietary intervention	2g/kg per day soybeans (~170mg/kg IF) or usual diet (control)	1 week	Mental rotation	No effects in either sex	Images only presented in 2 dimensions, effects may only be seen in 3 dimensions
Thorp et al. (2009)	34m 49y (10y) 30-80y RCT Crossover	116mg/d total IF or placebo	6 weeks	Spatial Working Memory Memory – Verbal Recall	Improved spatial working memory; No effect on any other	Included men as old as 80

				Memory – Episodic Memory – Auditory Executive function (mental flexibility, attention & planning) Visual-spatial Processing (mental rotation)	cognitive domain	
Vanata & Metzger (2007)	50 (13m; 37f) 20.1y (2.9y) Parallel groups RCT	50g ISP (54mg total IF) or whey protein control	Acute; 1 day in fasted state	Word recall & recognition Visual spatial memory	No effects	Gender balance not given. Findings suggest acute administration has no effect, therefore neuro-adaptation may be the mechanism through which cognition is improved

IF, Isoflavone(s); ISP, Isolated Soy Protein; m, Male; f, Female; y, Years; RCT, Randomised Controlled Trial

Table 4.4 *Summary of the effects of isoflavones on different cognitive domains in males and females of various ages.*

Study	Memory		Frontal lobe/ executive function	Psychomotor	Verbal Fluency
	Verbal	Non-Verbal/spatial			
Young females					
Celec *et al* (2004)		*			
Celec *et al* (2005)		*			
File *et al* (2001)		*	*		*
Hill *et al* (2004)	*				*
Islam *et al* (2008)	*	*			^
Ostatnikova et al. (2007)		*			
Pilsakova et al. (2009)					
Vanata & Metzger (2007)					
Early Post-menopausal Females					
Casini *et al* (2006)		*	*	*	
Duffy *et al* (2003)		*	*		
File *et al* (2005)		*	*		
Fournier *et al* (2007)	-				
Hill *et al* (2005)	^	-	^		
Henderson et al. (2012)		*			
Ho *et al* (2007)					
Hogervorst et al. (2011)		*			
Howes et al. (2004)	-	^			
Kritz-Silverstein *et al* (2003)	^		^		*
Maki *et al* (2009)					

Santos-Galduroz et al. (2010)		*		
Elderly Females				
Gleason *et al* (2009)	*	-	*	*
Kreijkamp-Kaspers et al. (2004)				
Kreijkamp-Kaspers et al. (2007)				
Hogervorst et al. (2011)				
Young males				
Celec *et al* (2004)	*			
Celec *et al* (2007)	*			
File *et al* (2001)	*	*		
Ostatnikova et al. (2007)	*			
Pilsakova et al. (2009)				
Thorp *et al* (2009)	*			
Vanata & Metzger (2007)				
Elderly Males				
Gleason et al. (2009)	*	-	*	*
Hogervorst et al. (2011)				
White *et al* (2000)	-	-		-

*, Significant improvement; ^, non-significant improvement; -, deterioration

Although the findings from human studies are somewhat contradictory, it appears that spatial memory, verbal fluency and frontal lobe functions are most likely to benefit from PE consumption. These effects may vary depending on age and gender, with young females most likely to see an improvement. Dose and duration of treatment may also be crucial factors in whether the effects are positive or negative, with higher doses administered for longer periods more likely to show an effect. More randomised, placebo controlled trials are needed to determine the optimal dose for different populations.

4.5. Potential Mechanisms of Action of Phytoestrogens

4.5.1. Actions on the Estrogen Receptors and Effects on Circulating Sex Hormones

Phytoestrogens have the ability to bind to ER and may elicit genomic (via nuclear ER) or non-genomic (via membrane ER) actions. As previously mentioned, PE have a greater affinity for ERβ than ERα, and ERβ are preferentially expressed in the frontal cortex, amygdala, hippocampus and hypothalamus, regions related to memory and learning (Desmond & Levy, 1997; Sherwin & McGill, 2003). Equol has been shown to concentrate in these regions following dietary isoflavone exposure (Lund et al., 2001). It therefore seems likely that cognitive effects of PE are mediated by ERβ, particularly since studies using knockout mice have shown ERβ to be crucial for estradiol-induced enhancements of object recognition and placement tasks (Walf, Koonce, & Frye, 2008). Estrogen has been shown to affect cognition through several receptor-mediated means, such as influencing cell survival, growth and plasticity (McEwen & Alves, 1999).

The affinity of isoflavones for ERβ may also explain some of the effects on aggressive behaviour. In studies using knockout mice, when ERα is disrupted aggressive behaviour becomes less frequent whereas when ERβ is disrupted aggression increases (Nomura et al., 2002; Ogawa et al., 1997; Ogawa et al., 1998). This suggests that ERβ may have an inhibitory effect on aggression. According to Kuiper (1998), depending on the dose of isoflavones and concentration of endogenous estrogen, isoflavones can have either estrogenic effects through activating ERs, or anti-estrogenic effects through blocking ERs and therefore reducing the effects of endogenous estrogen. Therefore isoflavones may either activate ERβ, thereby reducing aggression, or antagonise the effects of endogenous estrogen on ERβ and therefore increase aggression.

Isoflavones have been shown to inhibit the activity of 5α-reductase, which catalyses the conversion of testosterone (T) to 5α-dihydrotestosterone (5α-DHT), as well as aromatase P450 which is involved in the conversion of testosterone to estradiol, in peripheral tissues (Adlercreutz et al., 1993; Brooks & Thompson, 2005). This potentially results in lowered levels of circulating 5α-DHT and estradiol. However, it has also been reported that aromatase activity is only inhibited by low concentrations of isoflavones, whereas high concentrations increase aromatase activity (Almstrup et al.,

2002). In the brain, effects on aromatase P450 were not found, and reduced activity of 5α-reductase in the hypothalamus and amygdala was only found at low doses (Lephart, Thompson, Setchell, Adlercreutz, & Weber, 2000). This may explain some of the complex dose effects of PE.

The exact effects of isoflavones on circulating sex hormones are unknown as findings from several studies have been conflicting. Plasma T was found to be reduced in one study (Weber, Setchell, Stocco, & Lephart, 2001) whereas in others it was unchanged (Lephart et al., 2003; Lund et al., 2001). Free testosterone (FT) may be reduced in men by isoflavones (Berrino et al., 2001), although it has also been found that FT is not affected but serum T is increased (Celec, Ostatníková, Hodosy, Putz, & Kúdela, 2007). In women, isoflavones may decrease (Berrino et al., 2001) or have no effect on serum T (Celec et al., 2005).

It could be postulated that because the measures affected in some animal studies of the effects on aggression are androgen-dependent, such as the resident-intruder paradigm, the negative effects in males may be due to altered androgen production, however testes size and testosterone production were not affected by genistein (Wisniewski et al., 2005), suggesting the mechanism by which these effects occur, for genistein at least, may rather be via inhibition of enzymes required for androgen metabolism as has been previously shown (Fritz, Cotroneo, Wang, Eltoum, & Lamartiniere, 2003; Weber, Jacobson, Setchell, & Lephart, 1999). In human studies it has been suggested that with prolonged treatment, circulating levels of testosterone are lowered due to the ability of phytoestrogens to increase levels of SHBG, which may explain the poorer performance of males on male-positive cognitive tasks found in some studies.

The effects of isoflavones on female sex hormones in women are also not straightforward. In young women, either no effect (Celec et al., 2005) or a decrease in serum estradiol (E_2; Nagata, Takatsuka, Inaba, Kawakami & Shimizu, 1998) have been reported. In postmenopausal women the effects are even less clear, with one study reporting no effect (Petrakis et al., 1996), two reporting a decrease (Berrino et al., 2001; Low et al., 2005) and one reporting an increase in serum E_2 levels (Adlercreutz & Mazur, 1997). Furthermore, isoflavones have been shown to suppress luteinizing hormone (LH; Baker, Leitman & Jaffe, 2000; Cassidy, Bingham & Setchell, 1994) , and follicle stimulating hormone (FSH; Cassidy et al., 1994; de Sousa-Munoz & Filizola,

2009). These changes in steroidal hormones, particularly E_2, may account for the effects of PE on mood and cognitive function, and especially differential effects across the menstrual cycle (see chapter 2).

4.5.2. Neuroprotective effects

Several studies have suggested a neuroprotective effect of isoflavones in various models. This effect has been most studied in the hippocampus, where pyramidal dendritic spine density in the CA_1 region was found to be increased by 48% following a high PE diet compared to a low PE diet (Luine et al., 2006). Ischemia-induced loss of cells in this particular region was also fully prevented by genistein in male gerbils (Donzelli et al., 2010). It has been suggested that PE exert effects on the CA_1 region via the medial septum, through actions on the cholinergic system, as PE have been shown to increase cholinergic activity (see section 4.5.4).

Acute administration of soy extract even at low doses was shown to protect against neuronal loss in the dentate gyrus of the hippocampus following injury with the toxic kainic acid. Genistein alone was also investigated in that study, and protected against neuronal loss at the high dose of 10 mg/kg but not at lower doses, suggesting that other components in soy contribute to the effect (Azcoitia, Moreno, Carrero, Palacios, & Garcia-Segura, 2006). Indeed it has been shown that a combination of genistein, daidzein and equol had a greater effect on neuronal survival following toxic insult, and enhanced defence mechanisms against neurodegeneration such as mitochondrial function and β-amyloid degradation more than any of the compounds individually (Zhao, Mao, & Brinton, 2009).

Dendritic spine density was also increased in the PFC following a high PE diet, although not to the same extent as in the hippocampus (Luine et al., 2006). Genistein was found to protect primary cortical neurons from this region from toxicity induced by the calcium-ATPase inhibitor thapsigargin (Linford & Dorsa, 2002). It is thought that this effect is mediated by ER, since an agent that specifically binds to estrogen receptors blocked the attenuation of apoptosis by genistein (Linford & Dorsa, 2002).

It is believed that the neuroprotective effects of phytoestrogens may stem from their ability to influence brain derived neurotropic factor (BDNF). BDNF has been shown to

promote cell survival (Hartikka & Hefti, 1988) and is involved in synaptic plasticity (McAllister, Katz, & Lo, 1999). Phytoestrogens increase BDNF in the frontal cortex of OVX rats (Lephart et al., 2002; Pan, Anthony, & Clarkson, 1999a, 1999b), and in the hippocampus of female rats (Pan et al., 2010). Pan et al. (2010) found that phytoestrogen supplementation also increased BDNF gene expression in the hippocampus as well as increasing mRNA levels of tyrosine kinase receptor B (TrkB), the primary receptor for BDNF. Since the post-synaptic BDNF-TrkB pathway is crucial for long term potentiation (LTP) induction, an important synaptic connection model of learning and memory (Kovalchuk, Hanse, Kafitz, & Konnerth, 2002), this may explain some of the positive effects seen on cognitive function.

On the other hand, BDNF mRNA expression was lower in male rats fed phytoestrogens in the CA_4 and CA_3 regions of the hippocampus, and the cortex (File, Hartley, Alom, & Rattray, 2003), providing a possible explanation for the behavioural sex differences observed in cognitive function, although the reasons behind these differences are unclear. It is thought that the activation of cyclic adenosine monophosphate (cAMP)-response element-binding protein (CREB) mediates the effects of isoflavones on BDNF (Spencer, Vauzour, & Rendeiro, 2009) but further investigation is required to ascertain how this pathway might differ between males and females.

The sex differences observed in the effects of PE on cognitive function may also be explained by the reduction of levels of calbindin as well as increases in cyclooxygenase-2 (COX-2) in male rats, but no changes in female rats (Lephart et al., 2000; Lund et al., 2001). Calbindin is a calcium binding protein which protects against apoptosis, and the expression of COX-2 is associated with inflammation and neurodegeneration in Alzheimer's disease (O'Banion, 1999).

Modulation of synaptic formation proteins may also play a role in the neuroprotective effects of phytoestrogens. Dietary supplementation with 0.15 g/kg phytoestrogens was found to significantly increase mRNA levels of synaptophysin, synapsin 1, postsynaptic density protein 95 (PSD-95) and spinophilin in hippocampal tissue (Pan et al., 2010). Synaptophysin and synapsin 1 are presynaptic vesicle proteins which help modulate synaptic plasticity and therefore cognitive function. In fact, loss of synaptophysin in the hippocampus correlates with cognitive decline in Alzheimer's disease (Sze et al., 1997). PSD-95 and spinophilin are postsynaptic proteins that play a role in synapse

stabilisation and plasticity. Synaptic formation in the hippocampus is known to be important for learning and memory, therefore increases in synaptic formation proteins may explain the positive effects of phytoestrogens on cognition.

The inhibition of amyloid-β-peptide (Aβ) fibril formation has also been implicated in the neuroprotective effects of PE. Aβ is a peptide which increases the concentration of free calcium and generates free radicals, leading to impairments in cognitive function via neurodegeneration and increased oxidative stress (Butterfield, Reed, Newman, & Sultana, 2007). Isoflavone supplementation decreased Aβ formation by down-regulating Aβ-biosynthetic enzymes in male mice (Hsieh, Wu, & Hu, 2009). Genistein and equol have been shown to be the most effective inhibitors in vitro (Henry-Vitrac, Berbille, Mérillon, & Vitrac, 2010) and genistein in particular has been shown to reverse the negative effects of Aβ treatment in hippocampal rat cells (Sonee, Sum, Wang, & Mukherjee, 2004). Further research has shown that genistein protects human SH-SY5Y cells from Aβ-induced death, alleviate Aβ-induced DNA fragmentation and suppress Aβ-induced apoptosis (Bang et al., 2004).

4.5.3. Effects on Neurotransmitters

One of the potential mechanisms of action underlying the cognitive effects of phytoestrogens is via the cholinergic system. This system is thought to be particularly vulnerable to aging and the accompanying cognitive decline (Gibbs et al., 1994; Newman, Gupta, Climer, Monaghan, & Hasselmo, 2012), and is particularly important for attention and memory encoding (Gais & Born, 2004; Rogers & Kesner, 2003). Soy isoflavones have been shown to increase choline acetyltransferase (ChAT) activity in the cortex and basal forebrain (BF) as well as inhibiting acetylcholinesterase (AChE) activity in the cortex, BF and hippocampus, thus resulting in higher levels of acetylcholine (ACh). Furthermore, isoflavones increased the cell density of cholinergic neurons in the medial septum (MS) and hippocampus CA_1, as well as increasing ChAT immunoreactivity in these regions (Lee et al., 2004). Increased cholinergic activity may therefore underlie cognitive improvements following PE treatment. Since there are sex differences in the BF cholinergic system and in the response of the BF to estradiol (McEwen, 1987), this may explain some of the sex differences observed in the effects of PE.

It is believed that the effects of PE on the cholinergic system are not direct, rather indirect action via activation of ER leads to upregulation of ACh. Support for this theory comes from Kim et al. (2010) who discovered that daidzein reversed scopolamine induced spatial memory impairment, indicating restored cholinergic transmission, and that this effect was blocked by administration of the ER antagonist tamoxifen.

Modulation of the cholinergic system may also explain some of the effects of PE on mood. Genistein is believed to be an allosteric activator of the nicotinic ACh receptor α7 (nAChR α7; (Charpantier et al., 2005; Grønlien et al., 2007)). Positive activation of this receptor has been found to produce anxiolytic effects (Bencan & Levin, 2008; Feuerbach et al., 2009). Although this may explain some of the positive mood effects elicited by genistein administration it is unclear at this stage whether other phytoestrogens also act on nAChR α7.

Phytoestrogens also affect serotonergic neurotransmission. Soy protein isolate fed to OVX cynomolgus monkeys at a dose equivalent to 129 mg/kg in humans significantly increased tryptophan hydroxylase (TPH) in the dorsal raphe after 36 months. Serotonin transporter (SERT) protein was also significantly increased in this region compared with controls (Shively, Mirkes, Lu, Henderson, & Bethea, 2003). Increased TPH results in increased 5-HT synthesis, whereas increased SERT results in higher 5-HT removal. Although this seems paradoxical, the authors have suggested that this effect may demonstrate that when there is more 5-HT available there is also more uptake. It is therefore believed that the increase in SERT accompanies increased synthesis of 5-HT and neuronal firing.

Furthermore, 5-HT levels were found to be lower in female rats fed a low-PE diet compared with those fed a high PE-diet, and increased following injection with equol (Blake et al., 2011). Equol is an inhibitor of monoamine oxidase (MAO) in the rat liver, and since MAO is responsible for the deamination of monoamines, including 5-HT, this may explain the increased levels of 5-HT (Dewar, Glover, Elsworth, & Sandler, 1986). Serotonin is known to be implicated in the aetiology of depression (Cleare, 1997; Rilke et al., 1998) and increased serotonin is associated with more positive mood (Hamon & Blier, 2013).

The dopaminergic system may also be affected by phytoestrogens. Dopamine induced contraction of the rat vas deferens was inhibited by isoflavones, as was footshock-induced aggression in mice whereas haloperidol-induced catalepsy was potentiated, both effects known to be modulated by dopaminergic activity (Velis et al., 2008). This suggests an anti-dopaminergic effect of at least isoflavones, and perhaps other phytoestrogens, and explains some of the positive effects on mood observed following PE treatment. However, genistein was found to increase dopamine transporter (DAT) expression in the PFC (Neese et al., 2010). Dopamine release has been shown to be involved in verbal working memory and attention and may also be involved in other memory tasks (Aalto, Brück, Laine, Någren, & Rinne, 2005). These findings suggest that the effects of PE on neurotransmitters may depend on the specific tissue being investigated, and further studies will be required to extrapolate the exact effects on the dopaminergic system.

4.5.4. Other Potential Contributing Mechanisms

Changes in vascular function may account for some of the cognitive improvements observed following phytoestrogen consumption. In a comprehensive review Carlson, Peng, Prasain and Wyss (2008) reported that soy consumption lowered arterial pressure in several animal models as well as in post-menopausal women and age-matched men. This effect was also found in normotensive individuals (Teede et al., 2001; Welty, Lee, Lew, & Zhou, 2007). High arterial pressure is known to impair cognitive function in humans and is associated with AD and vascular dementia (Breteler, 2000). Reductions in blood pressure would result in more efficient cerebral blood flow (CBF), which is known to be vital for optimal brain function, and to facilitate neurogenesis in the hippocampus (Williams & Spencer, 2012). Isoflavones were also found to improve endothelial function and decrease arterial stiffness (Nestel, Fujii, & Zhang, 2007). It has been suggested that the vascular effects of isoflavones may be mediated by increases in nitric oxide bioavailability in the vasculature of the hippocampus following isoflavone-induced activation of endothelial nitric oxide synthase (eNOS), which leads to subsequent angiogenesis and neurogenesis (Williams & Spencer, 2012).

4.6. Chapter summary

Due to methodological differences between studies it is difficult to draw any firm conclusions about the effects of PE in humans. However, it appears that there may be positive effects on mood and some symptoms of PMS, particularly physical symptoms. Aggression may also be reduced although this may depend on dose and human trials are yet to be conducted in this area. Some domains of cognitive function, such as spatial memory, verbal fluency and frontal lobe functions are likely to be improved in females, but the effects in males are less clear. Again, dose may be a key factor here. PEs act via multiple mechanisms, all of which are most likely to be mediated by estrogen receptors, particularly ERβ. More RCTs are needed to determine the effects in humans and the optimal doses for different populations. In particular, the effects in younger women across different phases of the menstrual cycle warrant further investigation.

Chapter 5

Electrophysiological underpinnings of the effects of estrogens on response inhibition and face emotion processing

This chapter reviews the literature associated with the relationship between aggression, inhibition, and face emotion processing. The literature describing the neural and electrophysiological mechanisms underlying these behaviours will also be reviewed, as well as the effects of estrogens on inhibition and face emotion processing.

5.1. Inhibition, face emotion processing and aggression

Since the primary focus of this thesis is to understand the effects of estrogens on aggression, the cognitive activation tasks used for the electrophysiological part of the study were chosen as they are considered to be sensitive to changes in aggression. For example the go/nogo task is a measure of response inhibition, where participants are instructed to respond to certain stimuli but inhibit responses to others. The emotional salience of the stimuli can be manipulated in order to ascertain whether inhibition performance is affected by stimulus type (as described in section 5.3.2). Response inhibition has been repeatedly shown to be sensitive to differences in aggression whereby high levels of aggression have been related to poorer response inhibition and increased impulsiveness (Barratt et al., 1999; Fossati et al., 2004; Netter, Hennig, Rohrmann, Wyhlidal, & Hain-Hermann, 1998; Vigil-Colet & Codorniu-Raga, 2004). Indeed, performance of the go/nogo task has been shown to be impaired in violent offenders compared with controls when a time restriction is imposed (Chen, Muggleton, Juan, Tzeng, & Hung, 2008). In a recent study using the stop signal task, which has many experimental paradigm similarities to the go/nogo task, individuals with high levels of trait aggression exhibited impaired response inhibition compared with those who had low trait aggression, and moreover, the impairment was associated with higher motor impulsivity (Pawliczek et al., 2013).

Facial emotion expression is widely recognised as being of primary importance for communication and social functioning (Ekman, 1993). The processing of emotional cues has been shown to be disrupted in aggressive or hostile individuals, such that ambiguous or neutral stimuli tend to be perceived as threatening or angry (Hall, 2006), and individuals who score highly on trait anger show an attentional bias for angry faces (van Honk, Tuiten, & de Haan, 2001). Furthermore, hostile individuals tend to rate happy or neutral faces as less friendly than individuals with low hostility (Knyazev,

Bocharov, Slobodskaya, & Ryabichenko, 2008), a finding that is particularly pronounced in women (Knyazev, Bocharov, & Slobodskoj-Plusnin, 2009). On a neural level, the connectivity between the amygdala and the prefrontal cortex is of critical importance for processing negative emotions such as anger, and has been shown to be disrupted in aggressive populations (Coccaro, McCloskey, Fitzgerald, & Phan, 2007; Coccaro, Sripada, Yanowitch, & Phan, 2011; New et al., 2009), whilst other studies have found hyper-reactive responses to negative emotion processing in limbic regions such as the amygdala in aggressive individuals (Carré, Fisher, Manuck, & Hariri, 2012; Sebastian et al., 2012). The response of the amygdala may depend on other personality traits such as trait anxiety (Carre et al., 2012), however a hypothesis has been proposed which posits that enhanced amygdala response is characteristic of impulsive or reactive aggression, whereas blunted amygdala response may rather be more characteristic of instrumental aggression or associated with individuals with psychopathic traits (Coccaro et al., 2011). Taken together these findings suggest that performance on tasks of response inhibition and face emotion processing, as well as brain activity during performance of these tasks, may differ according to changes in aggression levels, particularly impulsive aggression.

5.2. Behavioural changes in inhibition and face emotion processing across the menstrual cycle

Several studies have investigated changes in impulsiveness, which is related to poorer response inhibition (Evenden, 1999), across the menstrual cycle (see review in Chapter 2). Briefly, impulsivity is generally found to increase during the luteal phase of the cycle, particularly in women who experience moderate to severe premenstrual symptoms, and is recognised as one of the more common and distressing symptoms of PMS (Canning et al., 2012; Hartlage & Arduino, 2002; Hsu, Liu, & Hsiao, 2007; Swann & Ussher, 1995). Changes in inhibition and face emotion processing across the menstrual cycle are summarised in Table 5.1.

Table 5.1 *Summary of behavioural changes in inhibition and face emotion processing across the menstrual cycle.*

Study	Sample (Mean age) Design	Task/Stimuli	Outcome
Amin et al.(2006)	14f (25.8 yrs) Within-group comparison of menses and mid-luteal phase	Verbal go/nogo task with neutral, positive and negative words	More errors discriminating between positive and neutral stimuli than negative and neutral stimuli regardless of phase. No effect of phase or stimulus type on reaction time.
Roberts et al. (2008)	15f (22.5 yrs) Within-group comparison of follicular and luteal phases	Go/nogo task with attractive male and female faces	Error of commission response times longer in follicular phase accompanied by reduced activity in right IFG to male faces only. No effect of phase on inhibition performance.
Pearson & Lewis (2005)	50f (20 yrs) Age range 18-22 yrs Between-group comparison of all phases	Face emotion recognition task with fearful, sad, happy, angry, disgusted and surprised faces	Enhanced recognition of fearful faces during late follicular phase compared with menses. No differences between any other phases or emotions.
Rubinow et al. (2007)	27 asymptomatic (33.5 yrs) 28 PMDD (37.9 yrs) Mixed between/within-groups comparison of follicular and luteal phases	Facial discrimination task including degrees of happy and sad emotions as well as neutral	Increased negative bias in luteal phase compared with follicular phase in PMDD sufferers only. No cycle effect for positive bias in either group.
Conway et al. (2007)	52f (19.3 yrs) Within-groups design, correlation between salivary progesterone and face recognition	Face emotion recognition with fearful, disgusted and happy faces	Fearful and disgusted faces perceived as more intense when progesterone levels are high. Effect not seen for happy faces.
Derntl, Kryspin-Exner, et al. (2008)	32f (23.84 yrs) Between-groups comparison of follicular	Emotion discrimination task with faces showing anger, disgust, fear, happiness and	Enhanced accuracy for several emotions in follicular compared with luteal phase. During luteal phase, negative emotions often mistaken for anger or disgust. Positive correlation between both estradiol and

	and luteal phases	sadness	progesterone and anger confusion, negative correlation between progesterone and neutral confusion.
Derntl, Windischberger et al. (2008)	22f (24.45 yrs) Age range 18-35 yrs Between-groups comparison of follicular and luteal phases	Emotion recognition task with angry, disgusted, fearful, happy, sad and neutral faces	Amygdala response to all faces stronger in follicular than luteal phase. Progesterone negatively correlated with amygdala response to fearful, sad and neutral faces. Enhanced emotion recognition in follicular compared to luteal phase when task difficulty high.
Derntl et al. (2013)	40f (25.3 yrs) Age range 19-34 yrs Between-groups comparison of follicular and luteal phases	Emotion recognition task with angry, disgusted, fearful, happy, sad and neutral faces	Enhanced emotion recognition in follicular than luteal phase. Faster reaction times to negative stimuli (sadness and anger) in luteal phase. Progesterone correlated negatively with emotion recognition in luteal phase, whereas correlated positively with affective responsiveness
Guapo et al. (2009)	30f (22.1 yrs) Age range 18-29 yrs Between-groups comparison of early follicular, ovulatory and luteal phases	Facial expression recognition task with angry, disgusted, fearful, happy, sad, surprised and neutral faces	Angry faces perceived more accurately in early follicular phase than all other groups, sadness more accurately perceived in follicular than luteal phase, fear more accurately perceived in follicular and ovulatory phases than in men. Estrogen negatively correlated with perception of angry male faces.

F, Female; IFG, Inferior Frontal Gyrus; PMDD, Premenstrual Dysphoric Disorder

It appears that the effects of menstrual cycle phase on inhibitory control may depend on the type of stimulus used. Whilst Amin et al. (2006) reported no variations in ability to inhibit responses to positive, neutral or negative words across phases, error of commission response times were reported to be longer during the follicular than the luteal phase when attractive faces were the stimuli (Roberts et al., 2008). This increase in response time was accompanied by reduced activity when inhibiting responses to male faces in the right inferior frontal gyrus (IFG), a core region for inhibitory control (Aron & Poldrack, 2006). Since inhibition accuracy did not vary across the cycle, the authors suggested that reduced IFG activity may represent a greater ease in inhibiting responses to reproductively salient stimuli during the follicular phase when conception is possible.

The above findings suggest that responses to face stimuli may vary across the cycle, and indeed changes in the ability to discriminate facial emotions have been documented. Studies have demonstrated enhanced face emotion recognition accuracy during the follicular phase compared with the luteal phase in women with premenstrual dysphoric disorder (PMDD; Rubinow, Smith, Schenkel, Schmidt, & Dancer, 2007) as well as asymptomatic women (Derntl, Kryspin-Exner, et al., 2008; Derntl, Windischberger, et al., 2008; Derntl et al., 2013). However, this effect may be restricted to perception of negative emotions since Guapo et al. (2009) reported the above pattern when negative face stimuli were used, but no variations across the cycle when happy faces were shown. Pearson and Lewis (2005) also reported superior recognition of fearful faces only during the late follicular phase compared with menses.

The involvement of female sex hormones in these performance variations has been demonstrated, with Conway et al. (2007) reporting that fearful and disgusted faces were perceived as being more intense during periods of high progesterone, whereas this effect was not found for happy faces. Estradiol and progesterone have also both been shown to correlate positively with mistaking other emotions for anger (anger confusion), whilst only progesterone correlated negatively with neutral confusion (Derntl, Kryspin-Exner, et al., 2008). Progesterone also correlated negatively with amygdala response to fearful, sad and neutral faces (Derntl, Windischberger, et al., 2008). One study found a negative correlation between estrogen and perception of angry male faces, suggesting that the effects of female sex hormones may be specific to the particular stimulus type.

Taken together, these findings suggest that in addition to cyclical changes in impulsiveness and response inhibition capabilities, face emotion processing may also be affected by the phase of the menstrual cycle. These effects likely depend on stimulus type and the emotional valence of the stimulus, with negative emotions more likely to be perceived less accurately during the luteal phase. Estrogen and progesterone may both play a role in these effects, however more research is needed and research on the effects of OCs and phytoestrogens are lacking.

5.3. Electrophysiological underpinnings of inhibition and face emotion processing

5.3.1. Basic Principles of Event Related Potentials (ERPs)

Electroencephalography (EEG) involves recording of voltage changes across the scalp by electrodes positioned on the scalp surface (Rugg & Coles, 1995). This electrical activity arises from the postsynaptic potentials of large populations of synchronously active pyramidal cells that are typically arranged perpendicular to the cortical surface. Because EEG measures postsynaptic potentials rather than action potentials, it is more informative regarding the afferent input of the populations of neurons than their efferent output. Furthermore, since electrical activity at the scalp can only be detected from neurons arranged perpendicular to the cortical surface, some deeper neural processes may not be detected by EEG.

The presentation of a stimulus during EEG recording results in a summated electrical potential from the neurons. The potentials evoked by stimulus presentation are contained within an epoch that is time-locked to the stimulus, and within this epoch voltage changes in response to the stimulus (event) are known as the event related potential (ERP). Typically numerous repetitions of each stimulus type are averaged, and the 'baseline' is calculated between a time interval prior to stimulus onset (e.g. 200 ms) to the event onset (0 ms) such that the mean voltage during this baseline period is zero. Group averages are then computed from these individual averages. Although use of the ERP technique has poor spatial resolution, the advantage to this technique is that it exhibits fine temporal resolution (in the order of milliseconds) compared with methods

such as fMRI which has temporal resolution of around one second, enabling accurate examination of the time-course of event related cognitive processes.

ERP components can either be positive (P) or negative (N) in polarity and are measured in terms of peak or mean amplitude (in microvolts, μv) and peak latency (in ms) from stimulus onset. For example, the N170 is a negative component that occurs approximately 170 ms following stimulus onset, and the P3 is the third positive peak. Different components may reflect processes from the same neural region, as will be discussed in section 5.3.2. One or more components are often examined from specific electrode sites based on findings of previous research rather than from all electrodes, in order to simplify interpretation. The main components associated with the go/nogo task and response inhibition are the N2 and P3, commonly referred to as the N2/P3 complex, in frontal regions (refer to Figure 5.1). The N2 is maximal over frontal-midline electrodes such as Fz, Fcz and Cz, whereas the P3 is maximal over fronto-central to centro-parietal electrodes such as Fcz, Cz and Pz. Other components are commonly described in studies of face emotion processing, such as the P1, N170/N200, vertex positive potential (VPP) and N2, however the focus of the current study regards whether inhibition-related components are modulated by the emotional valence of the stimulus presented. Therefore the N2/P3 complex will be discussed in more detail in the following sections, with brief reference to face-specific components where appropriate.

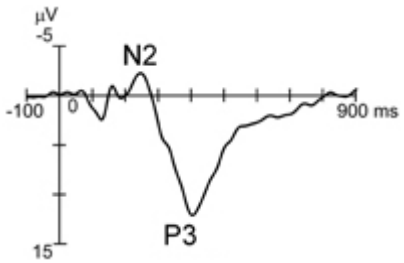

Figure 5.1 Typical representation of the N2/P3 complex waveform at the Fcz electrode site.

5.3.2. ERP components associated with the go/nogo task and response inhibition

In this thesis, response inhibition is assessed using the go/nogo paradigm, hence the literature related specifically to this task will be reviewed here, however literature concerning other tasks that operationalize response inhibition will be included as appropriate. The go/nogo paradigm consists of two tasks that are performed concurrently. The go task involves presentation of a 'go stimulus' that instructs a response, whereas the nogo task involves presentation of a 'nogo stimulus' that instructs no response. Typically more go than nogo trials are presented to increase prepotency of responses and therefore increase the difficulty of inhibiting responses.

Electrophysiological responses to nogo stimuli are generally interpreted as indicators of inhibitory processes due to the suppression of prepotent go task response, however whether this is an accurate interpretation will be discussed below. In terms of behavioural data for this task, dependent variables of interest typically include the mean reaction time to go stimuli, and the number of false alarms or errors of commission, in other words failure to appropriately inhibit the response to nogo stimuli (Huster, Enriquez-Geppert, Lavallee, Falkenstein, & Herrmann, 2013).

Two event-related potentials (ERPs) are consistently evoked by no-go stimuli: a frontal-midline N2, observed around 200-300 ms following stimulus onset, and a P3, which peaks approximately 150 ms later in the fronto-central to centro-parietal regions (Michael Falkenstein, Hoormann, & Hohnsbein, 2002; Johnstone et al., 2007). Due to the consistent observation of these components they are often referred to as the N2/P3 complex (De Jong, Coles, Logan, & Gratton, 1990; Simson, Vaughan, & Ritter, 1977). The P3 is commonly divided into two subcomponents, the P3a and the P3b, which have different scalp distributions and different functional correlates. The P3a is maximal at frontal-central electrodes and is thought to reflect the orienting of attention to unexpected events, whereas the P3b is largest at parietal electrodes and is thought to reflect the updating of working memory (Donchin, 1981; Donchin & Coles, 1988). The N2 is sometimes divided into the subcomponents of mismatch negativity (MMN), N2b which has an anterior distribution and is larger to nogo than go stimuli, and N2c which has a more posterior scalp distribution and is larger to go than nogo stimuli (Folstein &

van Petten, 2008). Theories of the functional correlates of N2 and P3 are discussed in more detail in section 5.3.3.

Several authors have attempted to delineate the neural networks underpinning the components relating to response inhibition through use of functional magnetic resonance imaging (fMRI), EEG source modelling or using a combination of imaging techniques. Evidence from fMRI studies suggests that two cortical regions are activated during response inhibition: an inferior frontal region including the insular cortex, and a medial region spanning the pre-supplementary area (preSMA), both of which have previously been implicated in the control of motor inhibition (Aron, 2011). These regions are strongly connected to basal ganglia networks, which are crucial for movement cancellation (DeLong & Wichmann, 2007; Wichmann & DeLong, 1996). Studies employing EEG source modelling techniques have reported that the frontal-midline N2 is generated in the inferior frontal cortex (Lavric, Pizzagalli, & Forstmeier, 2004), or the mid-cingulate cortex (MCC; Bekker, Kenemans, & Verbaten, 2005; Nieuwenhuis, Yeung, Van Den Wildenberg, & Ridderinkhof, 2003). Recently, Huster, Westerhausen, Pantev, and Konrad (2010) reported a frontal-midline N2 source in the anterior MCC and a left inferior frontal source, however the amplitude differences between go and nogo conditions were driven solely by the left dorsal anterior MCC.

Surface P3 potentials have also been reported to be generated by the MCC, although contributions from the precentral cortex have also been reported (Kok, Ramautar, De Ruiter, Band, & Ridderinkhof, 2004; Ramautar, Kok, & Ridderinkhof, 2006). Furthermore, Huster et al. (2010) found bilateral contributions from the middle frontal cortex and insulae, although as with the N2 component, differences in P3 amplitude between conditions were driven by the MCC. In a study that utilised a combination of EEG and fMRI techniques, P3 amplitude was associated with activity in a medial frontal source, which the authors suggested may correspond to the preSMA, as well as a right temporo-parietal and insular regions (Karch et al., 2008). A more recent study using a combination of EEG and fMRI reported that the N2/P3 complex for successful stop trials in a stop-signal paradigm was associated with one network spanning the dorsal MCC, preSMA and anterior insulae, as well as a second network mostly involving the basal ganglia (Huster et al., 2011). In the same study, N2 was also evoked on go trials and showed the same fronto-medial topography as the stop-related N2,

although it was linked to networks spanning anterior and posterior MCC, occipital regions, and supplementary motor areas, but not preSMA.

5.3.3. Theories of N2 and P3 components associated with the go/nogo task

Although the components evoked by nogo trials are usually interpreted as representing response inhibition processes, other theories have been proposed to explain the enhanced N2 and P3 following nogo compared with go stimuli. It has been suggested that the frontal-midline N2 may rather reflect a general evaluative or executive control mechanism (Nieuwenhuis et al., 2003) such as response conflict monitoring (Botvinick, Cohen, & Carter, 2004). Conflict arises when an infrequent or deviant type of response is required in the context of frequent responses, due to the simultaneous activation of competing response tendencies (Braver, Barch, Gray, Molfese, & Snyder, 2001). Indeed, the frontal-midline N2 amplitude is increased when the probability of nogo trials is reduced, as has been demonstrated in numerous studies (Enriquez-Geppert, Konrad, Pantev, & Huster, 2010; Nieuwenhuis et al., 2003; Ramautar, Kok, & Ridderinkhof, 2004), and in fact when the nogo stimulus is the more common trial type the N2 is smaller in amplitude than that following the less common go stimulus (Enriquez-Geppert et al., 2010). However, a study using unfamiliar cartoon images as stimuli that were either novel, conflicting (images belonging to a category but showing different features than those seen during training) or typical (images that looked the same as those during training), the N2 elicited was not affected by response conflict (Folstein, Van Petten, & Rose, 2008).

It has also been proposed that these effects may reflect a simple reaction to novel stimuli (Dimoska & Johnstone, 2008). Folstein et al. (2008) reported that the anterior N2 was affected by the mismatch between a stimulus and a mental representation of the category, in other words the novelty of the stimulus. This is partially refuted by studies demonstrating that the frontal-midline N2 is modulated by stimulus similarity, with N2 amplitude being increased when stimuli are more similar (Nieuwenhuis, Yeung, & Cohen, 2004; Smith & Douglas, 2011; Szmalec et al., 2008), which provide support for the conflict monitoring theory. In addition, Pfefferbaum, Ford, Weller, and Kopell (1985) first demonstrated that when go and nogo stimuli were presented with equal

probability, nogo trials elicited a larger N2 compared to go trials at a frontal midline electrode site despite the lack of novelty.

Support for the notion that the nogo N2 does reflect response inhibition comes from studies demonstrating that although nogo stimuli elicit larger N2s than go stimuli even in tasks requiring silent counting rather than physical responses, it does tend to be larger when an overt response must be withheld (Bruin & Wijers, 2002; Pfefferbaum et al., 1985). Furthermore, nogo N2 amplitude is increased when the task involves pressure to respond quickly (Jodo & Kayama, 1992) and is also larger and earlier in participants who have lower error of commission rates than those with more errors of commission, suggesting that N2 amplitude is related to successful response inhibition (Falkenstein, Hoormann, & Hohnsbein, 1999).

With regard to the P3, it has long been accepted that this component reflects individual differences in cognitive capacity (Intriligator & Polich, 1995). In terms of response inhibition, although some studies have demonstrated that P3 amplitude varies with nogo probability (Ramautar et al., 2004), it has also been shown that P3 amplitude is most often larger when a response has to be suppressed regardless of whether or not this is the most common trial type (Enriquez-Geppert et al., 2010). This suggests that the novelty of the stimulus does not evoke the P3. Comparison of behavioural data with electrophysiological findings has demonstrated that fast responders show higher frontal-midline P3 amplitudes with successful stop trials, whereas N2 is attenuated (Dimoska, Johnstone, & Barry, 2006; Smith, Johnstone, & Barry, 2006). This suggests that the P3 but not the N2 may reflect response inhibition processes. However, in a review of the relevant literature Huster et al. (2013) concluded that the frontal-midline P3 probably occurs too late to correspond to an actual motor inhibition process. Rather, it may reflect a post-inhibition effect, such as the evaluation of inhibitory performance (Bruin, Wijers, & Van Staveren, 2001).

The P3 has also been explained in terms of a context updating theory (Polich, 2003) which posits that when a stimulus is processed an attention driven comparison is made between this stimulus and the representation of the previous stimulus in working memory. If there is no difference in stimulus context, the schema is maintained, however if a new stimulus is detected, attentional processes update the stimulus representation resulting in a P3. In a review of the P3 literature, Polich (2007) reported

that this theory is supported by evidence that P3 latency is longer and amplitude is smaller during tasks that require greater attentional resources (Kok, 2001), as well as an improvement in memory for stimuli that elicit a P3 compared with those that do not (Rushby, Barry, & Johnstone, 2002), and that stimuli that receive full attention are recognised more easily and are associated with greater P3 amplitude (Curran, 2004; Curran & Cleary, 2003). However, Polich (2007) concluded that whilst this component is attention-driven, it may also reflect neural inhibitory activity by focusing attention and inhibiting responses to task-extraneous events.

5.3.4. Association between neurotransmitters and inhibition-related components

There is evidence to suggest that the dopaminergic system may be implicated in eliciting both the N2 and the P3 components. In patients with Parkinson's disease, a condition associated with dopaminergic deficits, P3 was almost non-existent in a three-stimulus oddball paradigm known to elicit large P3s in healthy individuals (Polich & Criado, 2006). More recently N2 was also found to be attenuated in patients with Parkinson's in a go/nogo task (Beste, Willemssen, Saft, & Falkenstein, 2010). In addition, administration of the dopamine antagonist sulpiride was shown to increase the P3 in low-amplitude subjects and decrease it in high-amplitude subjects (Takeshita & Ogura, 1994). Taken together these findings suggest that increased levels of dopamine result in larger N2 and P3 amplitudes.

Estrogen and progesterone have opposing actions on neurotransmission of dopamine. Estrogen increases dopamine synthesis as well as its turnover and release (Becker & Beer, 1986). Dopamine receptor density is also increased by estrogen (Hruska & Silbergeld, 1980), whereas monoamine oxidase (MAO) activity, which reduces the degradation of dopamine, is decreased (Luine, Khylchevskaya, & McEwen, 1975). On the other hand, progesterone has been shown to down-regulate dopaminergic systems (Shimizu & Bray, 1993). This suggests that when estrogen is high and progesterone is low, inhibitory control and N2 and P3 amplitude may be enhanced through increases in dopaminergic function, whereas when progesterone is also high, inhibitory control and the N2 and P3 may be reduced due to down-regulation of dopaminergic activity.

5.3.5. Evidence for a relationship between aggression levels and ERP components associated with response inhibition

Several studies have shown that the N2 and P3 components are modulated by aggressive tendencies. One such study found reduced N2 to nogo stimuli in violent offenders compared with matched controls, resulting in a smaller difference between nogo and go conditions in this population (Chen, Tien, Juan, Tzeng, & Hung, 2005). The authors concluded that since attenuated N2 is associated with poorer response inhibition, the violent offenders may have difficulties with inhibiting responses, which provides support for the notion that increased impulsiveness is associated with increased aggression (e.g. Barratt et al., 1999). No differences in P3 amplitude or latency were found between violent offenders and control subjects (Chen et al., 2005).

Similar findings were reported in a study of children with operational defiant disorder or conduct disorder, who display increased levels of aggression (Albrecht, Banaschewski, Brandeis, Heinrich, & Rothenberger, 2005). N2 mean amplitudes in a stop signal task were reduced in children with these disorders compared with children with no behavioural disorders, and performance deficits were also evident in the clinical group of children (Albrecht et al., 2005).

In contrast to the findings of Chen et al. (2005), Munro et al. (2007) reported no differences in either N2 or P3 amplitude between violent offenders and controls, nor did they find any relationship between the level of psychopathic tendencies and N2 or P3 amplitude. Interestingly, this lack of effect was evident despite a higher rate of errors of commission in offenders compared with non-offenders.

5.3.6. ERP components specifically associated with face emotion processing

The N2 and P3 components were specifically chosen for the current study to examine the effects of estrogen on response inhibition and whether these effects are modulated by the emotional salience of the stimulus presented. However, other components thought to be associated with face emotion processing are briefly presented here for completeness.

The P1 is an early positive component that is evoked by all visual stimuli and is often examined in studies of face processing. It is typically observed over parieto-occipital electrodes (Batty, Meaux, Wittemeyer, Rogé, & Taylor, 2011; Luo, Feng, He, Wang, & Luo, 2010). P1 amplitudes are larger in tasks requiring increased attention, therefore it is thought that the P1 reflects the early allocation of attention (Di Russo, Martinez, & Hillyard, 2003).

One of the most commonly described components evoked by face emotion stimuli is the N170/N200, which is a negative potential occurring over occipito-temporal sites and peaking from 140-240ms post stimulus onset (Allison et al., 1994; Batty & Taylor, 2003; Sprengelmeyer & Jentzsch, 2006; Wheaton, Pipingas, Silberstein, & Puce, 2001). The N170/N200 is a face-specific component, however whether it is sensitive to the emotional valence of the face stimulus is disputed. Some studies have reported larger N170/N200 amplitudes elicited by emotional faces compared with neutral faces (Batty & Taylor, 2003; Blau, Maurer, Tottenham, & McCandliss, 2007; Luo et al., 2010) whereas others have found no such effect (Eimer & Holmes, 2002; Herrmann et al., 2002). Accompanying the N170/N200 at a similar latency is a positive-going potential maximal over mid-central sites termed the vertex positive potential (VPP), which is thought to be the positive moment of the same dipole generating the N170/N200 (Rossion et al., 1999). The VPP has been shown to be sensitive to the emotional valence of face stimuli (Ashley, Vuilleumier, & Swick, 2004; Blau et al., 2007; Eger, Jedynak, Iwaki, & Skrandies, 2003; Eimer & Holmes, 2007). In particular, the VPP/N170 complex may be enhanced for fearful or angry faces compared to neutral faces (Ashley et al., 2004; Batty & Taylor, 2003; Foti, Olvet, Klein, & Hajcak, 2010), and the N170 may peak later for negative emotions compared to positive emotions and neutral faces (Batty & Taylor, 2003).

The fronto-central N2, on the other hand, is generally accepted as being associated with processing of specific facial emotions. Several studies have shown a decrease in N2 amplitude in response to fearful expressions compared with neutral expressions (Eimer & Holmes, 2002; Eimer, Holmes, & McGlone, 2003; Kiss & Eimer, 2008), and N2 is also attenuated to other face emotions such as anger and happiness in studies where participants are required to perform tasks of explicit emotional processing (Paulmann & Pell, 2009; Streit, Wölwer, Brinkmeyer, Ihl, & Gaebel, 2001).

In addition, larger positivities at frontocentral sites from 250-1000 ms post stimulus have been found for emotional compared to neutral face stimuli (Eimer & Holmes, 2002). The authors suggested that these very long latency positive potentials may indicate sustained attention being directed towards emotionally relevant stimuli whereas earlier positive potentials may reflect rapid activation of prefrontal areas involved in the detection of emotionally relevant stimuli.

Several authors have investigated the effects of emotional stimuli, including face emotions, in tasks of response inhibition. In a recent study using an oddball paradigm, Chai et al. (2012) reported that angry faces elicited a smaller N2 than neutral faces and a longer P3b latency than happy faces, as well as a larger P3b amplitude than sad or neutral faces. Happy faces also elicited a larger P3b than neutral faces, and P3b latency to happy faces correlated negatively with the personality traits passive aggressiveness and callousness, suggesting differential processing of emotional faces in individuals with aggressive tendencies. These findings suggest that emotional face stimuli in tasks of response inhibition may modulate inhibition-related components differently than the use of abstract stimuli.

Table 5.2 *Summary of the main ERP components associated with response inhibition and face emotion processing and theories of what these components reflect.*

Component	Scalp topography and latency of component	Theories of what the component may reflect
N2	200-300 ms post stimulus onset at frontal midline sites	Response conflict monitoring; Reaction to novel stimuli; Response inhibition processes.
P3	350-450 ms post stimulus onset at fronto-central to centro-parietal sites	Evaluation of inhibition performance; Updating of working memory: Attentional processes.
P1	100 ms post stimulus onset at parieto-occipital sites	Allocation of attention
N170/N200	140-240 ms post stimulus onset at occipito-temporal sites	Face specific – may reflect structural encoding of stimuli

5.4. Electrophysiological changes across the menstrual cycle and effects of female sex hormones

Several lines of evidence suggest that changes in female sex hormones may modulate electrophysiological responses to certain stimuli. Whilst only one study investigated N2 modulation across the cycle, finding shorter N2 latency during menses than the follicular or luteal phases (Walpurger, Pietrowsky, Kirschbaum, & Wolf, 2004), several studies have investigated changes in the P3 across the cycle. This research is summarised in Table 5.3.

Using various tasks consisting of frequent and infrequent stimuli, research has shown mixed findings regarding the effect of menstrual cycle phase on the P3. Increased P3 amplitude during the luteal phase compared with other phases of the cycle has been found by some researchers (Kluck et al., 1992; Sun, Qu, Zhoa, Yu, & Zheng, 2012), however others have reported no variations in P3 amplitude across the cycle (Baker & Colrain, 2010; Tasman, Hahn, & Maiste, 1999; Walpurger et al., 2004). Furthermore, P3 amplitude has been shown to be greater during menses than the ovulatory phase (O'Reilly, Cunningham, Lawlor, Walsh, & Rowan, 2004), and has also been reported to be reduced in women with premenstrual dysphoric disorder (PMDD) compared with controls (Baker & Colrain, 2010).

Findings regarding cyclical variations in P3 latency are also unclear, with Tasman, Hahn, and Maiste (1999) reporting longer P3 latency during the ovulatory phase than any other phase, whilst O'Reilly, Cunningham, Lawlor, Walsh, and Rowan (2004) reported shorter P3 latency during the ovulatory phase than menses. Others have found no cyclical variations in P3 latency (Ehlers, Phillips, & Parry, 1996; Walpurger et al., 2004), although Ehlers, Phillips, and Parry (1996) did report longer P3 latency in women with PMDD than controls, regardless of phase.

Interestingly, changes in reaction time across the cycle to the stimuli evoking these potentials have not been found (Kluck et al., 1992; Sun et al., 2012; Tasman et al., 1999), nor have changes in performance of other cognitive tasks (O'Reilly et al., 2004). However, improvements in mood accompanying reduced P3 amplitude and latency have led O'Reilly et al. (2004) to propose that the observed physiological changes may reflect changes in mood rather than cognitive capacity.

Table 5.3 *Summary of electrophysiological changes across the menstrual cycle, effects of female sex hormones and relationships with hostility.*

Study	Sample (Mean age) Design	Task/Stimuli	Outcome
Walpurger et al. (2004)	18f (26.5 yr) Age range 18-35 yr Within-groups comparison of menses, follicular and luteal phases	Auditory oddball paradigm	Shorter N2 latency during menses than follicular or luteal phases. No differences between phases in N2 amplitude, P3 latency or P3 amplitude.
Kluck et al. (1992)	6f (29.5 yr) Age range 27-33 yr Within-groups comparison of follicular, early luteal and late luteal phases	Visual-spatial task using infrequent targets and frequent non-targets	Increased P3 amplitude to infrequent target stimuli during late luteal phase (non-significant). No differences in reaction time.
Sun et al. (2012)	10m (22 yr) 23f (22 yr) Age range 18-30 yr Between-groups comparison of menses, luteal phase and men	Auditory oddball paradigm	Increase P3 amplitude to novel stimuli in luteal phase compared with menses and men. No differences with standard stimuli, and no differences in performance.
Tasman et al. (1999)	19f (28.5 yr) Age range 19-40 yr Within-groups comparison of follicular, ovulatory and luteal phases	Visual-spatial task using infrequent targets and frequent non-targets	Longer P3 latency at midline electrode sites in ovulatory phase than any other phase. No variation in amplitude or reaction time.
O'Reilly et al. (2004)	12f (23.5 yr) Age range 19-28 yr Within-groups comparison of menses and ovulatory	Animal words used as targets mixed with random non-targets	Greater P3 amplitude and longer P3 latency during menses than ovulatory phase at Pz electrode.

	phase		
Ehlers et al. (1996)	15 PMDD 15 asymptomatic controls Mixed between/within-groups comparison of follicular and luteal phases	Auditory oddball paradigm	Longer P3 latency to infrequent stimuli at central midline electrodes in women with PMDD than controls. No difference between phases for either group
Baker & Colrain (2010)	9 PMS/PMDD (28 yr) 8 asymptomatic controls (32 yr) Mixed between/within-subjects comparison of mid-follicular and late luteal phases	Auditory oddball paradigm	P3 amplitude reduced in women with PMDD compared with controls. No group differences in P3 latency. No differences across phases.
Zhang & Lu (2012)	11m; 9f Age range 18-20 yr Within-groups comparison of effects of different emotions	Emotional go/nogo task	Shorter N2 latencies and smaller N2 amplitudes to positive and negative than neutral facial go stimuli. No effect of stimulus valence on nogo N2 amplitude or latency. Higher P3 amplitudes and shorter P3 latency to emotional stimuli than neutral stimuli in both go and nogo conditions.
Johnston & Wang (1991)	30f (Age range 20-35 yr) Between-groups comparison of follicular, ovulatory and luteal phases	Viewing of pleasant, unpleasant or neutral stimuli	P3 amplitude changes largest in response to “pleasant” stimuli during periods of high progesterone. No effects of MC phase with neutral stimuli. Larger P3 to pleasant and unpleasant stimuli than neutral stimuli regardless of phase.
Wuttke et al. (1975)	16 NC 16 OC Age range 18-25 yr Mixed between/within-groups design	Resting with eyes closed	Peak of α-power shifts from slow to fast between late follicular phase and menstruation, with maximum acceleration 1-6 days before menses onset and sudden drop on first day of menses. No cyclical variations in OC users, but slower α-band activity in OC users than non-users across the cycle.
Creutzfeldt et al. (1976)	16 NC 16 OC Age range 20-28 yr Mixed between/within-	Resting with eyes closed	Lower α-frequency in OC users than NC women across the cycle. Acceleration of α-power during luteal phase in NC women.

	groups design		
Amin et al. (2006)	14f (25.8 yr) Within-groups comparison of early follicular and mid-luteal phases	Emotional go/nogo task with fMRI	Greater difference between go and nogo elicited activation to positive words during luteal phase than follicular phase in bilateral ACC, dorsolateral PFC and right putamen. No phase differences in activation to negative stimuli. Estradiol correlated positively with inhibition-related neural activation to positive stimuli but negatively with activation to negative stimuli during luteal phase.
Bannbers et al. (2012)	18 PMDD (34.9 yr) 14 asymptomatic controls (34.9 yr) Mixed between/within-groups comparison of mid-follicular and late luteal phases	Go/nogo task	PMDD show reduced activity during nogo response inhibition in parietal regions compared with controls regardless of phase. Increased insula activity during luteal phase in PMDD group, follicular phase in control group. No group differences in trait impulsivity.
Knyazev et al. (2009)	38m; 46f (20.7 yr) Age range 17-32 yr Between-groups design	Hostility ratings of emotional faces	Higher theta synchronisation and lower alpha desynchronisation in hostile individuals. Hostility-related differences more pronounced in females than males. Theta and alpha synchronisation most pronounced to angry faces in hostile females.

F, Female; M, Male; PMDD, Premenstrual Dysphoric Disorder; PMS, Premenstrual Syndrome; MC, Menstrual Cycle; OC, Oral Contraceptive users; NC, Normally Cycling women; ACC, Anterior Cingulate Cortex; PFC, Prefrontal Cortex

Due to the conflicting findings surrounding cyclical changes in the N2 and P3 components, it has been suggested that the emotional salience of the stimuli presented may modulate electrophysiological responses. P3 amplitudes elicited to emotional faces are higher than those to neutral faces (Zhang & Lu, 2012), and studies of the menstrual cycle have shown that P3 amplitudes in response to pleasant stimuli are largest during periods of high progesterone, whereas P3 amplitudes to neutral stimuli did not show cyclical variation (Johnston & Wang, 1991). It is thought that the enhanced P3 to pleasant stimuli (e.g. babies and male models) during periods of high progesterone may reflect the increased importance of these stimuli during periods of pregnancy or potential pregnancy (Johnston & Wang, 1991).

Estrogen has also been suggested as playing a role in the differential effects of emotionally laden stimuli across the cycle. Amin et al. (2006) demonstrated that differences in activation in response to positive nogo words relative to positive go words were greater during the luteal than the follicular phase in several brain regions associated with response inhibition, an effect that was not observed with negative stimuli. Since estradiol was found to correlate positively with neural activation to positive stimuli but negatively with neural activation to negative stimuli, it was suggested that these findings are consistent with estrogen being associated with positive mood (Amin et al., 2006).

Regarding the effects of oral contraceptives (OCs) on electrophysiological markers, whilst no studies have investigated the effects on the N2 and P3 components, it has been reported that α-band activity is slower in OC users than normally cycling (NC) women (Creutzfeldt et al., 1976; Wuttke et al., 1975). Both studies also found cyclical variations in α-frequency in NC women but not OC users. The authors suggested that these differences may reflect differing cognitive performance. No studies to date have investigated the electrophysiological effects of soy isoflavones.

5.5. Chapter Summary

The existing literature suggests that increased aggression is associated with poorer response inhibition as well as poorer face emotion recognition accuracy. Regarding the

effects of female sex hormones, response inhibition may be poorer during the luteal phase, however this also may depend on the emotional valence of the stimuli, with more salient stimuli being processed more efficiently during the follicular phase. Several studies have also shown that face emotion processing is enhanced during the follicular phase, particularly for negative emotions, which is accompanied by a stronger amygdala response to emotional stimuli during this phase. In terms of the electrophysiological correlates of response inhibition, the N2 and P3 may also be modulated by the emotional valence of the stimulus, with attenuation of the N2 to emotional compared with neutral faces, whereas the P3 has been shown to be enhanced to emotional stimuli. Research has also shown that these components are modulated by menstrual cycle phase, with shorter N2 latency during menses and a larger P3 during the luteal phase. These findings suggest that female sex hormones may modulate electrophysiological correlates of response inhibition, and that these effects may depend on the emotional salience of the stimulus. The use of electrophysiological measures may therefore provide some insight into the potential mechanisms of action underlying any effects of the menstrual cycle, OC and SIF on aggression, mood and cognition. To date, the effects of oral contraceptive use and soy isoflavone administration have not been studied in this context, therefore the experiment presented in the following chapters aimed to investigate the effects of OCs and SIF on premenstrual symptoms, aggression and cognition, as well as electrophysiological changes that may underlie these effects.

Chapter 6

Effects of Soy Isoflavones and Oral Contraceptive use on Mood, Aggression, Cognitive Function and Brain Activation across the Menstrual Cycle: Aims and Methods

6.1. Aims

The primary aim of this study was to investigate the effects of a soy isoflavone (SIF) supplement versus placebo on premenstrual symptoms, general mood, aggression and cognitive function across the menstrual cycle. The effects of SIF and placebo on brain electrical activity during tasks of response inhibition and face emotion recognition were also investigated as a potential aid to understanding the mechanisms involved in the effects of SIF on aggression and cognition. Further aims were to compare a group of OC users with normally cycling (NC) women on the above outcomes to explore the effects of OC use across the cycle in long-term OC users. Cyclical variations in all outcomes were also examined in an attempt to confirm findings that mood and cognition vary during different phases. A final aim was to test the hypothesis that the N2 and P3 components are elicited due to the novelty of the stimulus rather than the requirement of response inhibition, using a difference waveform approach to isolate components of interest by subtracting infrequent go waveforms from equally infrequent nogo waveforms.

6.2. Rationale and hypotheses

As summarised in Chapters 2-4, the effects of estrogens of various classes on PMS symptoms have been extensively studied, however there are some gaps in the literature. Few studies have specifically examined the effects of estrogens on aggression despite this and related symptoms causing distress for many women (e.g. Canning, Waterman, Simpson, & Dye, 2012; Endicott et al., 1999; Hallman, Oreland, Edman, & Schalling, 1987). Several studies have investigated the effects of oral contraceptives (OCs), however ethical issues regarding the administration of OCs in randomised, double-blind, placebo-controlled clinical trials make conducting these types of trials difficult, limiting research on the effects of OCs to mostly cross-sectional studies. The study of phytoestrogens allows the placebo-controlled investigation of the effects of an estrogenic compound that is taken for non-contraceptive reasons and effects can be more directly attributed to the chemical properties of the compound. Research on the effects of soy isoflavones has been largely restricted to older female populations, with

few studies focussing on younger females. No studies have yet investigated the effects of chronic SIF administration on mood, premenstrual symptoms, aggression or cognitive function across all phases of the menstrual cycle. Research has shown that in non-help-seeking women PMS symptoms are rarely confined to only the luteal phase and are also commonly experienced during menses (Romans, Clarkson, Einstein, Petrovic, & Stewart, 2012). Since the current study utilised a non-clinical sample, it was hypothesised that any premenstrual and mood symptoms observed at baseline in normally cycling (NC) women would be more severe in both the luteal phase and menses than the follicular and ovulatory phases. Furthermore, due to the findings from previous research (e.g. Poromaa & Segebladh, 2012; Toffol, Heikinheimo, Koponen, Luoto, & Partonen, 2011) it was expected that OC users would rate their mood and physical symptoms as less severe than NC women and would also experience less variation in these symptoms across the cycle. Regarding the effects of SIF on mood and premenstrual symptoms, since the few studies that have been conducted to date have found improvements in physical symptoms (e.g. Bryant et al., 2005) it was hypothesised that SIF supplementation would be associated with lower ratings of physical symptoms compared to placebo. Effects on mood were also hypothesised to be positive in comparison to placebo although it was expected that this effect would be smaller. Due to the estrogenic effects of SIF when endogenous estrogen is low it was hypothesised that these effects would be most pronounced during the late luteal phase and menses, when endogenous estrogen levels are at their lowest.

In light of previous findings that have demonstrated changes in different types of aggression depending on circulating estrogen levels (Brambilla, Speca, Pacchiarotti, & Biondi, 2010; Ritter, 2003) it was predicted that indirect aggression, such as verbal aggression, would be increased during times of increased estrogen (ovulatory and luteal phases in NC women), whereas direct aggression, such as physical aggression, would be increased during times of lower estrogen levels (menses in NC women). OC users have previously been shown to have lower levels of all forms of aggression than NC women (Perry, Canning, Scholey, & Dye, under review), therefore it was expected that OC use would be associated with lower levels of aggression, particularly during the luteal phase and menses when NC women were expected to have more severe symptoms. Human studies on the effects of SIF have not yet been conducted and animal studies have shown conflicting findings, however due to the evidence that activation of ERβ has

inhibitory effects on aggression and that SIF preferentially binds to ERβ it was hypothesised that women receiving SIF would have lower levels of aggression than women receiving placebo. As with effects on premenstrual symptoms, it was expected that these effects would be most pronounced during menses and the late luteal phase when levels of circulating estrogen are lowest.

Regarding cognitive function, due to the findings of studies demonstrating better performance of female-positive tasks during periods of high estrogen and better performance of male-positive tasks during periods of lower estrogen (e.g. Hampson, 1990a, 1990b) it was predicted that performance on tasks such as immediate and delayed word recall and recognition assessing verbal memory, as well as delayed picture recognition (Phillips & Sherwin, 1992) would be enhanced during the ovulatory and luteal phases, whereas performance on the corsi blocks task measuring spatial ability would be better during menses and the follicular phase. Since previous studies have demonstrated improved cognitive function in OC users compared with women in the early follicular phase (Gogos, 2013) it was hypothesised that in the current study, performance on cognitive tasks would be improved in OC users compared with NC women during menses and the follicular phase. Specifically, verbal memory as assessed in the immediate and delayed word recall tasks was expected to differ between OC users and NC women. It was also expected that there would be little difference between OC users and NC women in their luteal phase due to previous findings that these groups have similar cognitive outcomes (Gogos, 2013).

SIF use has been shown to improve various cognitive domains such as working memory and verbal learning during menses (Islam et al., 2008) as well as improving spatial memory, delayed picture recognition and aspects of executive function such as planning and rule learning and reversal when menstrual cycle phase was not controlled for (e.g. File et al., 2005; Thorp, Sinn, Buckley, Coates, & Howe, 2009). It was therefore hypothesised that SIF supplementation would result in improved performance compared with placebo on working memory tasks, word recall, spatial memory, and delayed picture recognition, as well as a card sorting task measuring rule learning and reversal, and a peg and ball task measuring planning ability. Since previous studies have not controlled for cycle phase it was expected that these improvements would be seen

across the cycle, but due to the findings of Islam et al. (2008) the difference between SIF and placebo was expected to be largest during menses.

Research has shown that response inhibition is impaired during the luteal phase of the menstrual cycle, when steroid hormone levels are high (e.g. Hsu, Liu, & Hsiao, 2007). It was therefore expected that performance of the go/nogo task, a task requiring response inhibition, would be poorer during the luteal phase compared with during menses, when endogenous hormones are at their nadir, in NC women. This impairment in response inhibition was expected to be indexed by reduced N2 and P3 peak amplitudes to nogo stimuli during this phase in accordance with previous literature showing that increased errors of commission are associated with reduced amplitudes of these components (Falkenstein et al., 1999). Due to the findings that face emotions are more accurately recognised during the follicular phase compared with the luteal phase (e.g. Derntl, Kryspin-Exner et al., 2008), and that salient stimuli are processed more efficiently during the follicular phase (Roberts et al., 2008) it was hypothesised that only go/nogo tasks requiring discrimination between facial emotions would show these performance differences between phases. Furthermore, since salient stimuli have been found to modulate ERP components across the cycle, whereas neutral stimuli do not (Johnston & Wang, 1991) it was expected that only emotional faces stimuli would show cyclical variation in N2 and P3 responses.

OC users have been reported to have lower levels of aggression and reduced impulsivity compared with NC women (e.g. Perry et al., under review), therefore it was hypothesised that OC users would make fewer errors of commission than NC women, and would have enhanced N2 and P3 amplitudes to nogo stimuli. It was also expected that these effects would be more pronounced when the task required discrimination of faces showing different emotions. The effects of SIF on aggression and impulsivity in humans have not yet been studied. However, due to the expectation that SIF would reduce aggression in the current study (see above), it was hypothesised that SIF would also be associated with improved response inhibition in terms of fewer errors of commission and increased N2 and P3 amplitude. In particular, women treated with SIF were hypothesised to have increased amplitudes and shorter latencies to nogo angry faces compared with women treated with placebo, as these stimuli may be more salient to aggressive individuals (Knyazev et al., 2009).

6.3. Methods

6.3.1. Overview of Experimental Methods

The current study utilised a mixed between-within groups design to examine the effects of isoflavones over three menstrual cycles. The first of these cycles was taken as a baseline, followed by two cycles of treatment with either 200 mg soy isoflavones (SIF) per day or placebo. A group of long term oral contraceptive (OC) users was also included and continued taking OC throughout the study. Measures of mood, cognitive function, aggression, and saliva samples were conducted once during each of four phases of each menstrual cycle (see Figure 6.1 for an overview of each experimental session).

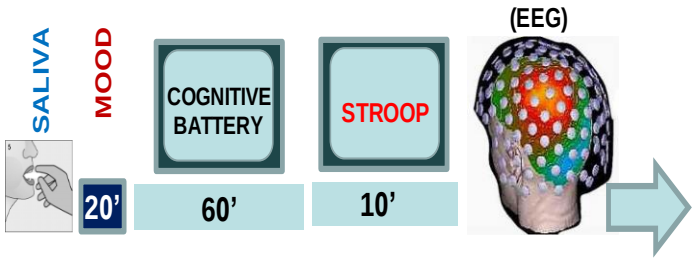

Figure 6.1. Overview of measures completed during experimental sessions in the order they were completed. Note that electrophysiological measures were completed in only four of the sessions.

The primary outcome measure for this study was total aggression scores as measured using the Buss-Perry Aggression Questionnaire (Buss & Perry, 1992). Secondary outcomes were total scores on the modified Daily Symptom Report (Canning et al., 2012), aggression scores as measured using the State-Trait Anger Expression Inventory (STAXI; Spielberger, 1991), and scores on the peg and ball, card sorting and Corsi blocks cognitive tasks. Electrophysiological measures were also conducted during menses and the luteal phase of the baseline and second treatment cycles only. Table 6.1 summarises the full study timeline.

Since no studies have previously examined the effects of isoflavones on aggression, a power calculation could not be conducted based on previous literature. However, according to Cohen (1992), a minimum of 21 participants per group are required to

detect a significant effect ($p<0.05$) with a large effect size in studies with three groups. In addition, Bryant et al. (2005) conducted post hoc power analyses in their study of the effects of isoflavones on premenstrual symptoms, and reported that with 23 participants their study was powered to detect significant effects. It was therefore decided that a minimum of 23 participants per group would be required to ensure adequate power.

Table 6.1 *Overview of the full study timeline.*

Cycle	Baseline				Treatment Cycle One				Treatment Cycle Two			
Phase	M	F	O	L	M	F	O	L	M	F	O	L
Aggression	+	+	+	+	+	+	+	+	+	+	+	+
Mood	+	+	+	+	+	+	+	+	+	+	+	+
Cognitive Battery	+	+	+	+	+	+	+	+	+	+	+	+
Saliva Samples	+	+	+	+	+	+	+	+	+	+	+	+
EEG	+			+					+			+

M, Menses; F, Follicular; O, Ovulatory; L, Luteal; EEG, Electroencephalograph

6.3.2. Participants

6.3.2.1 Selection Criteria

Participants were initially screened by telephone, and again with a face-to-face interview with the experimenter during the practice session to ensure they had no history of psychiatric disorders, anxiety or depression, had no neurological, gastrointestinal, endocrine or bleeding disorders, were not pregnant or lactating, were not taking any illicit drugs, supplements or any other medication other than a combined monophasic oral contraceptive, had no history of kidney, liver or heart disease, and had no food allergies. In order to be eligible participants must be female aged between 18 and 35 with a regular menstrual cycle (21-35 days) to ensure data could be collected during the correct phases of the cycle, and to ensure pre-menopausal status. In addition,

participants who reported being smokers were excluded due to possible confounding effects on central nervous system (CNS) cholinergic status.

During a practice session, participants were questioned regarding their contraceptive status to ensure that they were either not using hormonal contraception of any kind (if being allocated to soy or placebo), or that the oral contraceptive (OC) they were using was of the combined, monophasic variety (if being allocated to the OC group). Participants who had been using OCs for less than 3 months (if in the OC group), or who had been free from OC use for less than 3 months (if in the soy or placebo group) were excluded. Participants were also excluded if they reported consuming soy products more than 2 times per week as this may interfere with the outcomes of the study, and were provided with a list of phytoestrogen-containing foods to avoid. Height, weight and blood pressure were also measured, and only those with a blood pressure within the normal range and a Body Mass Index (BMI) of between 19 and 26 were included.

6.3.2.2. Sample Characteristics

A total of 72 female participants aged 18-34 years (mean = 23.7 years; SD = 4.2) completed the trial. Mean BMI for the whole cohort was 22.5, with an average of 16.3 years of education. Of the total sample, 23 were using oral contraceptives, 23 were randomised to receive soy isoflavones (SIF) and 26 were randomised to receive placebo throughout the course of the investigation. Demographics for each treatment group are shown in Table 7.1. Participants were recruited via media advertising (including newspapers, Facebook and Gumtree), internal advertising at Swinburne University of Technology, word of mouth, and registration with Clinical Trials Connect, a company specialising in recruiting for clinical trials (CTC, 2011). Figure 6.2 displays the number of participants who initially expressed interest in the trial, and how many were lost at each stage.

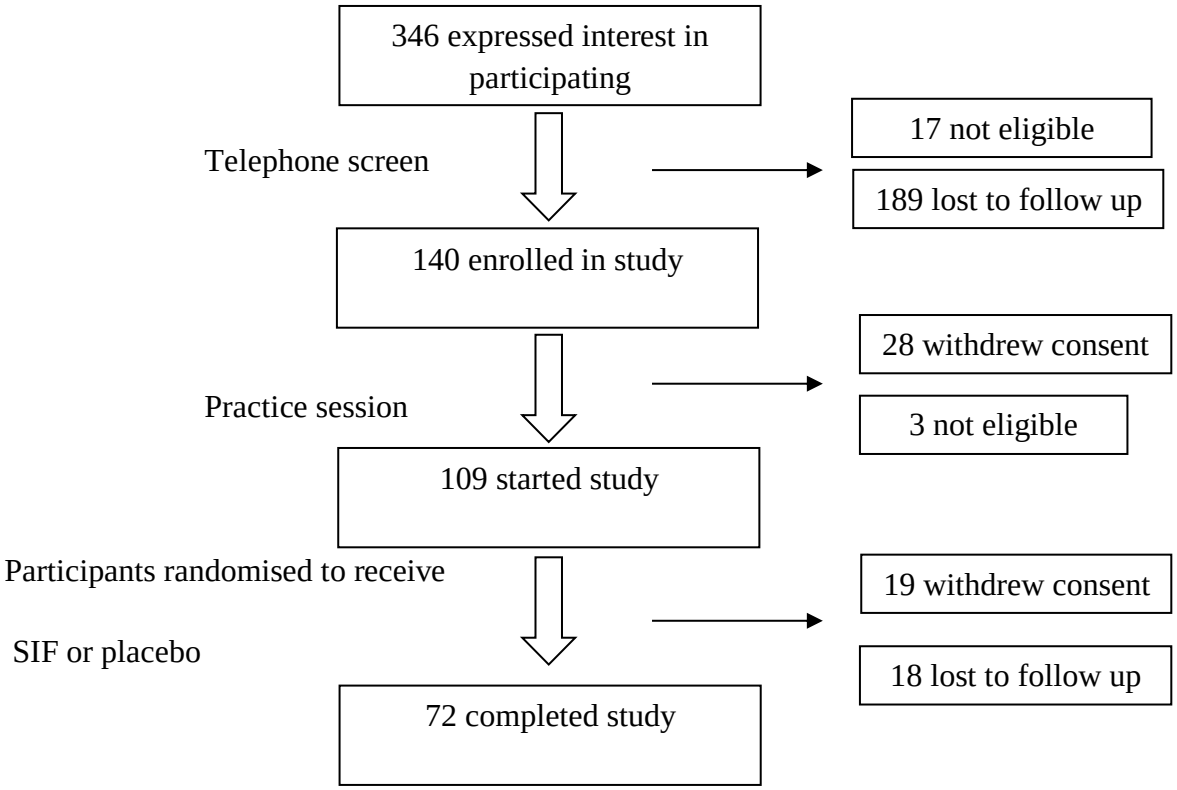

Figure 6.2. Summary of participants enrolled in the study and those who withdrew.

A subset of 36 right handed participants also completed electroencephalography recording sessions. Of these, 12 were in the OC group, 12 received SIF and 12 received placebo. Again, a power calculation could not be conducted based on the previous literature as this is the first study to investigate the effects of SIF on brain electrical activity. However, studies of changes in brain activity across the menstrual cycle have included similar numbers (e.g. Johnston & Wang, 1991; O'Reilly et al., 2004).

Full ethical approval for the study was granted by Swinburne University of Technology Human Research Ethics Committee (Approval number: SUHREC 2009/224, see appendix). Written and informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

6.3.2.3. OC Characteristics

Due to the many different brands of OC prescribed within Australia, it was not practical to recruit only participants using one specific brand. Therefore, participants were accepted into the trial provided the OC they were using was any of the available combined, monophasic OCs. The most commonly used OC in this trial was Levlen (n=9), which contains 0.15 mg levonorgestrel and 0.03 mg ethinyloestradiol (EE), and is a second generation OC. Six participants were using Yaz, which is a new generation OC and follows a 24/4 regimen as opposed to the conventional 21/7 regimen. Yaz contains 3 mg drospirenone (DRSP) and 0.02 mg EE. Yasmin was used by three participants in the study, and also contains 3 mg DRSP, with 0.03 mg EE administered in a 21/7 regimen. Three participants used Monofeme, which contains 0.15 mg levonorgestrel and 0.03 mg EE and is also a second generation OC. The remaining two participants were using the third generation OC Minulet, which contains 0.075 mg gestodene and 0.03 mg EE.

6.3.3. Treatment

SoyLife 40% capsules (Frutarom, Belgium) contain a glycine soy germ extract containing 40% isoflavones, with the active ingredients being daidzein (140-250 mg/g glucosidic weight), glycitein (70-160 mg/g glucosidic weight) and genistein (25-120 mg/g glucosidic weight). The product is extracted from the soy germ using ethanol and encapsulated in VCAPS capsules comprising hydroxypropyl methylcellulose, titanium dioxide and yellow iron oxide. Each 500 mg capsule (one to be taken per day, in the morning, until the end of the study) corresponds to 200 mg total soy isoflavones (SIF 200 mg), or 130 mg isoflavone aglycone equivalents. This is a slightly higher dose than has been used in previous studies (see chapter 4), however isoflavones have been shown to be safe and well tolerated in women up to 900 mg/day (Pop et al., 2008). Furthermore, animal studies have shown that higher doses may be more effective in reducing anxiety and depression in females. Since human studies have shown reduced physical, but not psychological symptoms at the doses administered, higher doses may also improve psychological symptoms. Although the half-life of isoflavones is relatively short, a cumulative effect was expected with sustained use, as daily consumption of SIF

has been shown to result in a steady state of high plasma concentrations (Setchell, Boriello, & Hulme, 1984). Placebo capsules contained 500 mg maltodextrin DE 15-20 (Baolingbao Biology Co., Ltd, China) and were matched for appearance, taste, weight and odour. In order to ensure blinding of the investigator, treatments were prepared and placed in clearly labelled dosette boxes by a third party who was not involved in any other aspect of the study.

6.3.4. Experimental Design

A mixed, partially-blinded, placebo-controlled design was employed. Within-subjects factors were menstrual cycle phase (menses, follicular, ovulatory and luteal; see section 6.3.4.1) and menstrual cycle (baseline, treatment cycle 1 and treatment cycle 2). The between-subjects factor was treatment (OC, SIF, or placebo). Participants completed one baseline menstrual cycle, with testing sessions during each of the four cycle phases to determine baseline performance, followed by two cycles receiving one of the treatments which they were randomly allocated to receive using a Latin square. Participants in the OC group were administered placebo only so as not to have any effect on the reliability of the contraception. For ethical reasons, this was known to themselves and the researchers. Participants not using oral contraception were randomly assigned to receive either isoflavone or placebo in a double-blind fashion, by a disinterested third party. Testing was conducted at the same time of day where possible to eliminate diurnal effects, and participants were asked to refrain from drinking alcohol or caffeine for 12 hours prior to testing sessions. The study was conducted according to Good Clinical Practice (GCP) standards.

6.3.4.1. Menstrual Cycle Timings

Participants were tested during the menses, follicular, ovulatory and luteal phases of each cycle. Cycle phase was determined using the counting backwards method from the first day of the following cycle as reported by the participant. For a 28 day cycle, menses was taken to be days 1-5 of the cycle, the follicular phase was days 6-11, ovulatory phase was days 12-16, and luteal phase was days 20-26. These timings were

chosen to enable comparison with previous studies as well as capturing maximal hormonal changes across the cycle.

Where participants did not have a 28-day cycle, day of ovulation was determined by counting backwards 14 days from the first day of the next menses since ovulation has been shown to occur almost exactly 14 days prior to the onset of menses (Lein, 1979). Luteal phase timing was also calculated using this method, and the following formula was applied in order to discern the equivalent day of a 28-day cycle for the follicular phase:

$$\text{Cycle day} = (\text{day of cycle}/\text{total no. days of cycle}) \times 28$$

Menses phase testing was always conducted during the menstrual bleed regardless of cycle length and therefore did not require calculation.

Circulating estradiol was also tested from saliva samples, and was used to confirm that participants had been tested during the correct cycle phase. Although OC users do not have menstrual cycles as such, the above testing days for each phase were adhered to as closely as possible for this group to ensure procedural consistency between groups..

6.3.5. Materials

Tables 6.2 and 6.3 summarise the materials used to assess aggression, mood and cognitive function, as well as relevant references where the instruments have been used in prior research. All measures were chosen based on their prior use in the investigation of the menstrual cycle, OCs or SIF unless otherwise stated.

6.3.5.1. Informed Consent Form

All participants completed and signed an informed consent form before commencing data collection, during a practice session at the Centre for Human Psychopharmacology.

This outlined the details of the study in lay terms, including participation requirements, time commitment, risks and benefits of participation and how participant data would be handled.

6.3.5.2. Case Report Form (CRF)

The CRF included questions about participant demographics (age, height, weight, blood pressure), current and previous medical conditions and medications received. It was also used during every session to record whether participants had complied with the study requirements of consuming a light meal before testing, no caffeine and no soy-containing foods.

6.3.5.3. Questionnaires

6.3.5.3.1. Telephone Screening Questionnaire

Participants were initially screened using a telephone screening questionnaire to assess eligibility for the study. The questionnaire included questions about participants' general health, medications and their willingness and ability to adhere to the study guidelines.

6.3.5.3.2. Food Frequency Questionnaire (FFQ; see Appendix B)

The FFQ was completed during the practice session in order to gather information about participants' regular eating habits, so as to control for effects of diet on the outcome variables. Participants indicated how often they ate certain foods, including dairy, bread and cereal, meat, fish and vegetables, during the past year, on a scale from "never" to "4+ times per day". This questionnaire also served to verify that participants were not regular soy consumers, as defined by consumption of soy foods twice per week or less.

Table 6.2 *Summary of materials used for the measurement of mood and aggression with relevant references.*

Material/Task	Outcome Measured	Comments	Example References
Daily Symptom Report (DSR-20)	PMS	Original DSR used extensively in MC research, DSR-20 relevant to this study due to inclusion of aggression-related items	Bryant et al. (2005); Schwartz et al. (2012)
Symptom Checklist	Side effects of treatment	Designed to assess side effects of natural medicines, therefore appropriate for examination of side effects of SIF	Stough et al. (2001)
State-Trait Anxiety Inventory	Mood (Anxiety)	MC and OC research	Gonda et al. (2008)
Buss-Perry Aggression Questionnaire	Aggression	MC research	Ritter (2003)
Barratt Impulsivity Scale (BIS-11)	Impulsiveness	MC research	Canning et al. (2010)
Profile of Mood States	Mood	MC and OC research	Wharton et al. (2008)
Schozotypal Personality Questionnaire	Schizotypal personality traits	Correlates differently with different forms of aggression, therefore may provide insight into aggressive behaviour	Seah & Ang (2008)
Beck Depression Inventory	Mood (Depression)	OC research	Greco et al, (2007); Toffol et al. (2011)
State-Trait Anger Expression Inventory	Anger	MC research	(Davydov et al., 2004)

Emotional Stroop Task	Aggression (Objective)	Has been shown to discriminate between aggressive and non-aggressive individuals	Smith & Waterman (2003)
Bond-Lader Mood Scales	Mood	Extensively used in studies of natural medicines	Scholey et al. (2012); Silvestrini et al. (2013)
Visual Analogue Scales	Mood (stress and mental fatigue)	Extensively used in studies of natural medicines	Scholey et al. (2012)

MC, Menstrual Cycle; OC, Oral Contraceptive

Table 6.3 *Summary of tasks used to assess different domains of cognitive function.*

Task	Outcome Measured	Comments	Example References
Immediate/ Delayed Word Recall	Secondary memory (verbal)	Used in MC research	Reed et al. (2008)
Delayed Word Recognition	Secondary memory (verbal)	Verbal memory sensitive to hormonal effects	Reed et al. (2008)
Delayed Picture Recognition	Secondary memory (visual)	MC research	Phillips and Sherwin (1992)
Sentence Verification	Secondary memory (semantic)		
Simple Reaction Time	Attention; Psychomotor speed	MC research	Wuttke et al. (1975)
Choice Reaction Time	Attention; Psychomotor speed	MC research	Wuttke et al. (1975)
Four Choice Reaction Time	Attention; Psychomotor speed	MC research	Wuttke et al. (1975)
Alphabetic Working Memory	Working memory	Working memory improved by SIF	Islam et al. (2008)
Numeric Working Memory	Working memory	As above	Islam et al. (2008)
N-Back (3-back)	Working memory	As above	Islam et al. (2008)
Corsi Blocks Forward/Reversed	Working memory (spatial)	Spatial memory affected by MC, OC and SIF	Thorp et al. (2009)
Card Sorting	Executive function (rule learning and reversal)	Rule learning and reversal improved in study of SIF	File et al. (2001)
Peg and Ball	Executive function (planning)	Planning improved in study of SIF	Duffy et al. (2003)
Serial Subtractions	Working memory; Attention; Executive function	Complex calculations affected by MC phase	Wuttke et al. (1975)

Rapid Visual Information Processing (RVIP)	Working memory; Attention; Executive function	Sustained attention varies with MC phase	Solis-Ortiz and Corsi-Cabrera (2008)

MC = Menstrual Cycle, SIF = Soy Isfolavone, OC = Oral Contraceptive

6.3.5.3.3. Modified Daily Symptom Report (DSR-20; Canning et al., 2012)

The DSR-20 is a modified version of Freeman, DeRubeis, and Rickels' (1996) 17-item daily diary for premenstrual symptoms, and contains an additional three items: aggression, anger and impulsiveness. Participants were asked to complete this measure every evening for the duration of the study. The 20 items are rated on a scale from 0 (not present at all) to 4 (Severe, symptom is overwhelming and/or unable to carry out daily activity). Factor analysis conducted by Freeman *et al* (1996) revealed four factors of the original measure: mood, behavioural, pain and physical symptoms. Reliability of the DSR was high, as was internal consistency of the four factors, with a reported Cronbach's alpha coefficient of 0.92 (Freeman, DeRubeis, & Rickels, 1996). Exploratory Principal Components Analysis of the modified DSR revealed 2 components describing psychological and physical symptoms. Internal consistency of these components was high (>0.90) as was internal consistency of the 20 items (0.95; Canning et al., 2012). Due to the relevance of the additional items in the DSR-20 to the current study data were analysed according to total scores and scores on the two components suggested by Canning et al. (2012).

Total scores for the DSR and the two factors were calculated for each cycle phase by summing the scores from individual items of a factor and dividing by the number of days in the phase. In order to maintain consistency with other studies that have utilised this measure, the phases calculated were menses (days 1-4), follicular (days 5-10), rest (i.e. rest of the cycle; any days that were not included in the other three phases) and late luteal (the six days preceding the next menses). Participants were asked to return completed DSRs during each testing session to prevent the identification of patterns of symptoms and avoid demand characteristics.

6.3.5.3.4. Symptom Checklist (SCL; Stough et al., 2001; see Appendix C)

The SCL was developed by Swinburne University's Brain Sciences Institute to assess the side effects of natural medicines. The checklist comprises 28 statements describing physiological and psychological states (e.g. I have stomach pains; I feel anxious more than usual), and participants rate how much each problem has bothered them over the past 7 days including the current day. Ratings are given on a 5-point scale where 1 = "not at all" and 5 = "very much so".

6.3.5.3.5. State-Trait Anxiety Inventory (STAI form Y; Spielberger, 1983)

The STAI measures participants' tendency to be anxiety prone (Trait-anxiety) as well as the intensity of their anxiety at the time of completion of the scale (State-anxiety). The State-anxiety scale consists of 20 items, and participants indicate how they feel "Right now, at this moment". The Trait-anxiety scale consists of 20 statements to which participants indicate how they generally feel. Participants respond using a 4-point scale (1 = not at all, 4 = very much so), with scores on both scales ranging from 20 to 80, and a higher score indicates a high level of anxiety. Internal consistency for both scales is high, with Spielberger (1983) reporting Cronbach's alpha between 0.86 and 0.95 for the State-anxiety scale, and between 0.89 and 0.91 for the Trait-anxiety scale.

6.3.5.3.6. Buss-Perry Aggression Questionnaire (BPAQ; Buss & Perry, 1992)

The BPAQ is a 29-item questionnaire with 4 subscales: Physical aggression, Verbal aggression, Anger, and Hostility. Items on the scale are assessed using a 5-point Likert scale (1 = extremely characteristic, 5 = extremely uncharacteristic). Items 17 and 23 are reverse scored, and a lower score indicates higher levels of aggression. The four subscales have high internal consistencies, with Buss and Perry (1992) reporting alpha coefficients between 0.72 and 0.85, and test-retest reliability on the scales was also high, with correlations of between 0.69 and 0.83.

6.3.5.3.7. The Revised Barratt Impulsiveness Scale (BIS-11; Patton, Stanford, & Barratt, 1995)

The BIS-11 is a 30-item questionnaire completed at every testing session, with each item being answered on a 4-point scale (1 = Rarely/never, 4 = almost always/always). Higher scores indicate higher impulsiveness. Principle Components Analysis produced 6 first-order factors: attention, motor, self-control, cognitive complexity, perseverance and cognitive instability; as well as 3 second-order factors: Attentional (attention and cognitive instability), Motor (motor and perseverance) and Non-planning Impulsiveness (self-control and cognitive complexity). Internal consistency was high, with Cronbach's alpha coefficient between 0.79 and 0.83 (Patton, Stanford, & Barratt, 1995).

6.3.5.3.8. The Profile of Mood States (POMS; McNair, Lorr & Droppleman, 1971)

The POMS is a 65-item self-report that provides an index of mood over the preceding week, where participants respond on a scale from 0 (not at all) to 4 (extremely). The POMS measures 6 identifiable mood dimensions: Tension-Anxiety; Depression-Dejection; Anger-Hostility; Vigour-Activity; Fatigue-Inertia; and Confusion-Bewilderment. A total Mood Disturbance Score is obtained by summing the scores from the six factors. Internal consistency was reported to be satisfactory (McNair, 1971).

6.3.5.3.9 Schizotypal Personality Questionnaire (SPQ; Raine, 1991)

The SPQ is a 74-item questionnaire measuring the nine traits of schizotypal personality disorder as stated in the DSM-III-R (APA, 1987): Ideas of reference, excessive social anxiety, odd beliefs or magical thinking, unusual perceptual experiences, odd or eccentric behaviour, no close friends, odd speech, constricted affect, and suspiciousness. Participants answer each item "yes" or "no" referring to whether or not they have experienced each situation. The questionnaire is scored by assigning the value 1 for "yes" and 0 for "no" and summing the responses. The SPQ has been shown to have high reliability and validity (Raine, 1991).

6.3.5.3.10 Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961)

The inventory consists of 21 items of depression, with each item describing a specific manifestation of depression. Each contains 4 or 5 statements which are rated absent (0) to severe (3), and participants indicate the extent to which they experience each symptom by marking the statement that best describes how they feel. Scores from 0-9 are considered to indicate minimal, 10-16 mild, 17-29 moderate and 30-63 severe depression. Internal consistency of the scale was high, with Beck *et al.* (1961) reporting a Cronbach's alpha coefficient of 0.93.

6.3.5.3.11 State-Trait Anger Expression Inventory (STAXI; Spielberger, 1991)

The STAXI is a 44-item measure comprising six scales and two subscales, where participants indicate responses from 1 (not at all/almost never) to 4 (very much

so/almost always). The first scale, State Anger (S-Anger), measures how angry the participant is feeling at the time of completion (10 items). The second scale, Trait Anger (T-Anger) assesses the participant's disposition to experience anger (10 items). This is further broken down into Angry Temperament (T-Anger/T), a 4-item subscale which assesses the individual's tendency to express anger without provocation, and Angry Reaction (T-Anger/R), a 4-item subscale measuring the individual's tendency to express anger when provoked. The anger expression section of the STAXI consists of four scales. Anger-In (AX/In) is an 8-item scale which measures how frequently the participant suppresses angry feelings. Anger-Out (AX/Out) measures how often the individual expresses their angry feelings. Anger Control (AX/Con) assesses how often the participant attempts to control their anger. Finally, Anger Expression (AX/EX) combines the previous three scales to give an overall index of the frequency of anger expression. A higher score indicates higher experiences of anger.

6.3.5.4. Emotional Stroop Task

As an objective measure of aggression, a variation of the original stroop colour naming task (Stroop, 1935) was completed during each experimental session. In the original version of this task, colour words are presented in either congruent ink colour (e.g. blue) or incongruent ink colour (e.g. blue) and participants are asked to name the font colour. It has been consistently shown that people respond slower to incongruent words, due to a conflict between the task and the automaticity of reading (MacLeod, 1991). The task has been adapted for use with computers in more recent studies, requiring either a verbal response by microphone, pressing coloured buttons on a keyboard/response box or using the mouse to identify the appropriate colour on the screen (MacLeod, 1991).

In this study, an emotional stroop task was used to measure response times to aggressive stimuli. This task has been shown to discriminate between aggressive and non-aggressive individuals, with violent offenders responding slower to aggression-related words than non-violent offenders and non-offenders (Smith & Waterman, 2003). Fifteen stimulus words were included in each of the categories (direct aggressive, indirect aggressive, positive affect, negative affect and neutral; see table 5.3 for stimuli). Stimuli for the two aggressive categories, as well as neutral stimuli, were taken from the MRC psycholinguistic database (Wilson, 1988) and were matched to within a score of

50 for imageability, within 10 words per million for written frequency and were matched exactly for length and number of syllables. Positive and negative affect stimuli were included in order to ensure that any delays in responses to the target words were not due to the emotions they elicited, but to the aggressive content. These words were taken from the empirical taxonomy of the affective lexicon (Clore, Ortony, & Foss, 1987), and all control stimuli were matched to the experimental stimuli using the same constraints as detailed above.

Table 6.4 *Stimuli used in the different categories of the emotional Stroop task.*

Direct Aggressive	Indirect Aggressive	Neutral	Positive Affect	Negative Affect
Savage	Hostile	Repair	Joyful	Solemn
Bludgeon	Besmirch	Billiard	Tranquil	Spiteful
Kick	Mock	Dill	Fond	Glum
Violent	Slander	Organic	Passion	Remorse
Brutal	Insult	Deluge	Amused	Horror
Batter	Betray	Manual	Humour	Dismay
Biting	Accuse	Kettle	Admire	Morose
Punch	Bitch	Ledge	Bliss	Grief
Homicide	Mischief	Youngest	Cheerful	Homesick
Vicious	Revenge	Thrifty	Gleeful	Ashamed
Bloody	Gossip	Jockey	Kindly	Dreary
Murderer	Prejudice	Cafeteria	Satisfied	Resentful
Hacked	Deceit	Rubble	Jovial	Regret
Throttle	Threaten	Blockade	Consoled	Contempt
Destruction	Blackmailer	Candlestick	Warmhearted	Embarrassed

Stimuli were presented using E-Prime version 2.0 Professional software (Psychology Software Tools, Inc). At the beginning of each session, participants completed the original stroop task as a practice, which involved the words “blue”, “green”, “yellow” and “red” being presented once in each colour, resulting in 16 trials. This was followed

by four experimental blocks, each containing 75 emotional stimuli (15 per category). The task was programmed such that each word was presented in a different colour in each block, and participants were given the opportunity to rest between each block to avoid eyestrain. The software presented the stimuli in a random order each time; ensuring participants never saw the same order of words twice. Stimuli remained on the screen until the participant had responded, and a fixation "+" appeared in the centre of the screen for 500ms between each trial. Responses were made using the keys C, V, B and N on the keyboard, which were marked with yellow, blue, red and green stickers respectively. The task was scored for accuracy (%) and reaction time (ms), and a mean score from the four blocks was taken as the score for each session.

6.3.5.5. Cognitive measures

The Computerised Mental Performance Assessment System (COMPASS) battery of cognitive tasks was used to assess the major cognitive domains of attention/concentration, reaction time, executive function, working memory, and secondary memory. The battery also includes computerised mood assessments. COMPASS is specifically designed to include tests which have been shown to be sensitive to nutritional manipulations (Reay, Kennedy, & Scholey, 2006). Whilst no psychometric data is available for this battery, the tasks included are almost identical to those used in the Cognitive Drug Research (CDR) battery, a very widely used cognitive assessment tool with good reliability and validity (e.g. Ward & Wesnes, 1999). Parallel forms of the tasks were presented during each testing session, and the tasks were computer-randomised. The duration of the COMPASS battery was around 45 minutes and was completed once during every testing session, and twice during the practice session in order to minimise practice effects. Participants were seated approximately one meter from the computer screen. Instructions for task completion and task stimuli were presented via the computer monitor. Participants responded using keyboard and/or mouse depending on the task. The tasks included in the battery are described below in their running order.

6.3.5.5.1. Bond-Lader Mood Scales (Bond & Lader, 1974)

The Bond-Lader visual analogue scales comprise sixteen 100mm scales anchored by antonyms (e.g. friendly-antagonistic, alert-drowsy), which are combined as recommended by the authors to form three factors: alertness, calmness and contentedness. The scales have been shown to have high reliability and validity (Ahearn, 1997).

6.3.5.5.2. Word Presentation

Participants were presented with 15 words which they were instructed to remember. The words were presented one at a time on the screen for 1500ms, with an inter-stimulus interval (ISI) of 1000ms. Words selected from http://www.math.yorku.ca/SCS/Online/paivio were matched for linguistic familiarity, concreteness and frequency, and were randomly selected for each session by the COMPASS program, which ensured no participant saw the same word twice for the duration of the study.

6.3.5.5.3. Immediate Word Recall

Participants were given 60 seconds to write down as many of the words as they could remember. The task was scored for number of correct answers.

6.3.5.5.4. Picture Presentation

Twelve black and white line drawings of everyday items were presented on the screen one at a time for a duration of 2000ms, with an ISI of 800ms. Participants were instructed to try to remember these pictures for recognition later on.

6.3.5.5.5. Simple Reaction Time

An upwards pointing arrow was presented 40 times on the screen, with an ISI varying randomly between 1000ms and 3000ms. Participants were asked to press the space bar

as quickly as possible whenever they saw the stimulus. Response times were recorded in ms, and the task was scored for accuracy and reaction time.

6.3.5.5.6. Choice Reaction Time

Arrows appeared on the screen one at a time pointing either to the left or the right. Participants were instructed to respond as quickly and accurately as possible to the stimulus by pressing the "z" key on the keyboard if the arrow was pointing left, and the "m" key if the arrow was pointing right. In total, 40 stimuli were presented with an ISI varying randomly between 1000ms and 3000ms. The task was scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.7. Four-Choice Reaction Time

An image of the four directional arrow keys on the computer keyboard was displayed on the screen. At random intervals of between 1000ms and 3000ms, and in a random order, the arrows lit up sequentially. There were 40 trials, and participants were instructed to respond by pressing the corresponding arrow key on the keyboard as quickly and accurately as possible to each stimulus. The task is scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.8. Sentence Verification

Twenty sentences were presented sequentially on the screen and remained there until a response was made. Half the sentences were true, and half false (see examples below). Participants responded by pressing "m" if they thought the sentence was true and "z" if they thought it was false. Participants were asked to respond as quickly and accurately as possible, and the task was scored for accuracy (% correct) and reaction time (ms).

Example True sentence: "Birds have wings"

Example False sentence: "Bikes are animals"

6.3.5.5.9. Alphabetic Working Memory

Five letters were presented sequentially for participants to hold in memory, appearing on the screen for 1500ms with an ISI of 500ms. This was followed by a series of 30 probe letters. Participants were instructed to indicate whether or not they thought the target letter had appeared in the original sequence by pressing "m" for yes, or "z" for no as quickly and accurately as possible. This was repeated 2 further times with different stimuli and probe digits. The task was scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.10. Numeric Working Memory

This task is similar to that of Alphabetic working memory, except that digits are presented rather than letters. Five digits were presented sequentially for participants to hold in memory. This was followed by a series of 30 probe digits. Participants indicated whether or not they thought the target digit was included in the initial sequence by pressing "m" for yes and "z" for no as quickly as possible. This was repeated 2 further times with different stimuli and probe digits. The task was scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.11 N-Back (3-back)

Thirty single letters were presented one at a time on the screen, including 10 target pairs. Participants were to decide whether the letter was the same as the one presented 3 times previously. If they thought it was, they pressed "m", if not they pressed "z". The task was scored for accuracy (% correct) and reaction time (ms).

T L H C H O **C** Q **L** **C** K **L** H C Q T R R K C H R

Figure 6.3 Schematic representation of 3-back task.

6.3.5.5.12. Card Sorting Task

Four images of cards were presented at the top of the screen, displaying different numbers, colours and shapes. The COMPASS program randomly chose a rule for the participant to follow when sorting the cards (e.g. colour). Cards were then presented one at a time at the bottom of the screen and the participant was instructed to sort the cards by clicking the appropriate card at the top of the screen. Participants were not told what the rule was and had to establish this by trial and error. The program randomly changed the rule and participants must again establish the new rule by trial and error. The task was scored for accuracy (% correct) and reaction time (ms).

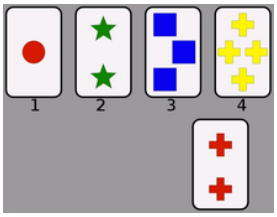

Figure 6.4 Schematic representation of Card Sorting task.

6.3.5.5.13. Delayed Word Recall

Approximately 25 minutes after the original word presentation, participants were instructed to write down as many words as they could remember from the list presented at the beginning of the battery within 60 seconds. The task was scored for number of correct items recalled.

6.3.5.5.14. Delayed Word Recognition

The 15 words initially presented were again presented one at a time, randomly interspersed with 15 distracter words. Participants were instructed to indicate whether or not each word had been presented in the original list by pressing “m” for yes and “z” for no. The task was scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.15. Delayed Picture Recognition

The twelve pictures originally presented were displayed on the screen sequentially, with an equal number of distracter pictures randomly interspersed. Participants were instructed to indicate whether or not each image had been included in the initial sequence by pressing "m" for yes and "z" for no. The task was scored for accuracy (% correct) and reaction time (ms).

6.3.5.5.16. Peg and Ball Task

Three "pegs" appeared on the screen with one blue, one red and one green ball positioned randomly on the pegs. The target position of the balls was presented at the top of the screen, and participants were instructed to use the mouse to move the balls in the fewest moves possible and as quickly as possible to achieve the displayed configuration. The task increased in difficulty with more moves being necessary to achieve the end point. There was no time limit to complete each configuration, however if the participant had not completed the entire task after ten minutes the task ended. The task was scored for accuracy (% trials completed in minimum moves), number of extra moves taken, and reaction time (thinking time; completion time; both in ms).

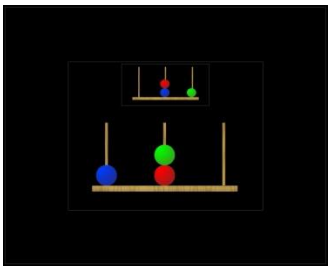

Figure 6.5 Schematic representation of Peg and Ball task.

6.3.5.5.17. Serial Subtractions

For the serial threes task, participants were required to count backwards in threes from a random starting number ranging from 800 to 999. They were instructed to use the numeric keypad to input their responses as quickly and accurately as possible, followed

by the "enter" key. Digits entered on the numeric keypad were represented on the screen as an asterisk, thereby preventing the participant from seeing their response and ensuring working memory was accessed for the task. The task was scored for total number of subtractions, accuracy (%) and reaction time (ms). If the participant made an incorrect response, subsequent responses were scored as correct if they were correct in relation to the new number. The serial sevens task is exactly the same except that participants count backwards in sevens. Comparison of serial threes and serial sevens allows assessment of the interaction of treatment with the cognitive load of the task.

6.3.5.5.18. Rapid Visual Information Processing (RVIP)

Digits were presented one at a time at a rate of 100 per minute. Participants were instructed to press the space bar whenever they saw strings of three consecutive odd or three consecutive even numbers. The task lasted for five minutes, with eight targets per minute (forty targets in total). The task was scored for accuracy (% correct), number of false alarms, and reaction time (ms).

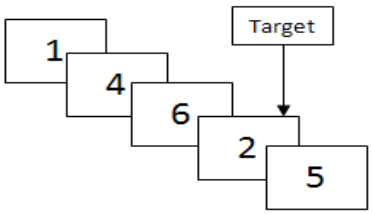

Figure 6.6 Schematic representation of RVIP task.

6.3.5.5.19. Corsi Blocks Task

Nine identical blue blocks (size 93 x 93 pixels) appeared in a random pattern on the screen. Four of the blocks were illuminated sequentially by changing colour to red, then back to blue, at the rate of one per second, creating a sequence of spatial locations for the participant to remember. Participants were instructed to use the mouse to identify the pattern of illumination as quickly and accurately as possible. Task difficulty was gradually increased by increasing the number of illuminated boxes in the sequence. The

task was scored according to the length of sequence the participant could correctly remember (span score) as well as reaction time (ms).

6.3.5.5.20. Reversed Corsi Blocks Task

This task was the same as the Corsi Blocks task, except that participants were instructed to identify the pattern in reverse order, beginning with the last box to be illuminated and working backwards to the first. This task was also scored for length of sequence correctly recalled (span score) and reaction time (ms).

6.3.5.5.21. Visual Analogue Scales (VAS)

Participants completed two VAS following the cognitive tasks in order to measure subjective levels of mental fatigue and stress. The questions "How stressed do you feel right now?" and "How mentally fatigued do you feel right now?" were followed by 100 mm rating scales anchored by "not at all" (at 0 mm) and "extremely" (at 100 mm), and participants were instructed not to respond at the absolute extremes as this equated to the least/most mentally fatigued/stressed they had ever felt.

6.3.5.6. Saliva Samples

One saliva sample was taken from each participant during each experimental session using a 9.7 ml salivette tube (Sarstedt, UK) as a non-invasive measure of free E_2. The saliva was collected by the participant placing a cotton dental swab in their mouth and chewing for a minimum of 30 seconds, then placing it back inside the salivette container. Saliva samples were immediately stored in a -20˚c freezer. Samples were thawed and centrifuged prior to E_2 concentrations being determined by colorimetric competitive enzyme immunoassay (EIA) at Swinburne University of Technology using a 17-β-estradiol EIA kit (Enzo Life Sciences, USA). Absorbance was read at 405 nm, and sensitivity of the kit was 15 pg/ml (range 29.3 – 30,000 pg/ml). Salivary analysis of estradiol has previously been shown to provide a reliable assessment of menstrual cycle profile (Gandara, Leresche, & Mancl, 2007). Laboratory personnel were blind to the treatment group as well as cycle phase.

6.3.6. Tasks and Stimuli used during EEG recording

6.3.6.1 Standard go/nogo task

As described in chapter 5, the go/nogo task is a measure of response inhibition, where participants are required to respond to certain stimuli but inhibit responses to others. This task was included in the study due to the close relationship between inhibition and aggression (Barratt et al., 1999; Fossati et al., 2004), one of the primary foci of this thesis. Indeed, performance of this task and the associated ERP components have been shown to be affected by aggression levels (see Chapter 5.3.5). Stimuli for the go/nogo task were black arrows measuring 4cm x 7.5cm pointing either up or down and presented on a white background. Experimental data were acquired in two blocks lasting 8 minutes each. In each block, 80 up arrows (25%) were presented along with 240 down arrows (75%), in random order. The presentation order of these blocks was counterbalanced between participants so as to negate any order effects of block type presentation. In the 25% nogo block, participants were instructed to respond only to down arrows (75% go stimuli) and not to up arrows (25% nogo stimuli), and in the 25% go block they responded only to up arrows (25% go) and not to down arrows (75% nogo). Stimuli appeared on the screen for a duration of 100 ms, with an inter-stimulus interval (ISI) of 1500 ms. Participants were instructed to respond to go stimuli as quickly and accurately as possible, and that responding to go stimuli should not be slowed in order to improve accuracy for nogo stimuli (Refer to Figure 6.7). Accuracy was recorded as the number of go stimuli correctly responded to. Errors of commission were also calculated as the number of button presses following nogo stimuli and were used as an index of impulsivity. Mean reaction times taken (recorded in milliseconds, ms) to respond to stimuli were also calculated for correct responses.

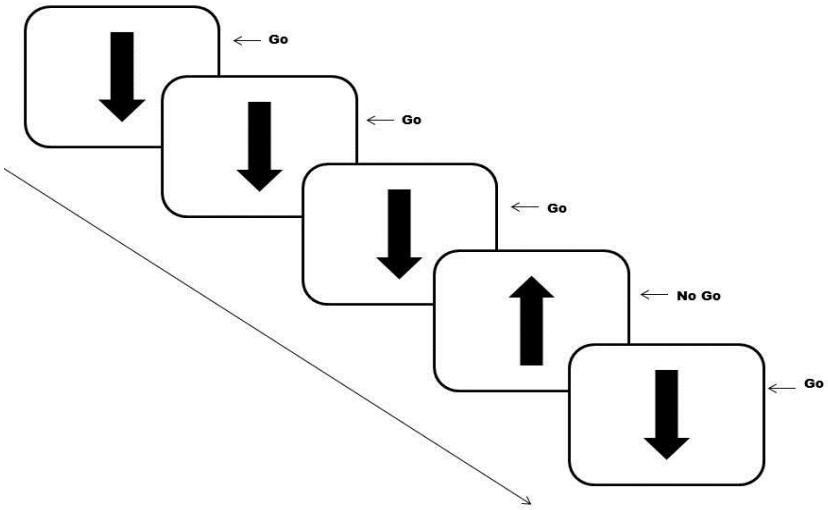

Figure 6.7 Schematic representation of the go/nogo task where down arrows (75%) were the go stimulus and up arrows (25%) were the nogo stimulus.

6.3.6.2. Go/nogo task with face emotion stimuli

This task was designed to be isomorphic with the previous task, in that participants were presented with 80 target stimuli and 240 distracter stimuli in 8 minute blocks. Stimuli used in this task were angry, happy and neutral faces of 10 males and 10 females taken from the NimStim series (Tottenham et al., 2009), although only angry and neutral faces were included as 25% stimuli in each condition. These three emotions were chosen due to the differences between aggressive and non-aggressive individuals in responses to these emotions (see Chapter 5.1). A black oval was placed around each image to reduce changes in luminance due to clothing or hair, and stimuli measuring 10cm x 14cm were presented on a black background. The task consisted of four blocks in total, with two emotions (angry and neutral) as targets in both nogo (75% go and 25% nogo) and go conditions (25% go and 75% nogo). For example, when angry faces were targets, in the nogo condition 120 neutral faces and 120 happy faces served as go stimuli and 80 angry faces served as nogo stimuli. Accuracy was recorded as the number of go stimuli correctly responded to. Errors of commission were also calculated as the number of button presses following nogo stimuli and were used as an index of impulsivity. Mean reaction times taken (recorded in milliseconds, ms) to respond to stimuli were also calculated for correct responses.

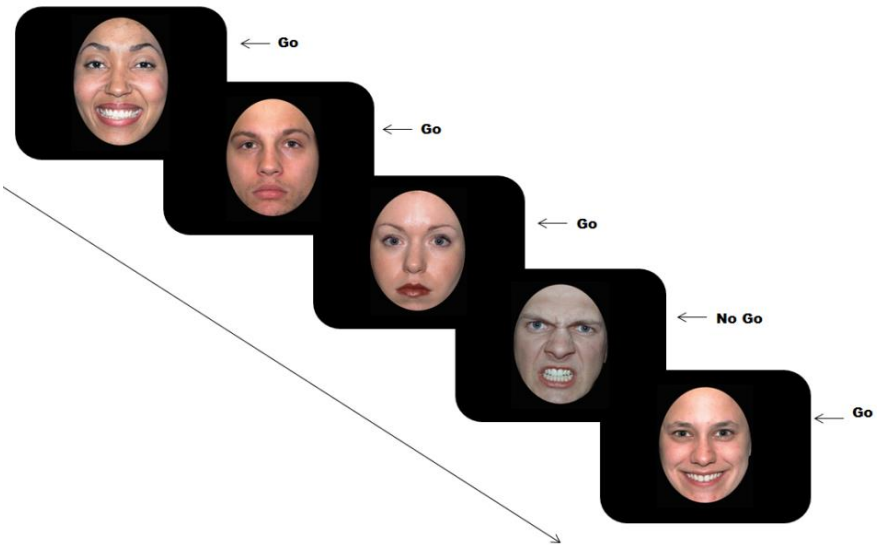

Figure 6.8. Schematic representation of the emotional go/nogo task where happy and neutral faces (75%) were the go stimuli and angry faces (25%) were the nogo stimuli.

6.3.6.3. Stimulus Presentation

During each of the four EEG recording sessions, participants were fitted with the electrode cap (Quik-Cap) and seated in a dimly-lit sound-attenuated and electrically-shielded testing room. Instructions for each task were presented on the monitor as well as being read aloud prior to actual testing. Participants were also instructed to remain as still as possible and to minimize eye movements. All aspects of stimulus presentation were controlled by STIM2 software. Participants were seated approximately one metre from the 17-inch computer monitor where stimuli were presented in central vision. The index finger of the right hand was positioned over a button on a button response box. Participants were instructed to press the button whenever they saw a go stimulus appear on the screen, and not to press anything when they saw a nogo stimulus. Four configurations of each task were constructed, to ensure that participants were presented with a different order of stimuli during each experimental session. These configurations and the order of task presentation were randomized and counterbalanced across participants to prevent order effects.

6.3.7. Electrophysiological Data Acquisition

Continuous EEG was recorded using a custom electrode cap (Quik-Cap) from 62 scalp electrodes positioned according to the international 10/20 system as shown in Figure 7.3 (O2, O1, OZ, PZ, P4, CP4, P8, C4, TP8, T8, P7, P3, CP3, CPZ, CZ, FC4, FT8, TP7, C3, FCZ, FZ, F4, F8, T7, FT7, FC3, F3, FP2, F7, FP1, PO3, P1, POZ, P2, PO4, CP2, P6, CP6, C6, PO8, PO7, P5, CP5, CP1, C1, C2, FC2, FC6, C5, FC1, F2, F6, FC5, F1, AF4, AF8, F5, AF7, AF3, FPZ). Vertical and horizontal electro-oculograms (EOG) were recorded via electrodes positioned above and below the left eye, and on the outer canthi of each eye, respectively. Data were acquired using Neuroscan version 4.3 with a high pass filter of 0.05 Hz and a low pass filter of 200 Hz, at a sampling rate of 1000 Hz. Impedances for the reference and ground electrodes were kept below 10 kΩ.

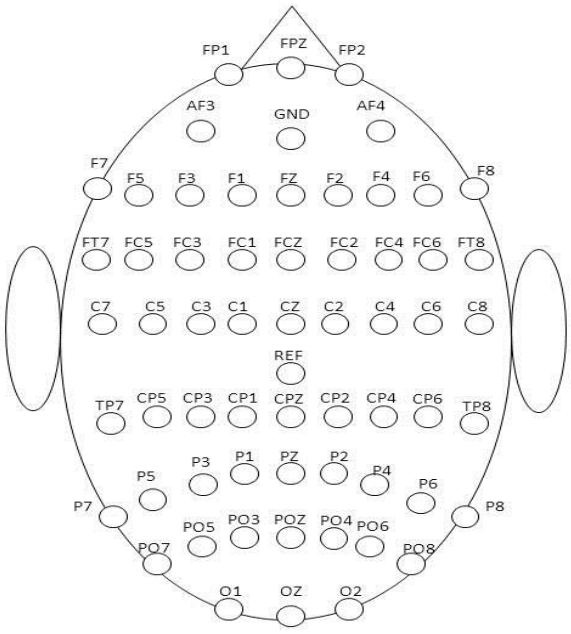

Figure 6.9. Placement of the 62 electrodes used in the current study. Electrodes correspond to the international 10-20 system.

6.3.8. EEG data preprocessing and trial averaging

6.3.8.1. Artefact Reduction

Preprocessing of ERP data was performed using Neuroscan software (v 4.5). Initially, the data were visually inspected for muscle and ocular artifact. Data recorded from individual electrodes that contained excessive artifact were replaced with the averaged data of its four nearest neighbours. EEG time-series segments containing gross artifact were excluded. Data were re-referenced to a global channel including all electrodes except for the EOG channels. Ocular artifact reduction was conducted through the Semlitsch method (Semlitsch, Anderer, Schuster, & Presslich, 1986) within the Neuroscan Edit software.

6.3.8.2. ERP Data Reduction

The majority of data were acquired in AC mode and did not require filtering, however a small proportion of recordings were acquired in DC mode, and these data were band pass filtered from 0.15-200 Hz (12dB/oct). Event files were created in order to extract data specifically for each stimulus type. Stimulus-locked epochs were then created to capture data from 200 ms prior to stimulus onset to 1000 ms post stimulus onset, resulting in an epoch length of 1200 ms. Epochs in which the amplitude exceeded +/- 100 µv were discarded. Nogo and go epochs for each stimulus type were averaged separately for each individual participant and baseline corrected over the pre-stimulus time period of -150 ms to -50 ms. Group averages were also computed for each stimulus type for nogo and go conditions, from all individual averages within a group.

6.3.8.3. ERP data extraction

For the standard go/nogo task, mean amplitude, peak amplitude and peak latency measures for N2 and P3 components were determined by first identifying appropriate time windows from the group averaged waveforms then extracted using Matlab (version 12; Mathworks). These measures were limited to the Fcz electrode consistent with previous research (e.g. Gajewski & Falkenstein, 2013; Niewenhuis, Yeung, Van Den Wildenberg, & Ridderinkhof, 2003). Amplitude was measured from the mean pre-

stimulus level to the peak within specified latency windows. Latency (ms) was defined as the time of the peak amplitude within these latency windows. Specifically, N2 mean amplitudes were extracted between 165-280ms, peak amplitudes between 120-300ms and peak latency between 120-300ms. P3 mean amplitudes were extracted between 250-500ms, peak amplitudes between 275-500ms and peak latency between 275-500ms. Difference waveforms (25% nogo minus 25% go) were also computed (termed 'N2d' and 'P3d') in order to examine the difference between these conditions when the probability of the stimulus type occurring was the same. N2 mean amplitude, peak amplitude and peak latency differences were extracted between 120-350ms. P3 mean amplitude, peak amplitude and peak latency differences were extracted between 250-600ms.

To maintain consistency across tasks, data from the go/nogo task with face emotion stimuli were treated in the same way as detailed above, and N2 and P3 data were extracted from the Fcz electrode site only. For angry faces, N2 peak amplitudes were extracted between 120-300ms, mean amplitudes between 150-270ms, and peak latency between 120-300ms. P3 peak amplitudes were extracted from 280-600ms, mean amplitudes between 300-600ms, and peak latencies between 280-600ms. For neutral faces, N2 peak amplitudes were extracted between 130-300ms, mean amplitudes between 140-270ms, and peak latencies between 130-300ms. P3 peak amplitudes were extracted between 280-630ms, mean amplitudes between 300-600ms and peak latencies between 280-630ms. Difference waveforms were also calculated, and for both angry and neutral faces N2 mean amplitude, peak amplitude and peak latency were extracted between 120-350ms. P3 mean amplitude, peak amplitude and peak latency were extracted between 250-650ms.

6.3.9. Statistical Analyses

All data were analysed using the IBM SPSS statistical package (v.21.0). Where variables were not normally distributed, they were transformed using appropriate transformation methods as reported in the results section. Values 3 standard deviations or more from the mean were considered outliers and were removed from the analysis. A

p value of 0.05 was used to determine significance, and to allow greater exploration of the data *p* values <0.1 were taken as trend level and are discussed in text.

6.3.9.1. Baseline comparisons

The primary outcome measure was total aggression score on the BPAQ. Due to the examination of multiple outcome variables, factors previously identified by authors of the various mood measures were analysed but not individual items in order to reduce the number of comparisons. For cognitive data, however, composite scores were not obtained due to the specificity of the effects of steroidal hormones on individual tasks (see Chapters 2-4). Ceiling effects for accuracy were expected for choice reaction time and stroop tasks (Pipingas et al., 2010) therefore only reaction times were analysed for these tasks. Correct responses and missed sequences on the RVIP task were also excluded from the analysis as they were included in an overall accuracy score. Pearson's correlations were conducted to determine whether any of the dependent variables correlated with age, BMI or years of education and where these factors were significantly correlated with the dependent variables they were included as covariates in the model.

Linear mixed models (LMMs) with a random intercept for each participant were run for all cognitive, mood and hormonal measures. LMMs were chosen for their power to handle repeated measures data and data missing at random. Due to expected menstrual cycle-related variations in the outcomes (see Chapter 1), data from all phases of the first cycle were included in the baseline analysis (with the exception of EEG data which were only collected during menses and the luteal phase). The two NC groups were collapsed in order to compare OC users with NC women. Therefore treatment group, cycle phase and their interaction were included as fixed effects in all models. A variance components covariance matrix and the default identity structure of the residual matrix were used in all models. Restricted maximum likelihood method (REML) estimations were used to produce unbiased estimates of variance and covariance parameters. Post hoc pairwise comparisons were used to determine significant differences between phases and to explore interactions. Cohen's *d* effect sizes were calculated for significant pairwise comparisons.

6.3.9.2. Post-treatment comparisons

Primary outcome measures were the same as for baseline comparisons, with LMMs used to analyse data. Mixed marginal models were used for each post-treatment cycle with treatment (OC, SIF, placebo), phase (menses, follicular, ovulatory, luteal) and their interaction as fixed effects, and baseline scores for each phase as a covariate. The REML method was used with an unstructured residual covariance matrix and phase specified as the repeated term. As with baseline analyses, post hoc pairwise comparisons were used to explore significant main effects and interactions.

Chapter 7

Baseline Results: Effects of Menstrual Cycle Phase and Oral Contraceptive use

7.1. Participant Demographics

Sixty-eight of the 72 participants completed the entire trial. Of the four who did not, all completed at least the baseline cycle plus one treatment cycle and agreed to the use of their data in the analysis. No participants withdrew due to side effects. Of those who did not complete the trial, one was in the OC group, one was in the SIF group and two were in the placebo group. One way analysis of variance (ANOVA) revealed no significant differences between groups on the demographic characteristics of age, BMI, years of education or level of education ($p>0.05$; see Table 7.1). All groups also scored similarly on the food frequency questionnaire, with a one way ANOVA revealing no significant group differences.

Table 7.1 *Subject demographics and characteristics for each treatment group in the full study and those included in the EEG analysis.*

Treatment Group	N	Age range	Mean age (SD)	Education (years)	BMI (kg/m^2)
Full study					
OC	23	18-29	23.4 (3.3)	16.3	23.4
Placebo	26	18-32	23.8 (4.8)	16.4	22.2
SIF	23	18-34	23.8 (4.5)	16.1	22.0
EEG Cohort					
OC	12	18-29	23.7 (3.3)	15.9	23.4
Placebo	12	18-32	24.1 (5.0)	16.7	21.1
SIF	12	18-34	24.1 (5.2)	16.3	21.8

BMI = Body Mass Index, SIF = Soy Isoflavone, OC = Oral Contraceptive, EEG = Electroencephalography

7.2. Excluded Participants

Cognitive data: Participants with reaction times and accuracy scores 3 or more standard deviations from the mean were excluded from the relevant analysis.

Daily Symptom Reports: Participants were only included in the analysis if they had completed at least 3 days of reports for each phase during every cycle.

Missing Data: Cycle phase was calculated by confirming the date of the start of the next menses and counting backwards. Where there was doubt about whether a participant had been tested during the correct phase, data from that session were not included in the analysis. Appropriate transformations were applied to skewed data in order to normalise the data prior to analysis.

7.3. Salivary Estradiol

Inspection of the data revealed unexpectedly large variability in estradiol levels. This variability was evident in OC users as well as NC women, therefore it was decided that the use of this measure to verify cycle phase would be unreliable. Exploratory analysis was conducted, however, to determine whether OC users and NC women differed in their estradiol levels. Values more than 3 standard deviations from the mean were excluded from the analysis. As expected, OC users had significantly lower levels of circulating estradiol than NC women ($F(1,64)=9.62$, $p=0.003$, $d=0.80$).

7.4. Mood measures

Means, standard deviations and statistical values for all mood outcomes are shown in table 7.2. Where significant main effects of phase or significant interactions were found, results of post hoc pairwise comparisons of estimated marginal means are reported in table 7.3. Significant effects of covariates (age, body mass index and years of education if correlated with the dependent variable) are reported in text along with main effects of treatment. For the sake of brevity p values associated with significant and trend differences only are reported.

Beck Depression Inventory (BDI): Logit transformation was applied to BDI scores in order to normalise the data prior to analysis. NC women had significantly higher depression ratings than OC users ($d=0.53$) and depression scores were significantly higher during menses than the ovulatory or luteal phases. There was also a significant main effect of body mass index (BMI) ($F(1,67)=6.59$, $p=0.013$, $d=0.63$), with higher BMI being associated with higher scores on the BDI.

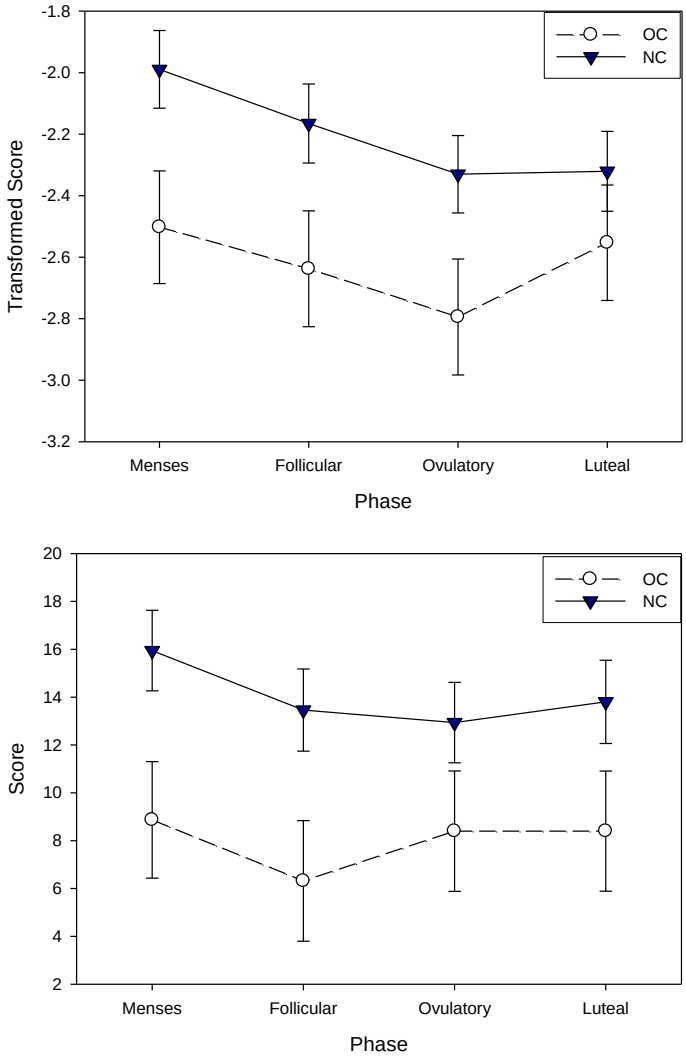

Figure 7.1 Depression scores in OC users and NC women during different phases of the baseline menstrual cycle as measured using the BDI (top; transformed scores presented) and the depression-dejection subscale of the POMS (bottom).

Table 7.2 *Means and standard deviations for mood outcomes during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,178	Treatment Group (T); df=1,68	P x T interaction; df=3,178
BDI	OC	8.05 (6.52)	7.20 (7.43)	6.50 (7.19)	8.65 (8.39)	4.70**	4.33*	NS
	NC	12.62 (9.97)	11.00 (9.07)	9.33 (10.92)	9.59 (9.43)			
BIS: *Total*	OC	66.79 (14.70)	61.57 (12.96)	61.38 (12.92)	61.55 (16.14)	5.69**	3.32^	NS
	NC	68.05 (8.92)	65.80 (11.46)	66.40 (10.90)	64.75 (11.83)			
Attentional Impulsiveness	OC	16.74 (4.54)	15.44 (4.67)	15.15 (3.76)	15.85 (5.58)	4.23**	3.73^	NS
	NC	18.07 (3.75)	17.38 (4.98)	16.70 (4.30)	16.79 (5.02)			
Motor Impulsiveness	OC	23.62 (5.88)	21.97 (5.32)	23.35 (5.36)	21.95 (5.56)	3.57*	NS	NS
	NC	23.54 (4.28)	22.43 (4.55)	22.54 (4.24)	21.95 (4.30)			
Non-Planning Impulsiveness	OC	26.43 (5.57)	24.17 (5.93)	22.88 (6.00)	23.75 (6.88)	2.18^	3.66^	3.87*
	NC	26.44 (4.35)	26.00 (5.98)	27.16 (5.54)	26.01 (5.45)			
Attention (first order)	OC	11.04 (2.92)	10.39 (3.22)	9.50 (2.96)	10.35 (3.86)	3.20*	NS	NS
	NC	11.60 (2.70)	11.33 (3.42)	11.09 (2.92)	10.87 (3.71)			
Motor (first order)	OC	16.86 (4.85)	15.78 (4.49)	16.50 (4.32)	15.25 (4.99)	2.23^	NS	NS
	NC	16.04 (3.72)	15.63 (3.97)	15.67 (4.05)	15.08 (3.52)			
Self-control	OC	14.35 (3.75)	12.65 (3.60)	11.99 (4.04)	12.45 (4.24)	3.42*	NS	2.42^
	NC	13.91 (3.25)	13.60 (3.97)	13.84 (3.75)	13.33 (3.75)			
Cognitive Complexity	OC	12.09 (2.43)	11.53 (3.31)	10.89 (2.54)	11.30 (3.36)	NS	4.36*	3.40*
	NC	12.53 (2.45)	12.40 (2.73)	13.33 (2.39)	12.68 (2.78)			
Perseverance	OC	6.78 (2.02)	6.20 (1.64)	6.85 (1.98)	6.70 (1.92)	2.35^	NS	NS
	NC	7.50 (1.86)	6.80 (1.52)	6.87 (1.63)	6.87 (1.63)			
Cognitive Instability	OC	5.65 (2.08)	5.05 (2.21)	5.65 (1.93)	5.50 (2.44)	2.53^	4.22*	NS
	NC	6.47 (2.03)	6.05 (2.24)	5.61 (1.79)	5.92 (2.09)			
STAI: *State*	OC	33.00 (10.45)	32.74 (10.59)	35.05 (10.28)	35.80 (10.49)	NS	NS	NS

	NC	37.64 (11.91)	35.71 (10.75)	36.35 (11.16)	35.49 (12.06)			
Trait	OC	38.17 (10.50)	36.92 (10.23)	37.75 (11.68)	38.93 (11.39)	NS	4.33*	NS
	NC	43.20 (11.13)	42.46 (10.59)	40.70 (10.32)	40.30 (11.66)			
Bond-Lader: *Alert*	OC	51.96 (16.91)	51.73 (16.17)	56.87 (16.03)	47.61 (20.52)	NS	NS	
	NC	52.08 (14.07)	50.60 (14.98)	48.91 (15.93)	49.89 (15.15)			
Content	OC	64.93 (14.17)	65.97 (15.77)	63.52 (15.97)	65.44 (16.29)	NS	NS	NS
	NC	63.87 (16.04)	64.25 (14.55)	61.09 (14.78)	61.11 (14.07)			
Calm	OC	65.98 (13.04)	67.78 (12.32)	63.33 (14.44)	62.10 (14.99)	NS	NS	NS
	NC	61.90 (13.99)	61.14 (12.92)	64.59 (12.66)	61.94 (14.15)			
VAS: *Stress*	OC	38.91 (19.97)	35.90 (16.98)	36.05 (18.03)	31.65 (14.99)	NS	4.22*	NS
	NC	48.80 (23.26)	42.25 (21.11)	41.83 (19.51)	39.13 (21.80)			
Fatigue	OC	68.83 (17.34)	55.05 (19.19)	57.75 (18.17)	57.30 (21.41)	5.84**	NS	NS
	NC	63.50 (18.87)	56.33 (19.95)	60.15 (20.28)	51.23 (20.84)			
POMS: *Total*	OC	31.70 (34.81)	25.19 (32.29)	31.00 (35.54)	34.03 (40.57)	2.33^	3.15^	NS
	NC	50.42 (36.96)	40.65 (36.89)	33.63 (40.12)	39.46 (39.01)			
Tension-Anxiety	OC	9.22 (7.81)	9.30 (7.72)	11.05 (7.49)	10.30 (7.82)	NS	NS	3.76*
	NC	12.60 (7.07)	10.73 (6.55)	9.57 (7.57)	11.18 (7.18)			
Depression-Dejection	OC	9.83 (9.10)	7.05 (7.48)	10.05 (10.76)	9.78 (12.20)	NS	5.24*	NS
	NC	15.60 (13.60)	13.27 (12.83)	11.59 (12.87)	12.87 (12.32)			
Anger-Hostility	OC	7.87 (7.96)	7.10 (7.53)	8.35 (8.36)	8.50 (9.87)	NS	3.51^	NS
	NC	12.29 (9.82)	10.34 (8.86)	8.61 (8.53)	10.44 (9.26)			
Vigour-Activity	OC	14.04 (8.12)	14.26 (8.10)	15.10 (7.05)	12.90 (8.03)	NS	NS	NS
	NC	14.31 (6.21)	14.59 (6.59)	14.78 (6.11)	14.36 (6.31)			
Fatigue-Inertia	OC	10.52 (6.93)	8.70 (5.16)	8.65 (6.24)	10.75 (6.09)	4.48**	NS	NS
	NC	13.33 (6.20)	11.24 (5.89)	9.73 (6.89)	9.77 (6.29)			
Confusion-Bewilderment	OC	8.30 (4.20)	7.30 (4.16)	7.70 (4.93)	7.60 (5.46)	3.00*	4.19*	NS
	NC	10.91 (4.89)	9.65 (4.89)	8.91 (4.82)	9.56 (5.13)			
SPQ: *Total*	OC	15.70 (12.25)	14.15 (15.61)	14.20 (16.26)	14.61 (15.06)	8.94**	7.72**	NS
	NC	23.55 (14.08)	25.05 (15.44)	20.30 (15.29)	19.38 (12.33)			
Ideas of reference	OC	2.00 (2.13)	1.75 (2.43)	1.55 (2.09)	1.75 (2.22)	2.19^	3.31^	NS
	NC	2.78 (2.44)	3.22 (2.76)	2.48 (2.49)	2.23 (2.24)			
Excessive social	OC	2.74 (2.38)	2.55 (2.65)	2.45 (2.63)	2.70 (2.64)	NS	NS	NS

anxiety	NC	3.28 (2.58)	3.46 (2.64)	2.67 (2.34)	2.71 (2.33)			
Odd beliefs/ magical thinking	OC	0.57 (0.95)	0.25 (0.44)	0.35 (0.93)	0.40 (0.82)	3.08*	8.16**	NS
	NC	1.62 (1.79)	1.92 (2.07)	1.43 (1.89)	1.10 (1.37)			
Unusual perceptual experiences	OC	1.61 (1.70)	1.65 (2.08)	1.15 (1.69)	0.95 (1.36)	6.38**	3.71^	NS
	NC	2.14 (2.02)	2.48 (2.58)	1.93 (2.36)	1.88 (2.15)			
Odd/eccentric behaviour	OC	1.91 (2.47)	1.60 (2.16)	1.95 (2.50)	2.05 (2.35)	2.39^	NS	NS
	NC	2.35 (2.15)	2.27 (2.17)	2.15 (2.28)	1.81 (2.30)			
No close friends	OC	1.39 (1.75)	1.00 (1.69)	1.35 (2.13)	1.56 (2.01)	NS	NS	NS
	NC	2.51 (2.50)	2.68 (2.44)	2.07 (2.28)	2.23 (2.39)			
Odd speech	OC	2.98 (2.21)	2.70 (2.98)	2.95 (3.00)	2.35 (2.85)	5.80**	8.42**	NS
	NC	4.33 (2.44)	4.26 (2.69)	3.90 (2.70)	3.79 (2.53)			
Constricted affect	OC	1.28 (1.73)	1.00 (1.65)	0.90 (1.74)	1.05 (1.67)	3.33*	4.09*	NS
	NC	2.20 (2.01)	2.17 (2.14)	1.65 (1.95)	1.52 (1.75)			
Suspiciousness	OC	1.22 (1.41)	1.65 (2.37)	1.55 (2.26)	1.80 (2.44)	NS	NS	NS
	NC	2.33 (2.22)	2.59 (2.59)	2.02 (2.39)	2.10 (2.26)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Revised Barratt Impulsiveness Scale (BIS-11): Logit transformation was applied to scores on the perseverance subscale, all other scores were normally distributed. There was a trend for total impulsivity scores to be higher in NC women than OC users, as well as a significant effect of phase with increased impulsivity in menses compared with all other phases. Age also contributed significantly to the model for total BIS scores ($F(1,67)=5.92$, $p=0.018$, $d=0.60$), with increased age being associated with lower impulsivity. For the attentional impulsiveness factor there was a trend for NC women to have higher ratings than OC users, as well as a significant effect of phase with higher attentional impulsiveness during menses than all other phases. Scores on the motor impulsiveness factor were significantly higher in menses than the follicular and luteal phases. Age also contributed significantly to the model for motor impulsiveness ($F(1,67)=6.74$, $p=0.012$, $d=0.63$), having a negative association with ratings. There was a trend for NC women to have higher ratings on the non-planning impulsiveness factor than OC users, as well as a trend towards an effect of phase with pairwise comparisons revealing significantly higher scores in menses than the follicular and ovulatory phases. Moreover, there was a significant phase x treatment interaction with NC women having significantly higher ratings than OC users during the ovulatory phase only and with phase effects only being significant in OC users where scores were higher in menses than all other phases. Age also contributed negatively to the model for non-planning impulsiveness ($F(1,68)=5.38$, $p=0.023$, $d=0.56$).

Regarding first-order factors, attention scores were significantly higher during menses than the ovulatory phase. For the motor subscale there was a trend towards an effect of phase with pairwise comparisons revealing significantly higher scores in menses than the follicular and luteal phases. Age also contributed significantly to the model for motor ($F(1,67)=8.61$, $p=0.005$, $d=0.72$), with increased age being associated with lower motor impulsiveness. For self-control there was a trend towards an interaction between treatment and phase, with NC women having significantly poorer self-control during the ovulatory phase than OC users, and significant phase effects only for the OC group where self-control was significantly poorer during menses than all other phases. There was also a significant main effect of phase with higher scores in menses than all other phases. Age contributed significantly to the model for self-control ($F(1,69)=4.67$, $p=0.034$, $d=0.52$). There was a significant phase x treatment interaction for cognitive complexity, with pairwise comparisons revealing significantly higher scores in the NC

group than the OC group during only the ovulatory phase. The NC group also had significantly higher scores than the OC group overall (d=0.54). NC women had significantly higher cognitive instability than OC users (d=0.53), and there was a trend towards an effect of phase with pairwise comparisons revealing significantly higher scores in menses than all other phases. For perseverance there was a trend towards a main effect of phase, with significantly higher scores during menses than the follicular phase.

Profile of Mood States (POMS): For total scores on the POMS there was a trend towards a main effect of phase with pairwise comparisons revealing higher total mood disturbance scores in menses compared with the follicular and ovulatory phases. There was also a trend for NC women to have higher total mood disturbance scores than OC users. For the tension-anxiety factor there was a significant phase x treatment interaction with NC women experiencing higher levels of tension-anxiety than OC users during menses only. Significant phase effects were also only found for NC women where scores were higher during menses than the follicular and ovulatory phases, and higher during the luteal phase than the ovulatory phase (t=1.98, p=0.049, d=0.66). NC women also had significantly higher scores on the depression-dejection subscale than OC users (d=0.59), as well as a trend towards higher anger-hostility scores. For fatigue-inertia there was a significant main effect of phase with higher ratings during menses than all other phases. For confusion-bewilderment NC women had significantly higher ratings than OC users (d=0.53), and a main effect of phase was found with higher ratings during menses than all other phases.

State-Trait Anxiety Inventory (STAI): Logit transformation was applied to scores on the STAI. Whilst there were no significant effects of treatment, phase or their interaction on scores on the state subscale, OC users had significantly lower trait anxiety than NC women (d=0.53). Higher BMI was also significantly associated with higher trait anxiety (F(1,67)=4.50, p=0.038, d=0.52).

Table 7.3 *Results of post hoc comparisons of estimated marginal means where significant main effects of phase and significant interactions were found on mood outcomes.*

Outcome	Main effect of Phase	Treatment x Phase Interaction
Beck Depression Inventory	M > O (t=3.72, p<0.001, d=0.88) M > L (t=2.20, p=0.029, d=0.52)	NS
Revised Barratt Impulsiveness Scale (BIS-11) Total Scores	M > F (t=3.47, p=0.001, d=0.82) M > O (t=3.18, p=0.002, d=0.76) M > L (t=3.27, p=0.001, d=0.78)	NS
BIS-11 Attentional Impulsiveness	M > F (t=2.54, p=0.012, d=0.60) M > O (t=3.30, p=0.001, d=0.78) M > L (t=2.47, p=0.014, d=0.59)	NS
BIS-11 Motor Impulsiveness	M > F (t=2.83, p=0.005, d=0.67) M > L (t=2.72, p=0.007, d=0.65)	NS
BIS-11 Non-Planning Impulsiveness	M > F (t=2.11, p=0.036, d=0.50) M > O (t=2.10, p=0.037, d=0.50)	Ovulatory phase only: NC > OC (t=3.20, p=0.002, d=0.82) OC only: M > F (t=2.17, p=0.031, d=0.52); M > O (t=3.34, p=0.001, d=0.79); M > L (t=2.16, p=0.032, d=0.51)
BIS-11 Attention	M > O (t=3.01, p=0.003, d=0.71)	NS
BIS-11 Motor	M > F (t=1.98, p=0.049, d=0.47) M > L (t=2.37, p=0.019, d=0.56)	NS
BIS-11 Self Control	M > F (t=2.31, p=0.022, d=0.55) M > O (t=2.75, p=0.007, d=0.65) M > L (t=2.59, p=0.01, d=0.62)	Ovulatory phase only: NC > OC (t=2.29, p=0.023, d=0.59) OC only: M > F (t=2.35, p=0.02, d=0.56); M > O (t=3.29, p=0.001, d=0.78); M > L (t=2.40, p=0.017, d=0.57)
BIS-11 Cognitive Complexity	NS	Ovulatory phase only: NC > OC (t=3.32, p=0.001, d=0.85)
BIS-11 Cognitive Instability	M > F (t=2.31, p=0.022, d=0.55) M > O (t=2.24, p=0.026, d=0.53) M > L (t=2.05, p=0.042, d=0.49)	NS
BIS-11 Perseverance	M > F (t=2.57, p=0.011, d=0.61)	NS
Profile of Mood States (POMS) Total Mood Disturbance	M > F (t=2.29, p=0.023, d=0.61) M > O (t=2.22, p=0.028, d=0.57)	NS
POMS Tension-Anxiety	NS	Menses only: NC > OC (t=2.26, p=0.026, d=0.59) NC only: M > F (t=2.35, p=0.02, d=0.78); M > O (t=3.48, p=0.001, d=1.09); L > O (t=1.98, p=0.049, d=0.66)
POMS Fatigue-	M > F (t=2.39, p=0.018, d=0.64)	NS

Inertia	M > O (t=3.45, p=0.001, d=0.88) M > L (t=2.59, p=0.01, d=0.71)	
POMS Confusion-Bewilderment	M > F (t=2.20, p=0.029, d=0.59) M > O (t=2.69, p=0.008, d=0.69) M > L (t=2.23, p=0.027, d=0.61)	NS
Schizotypal Personality Questionnaire (SPQ) Total Scores	M > F (t=2.02, p=0.043, d=0.54) M > O (t=4.61, p<0.001, d=1.18) M > L (t=4.09, p<0.001, d=1.12) F > O (t=2.47, p=0.014, d=0.65) F > L (t=1.97, p=0.05, d=0.58)	NS
SPQ Ideas of Reference	F > O (t=2.18, p=0.031, d=0.57)	NS
SPQ Odd Beliefs and Magical Thinking	M > O (t=2.26, p=0.027, d=0.58) M > L (t=2.67, p=0.009, d=0.73)	NS
SPQ Unusual Perceptual Experiences	M > O (t=3.83, p<0.001, d=0.98) M > L (t=3.24, p=0.002, d=0.89) F > O (t=2.66, p=0.009, d=0.70) F > L (t=2.05, p=0.043, d=0.61)	NS
SPQ Odd or Eccentric Behaviour	M > L (t=2.55, p=0.011, d=0.70)	NS
SPQ Odd Speech	M > F (t=2.48, p=0.015, d=0.66) M > O (t=2.37, p=0.018, d=0.61) M > L (t=4.13, p<0.001, d=1.14)	NS
SPQ Constricted Affect	M > O (t=2.89, p=0.004, d=0.74) M > L (t=2.44, p=0.016, d=0.67)	NS
Bond-Lader Alertness	NS	Ovulatory Phase Only: OC > NC (t=2.16, p=0.032, d=0.59) OC only: O > L (t=2.51, p=0.013, d=1.22)
Visual Analogue Scales Mental Fatigue	M > F (t=3.18, p=0.002, d=0.85) M > O (t=2.13, p=0.034, d=0.55) M > L (t=3.87, p<0.001, d=1.06)	NS

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; NS, Non-significant; OC, Oral Contraceptive Users; NC, Normally Cycling Women

Schizotypal Personality Questionnaire (SPQ): Total scores on the SPQ as well as the subscales social anxiety, no close friends and odd speech were transformed according to the logit method, and scores on the odd beliefs, unusual perceptual experiences, odd behaviour, constricted affect and suspiciousness subscales were transformed using square root transformation.

There was a significant main effect of phase on total SPQ scores, with higher schizotypal personality traits during menses than all other phases, and higher scores in the follicular phase than the ovulatory and luteal phases. OC users also had significantly lower total SPQ scores than NC women (d=0.71). For ideas of reference there was a trend for NC women to have higher scores than OC users, as well as a trend towards a main effect of phase with pairwise comparisons revealing significantly higher scores in the follicular than the ovulatory phase. For excessive social anxiety there was a significant negative association with age ($F(1,68)=5.90$, $p=0.018$, $d=0.59$). NC women had significantly more traits of odd beliefs and magical thinking than OC users (d=0.73), and there was also a significant main effect of phase with higher scores during menses than the ovulatory and luteal phases. There was a trend for NC women to report more unusual perceptual experiences than OC users, as well as a significant effect of phase with more experiences during menses than the ovulatory and luteal phases, and higher scores during the follicular than the ovulatory and luteal phases. There was also a trend towards a main effect of phase on odd or eccentric behaviour, with higher scores during menses than the luteal phase. NC women reported significantly more odd speech than OC users (d=0.74), as well as there being a main effect of phase with increased odd speech during menses compared with all other phases. There was also a significant negative association between age and odd speech ($F(1,68)=4.26$, $p=0.043$, $d=0.50$). NC women reported significantly more constricted affect than OC users (d=0.52), as well as there being a main effect of phase with higher constricted affect during menses than the ovulatory and luteal phases. Increased age was associated with lower suspiciousness ($F(1,68)=4.25$, $p=0.043$, $d=0.50$).

Bond-Lader: There was a trend towards a phase x treatment interaction for alertness, with pairwise comparisons revealing higher scores in OC users during the ovulatory phase than in NC women, and significant phase effects for OC users only where alertness was significantly higher during the ovulatory phase than the luteal phase.

VAS: For mental fatigue, there was a significant main effect of phase with pairwise comparisons revealing significantly increased mental fatigue during menses compared

with all other phases. OC users had significantly lower stress ratings than NC women (d=0.53).

7.5. Premenstrual symptoms and general symptoms

Means, standard deviations and statistical values associated with all measures of premenstrual symptoms at baseline are given in Table 7.4. Where significant main effects of phase or significant interactions were found, results from post hoc pairwise comparisons of estimated marginal means are shown in Table 7.5.

Symptom Checklist (SCL): Following natural log transformation OC users had significantly less severe symptoms than NC women (d=0.63). There was also a significant main effect of phase with more severe symptoms during menses than all other phases. Whilst there was no significant phase x treatment interaction, pairwise comparisons of estimated marginal means did reveal significantly more severe symptoms in NC women than OC users during menses and the follicular phaseonly, as well as phase effects being only significant for the NC group where symptoms were significantly more severe during menses than all other phases.

Modified Daily Symptom Report (DSR-20): Square root transformation was applied to total DSR scores. Logit transformation was applied to psychological and physical factors, and box-cox transformation (Lambda=0.2) was applied to impulsiveness scores. There was a significant phase x treatment interaction for total scores on the DSR, with pairwise comparisons revealing less severe symptoms in OC users than NC women during menses and the luteal phase. There was also a significant main effect of treatment with NC women experiencing more severe symptoms (d=0.64), and a significant main effect of phase with more severe symptom ratings during menses than all other phases, and higher symptom scores during the luteal phase than the "rest" phase (t=2.16, p=0.032, d=0.59).

Table 7.4 *Means and standard deviations for measures of premenstrual and general symptoms during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Rest[+]	Luteal	Cycle Phase (P); df=3,159	Treatment Group (T); df=1,52	P x T interaction; df=3,159
SCL	OC	45.80 (13.61)	39.70 (8.94)	42.70 (11.87)	43.05 (11.09)	6.57**	6.05*	NS
	NC	58.53 (19.40)	50.58 (18.12)	48.34 (17.11)	49.40 (15.28)			
DSR: *Total*	OC	12.14 (10.77)	8.78 (7.45)	6.18 (4.14)	6.04 (4.75)	21.26**	4.72*	5.69**
	NC	17.43 (11.02)	9.99 (8.00)	9.73 (8.35)	13.83 (9.39)			
Psychological factor	OC	5.68 (5.60)	3.95 (4.01)	2.89 (2.54)	2.88 (3.14)	8.92**	4.39*	NS
	NC	7.50 (5.86)	5.19 (4.41)	4.99 (4.55)	6.55 (5.37)			
Physical factor	OC	6.46 (6.34)	4.83 (4.00)	3.29 (2.21)	3.16 (2.02)	24.47**	3.47^	8.44**
	NC	9.93 (6.27)	4.80 (3.88)	4.73 (4.02)	7.27 (4.90)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant; [+], for SCL value is for the ovulatory phase

For the psychological factor, symptoms were significantly more severe for NC women than OC users (d=0.61), and there was also a significant main effect of phase with significantly higher scores in menses than all other phases. Although no significant interaction was found, pairwise comparisons revealed that differences between groups were only significant during the luteal phase and that for the NC group only scores were higher during the luteal phase than the "rest" phase. For scores on the physical factor, there was a significant phase x treatment interaction with NC women rating their symptoms as significantly more severe than OC users during menses and the luteal phase. There was also a significant main effect of phase, with more severe physical symptoms during menses than all other phases, and more severe symptoms during the luteal phase than the "rest" phase. There was a trend for NC women to have more severe physical symptoms.

Table 7.5 *Results of post hoc comparisons of estimated marginal means where significant main effects of phase and significant interactions were found on outcomes of premenstrual symptoms.*

Outcome	Main effect of Phase	Treatment x Phase Interaction
Symptom Checklist (SCL)	M > F (t=4.00, p<0.001, d=1.07) M > O (t=3.47, p=0.001, d=0.89) M > L (t=3.06, p=0.002, d=0.84)	Menses: NC > OC (t=3.12, p=0.002, d=0.81) Follicular: NC > OC (t=2.26, p=0.025, d=0.60) NC only: M > F (t=4.15, p<0.001, d=1.38); M > O (t=4.95, p<0.001, d=1.55); M > L (t=3.80, p<0.001, d=1.32)
Modified Daily Symptom Report (DSR-20) Total Scores	M > F (t=5.85, p<0.001, d=1.59) M > R (t=7.54, p<0.001, d=2.05) M > L (t=5.38, p<0.001, d=1.46) L > R (t=2.16, p=0.032, d=0.59)	Menses: NC > OC (t=2.32, p=0.023, d=0.68) Luteal: NC > OC (t=3.41, p=0.001, d=1.00)
DSR-20 Psychological Factor	M > F (t=3.84, p<0.001, d=1.05) M > R (t=4.75, p<0.001, d=1.29) M > L (t=3.75, p<0.001, d=1.02)	Luteal only: NC > OC (t=2.95, p=0.004, d=0.76) NC only: L > R (t=2.54, p=0.012, d=0.73)
DSR-20 Physical Factor	M > F (t=6.24, p<0.001, d=1.70) M > R (t=8.16, p<0.001, d=2.22) M > L (t=5.53, p<0.001, d=1.51) L > R (t=2.64, p=0.009, d=0.72)	Menses: NC > OC (t=2.58, p=0.012, d=0.76) Luteal: NC > OC (t=3.18, p=0.002, d=0.93)

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; R, Rest of the cycle; NS, Non-significant; OC, Oral Contraceptive Users; NC, Normally Cycling Women

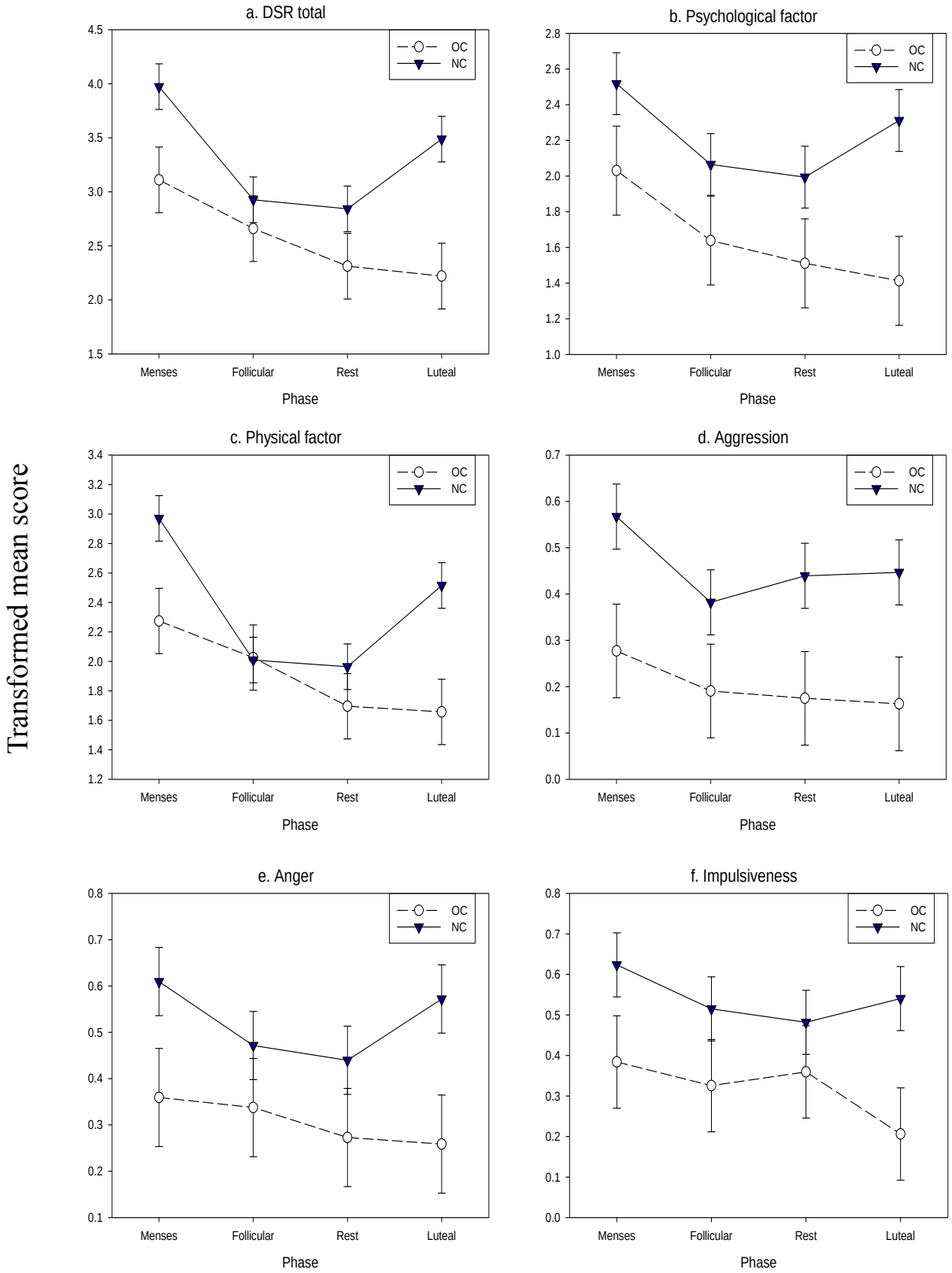

Figure 7.2 Mean ratings of premenstrual symptoms on the Modified Daily Symptom Report (DSR-20) in normally cycling (NC) women and oral contraceptive (OC) users across the cycle at baseline. Graphs depict mean (±SEM) transformed scores for **a.** Total DSR scores, **b.** Psychological factor scores, **c.** Physical factor scores, **d.** Aggression, **e.** Anger and **f.** Impulsiveness

7.6. Aggression Measures

Table 7.6 shows means, standard deviations and statistical values for all outcomes associated with aggression measures at baseline. Results of post hoc pairwise comparisons of estimated marginal means for significant main effects of phase and significant interactions are presented in Table 7.7.

Buss-Perry Aggression Questionnaire (BPAQ): Box-cox transformation was applied to all subscales (Lambda=2) but not for total scores which were normally distributed. There was a marginally significant effect of treatment on total scores with OC users being less aggressive (higher scores) than NC women (d=0.51). There was also a significant main effect of phase, with pairwise comparisons revealing increased aggression during menses compared with all other phases. Whilst there was no significant interaction, pairwise comparisons showed that the difference between groups was only significant during menses and that phase effects were only significant for the NC group where women were more aggressive during menses than all other phases. Higher BMI was also significantly associated with increased aggression (F(1,67)=4.27, p=0.043, d=0.51).

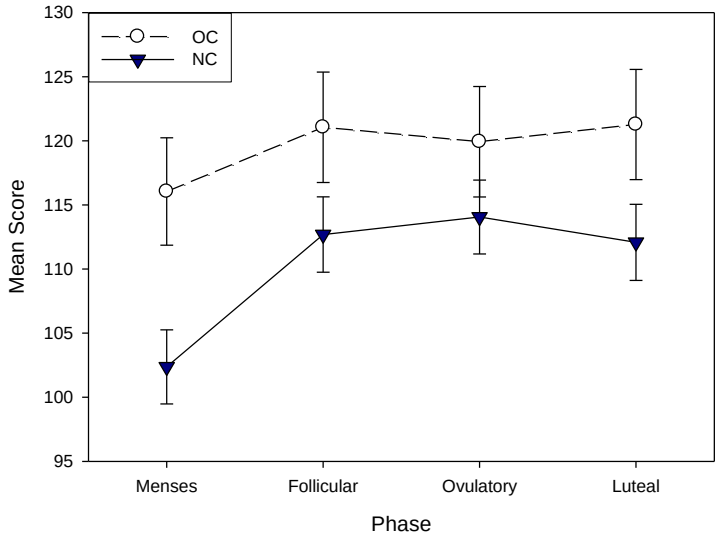

Figure 7.3 Total mean ratings of aggression on the BPAQ for OC users and NC women during different phases of the baseline menstrual cycle. A lower mean score indicates a higher level of aggression.

For the anger subscale, there was a significant main effect of phase with women being more angry during menses than all other phases. Higher BMI was also significantly associated with increased anger ($F(1,67)=4.92$, $p=0.03$, $d=0.54$). A significant main effect of phase was also found for hostility scores, where women were more hostile during menses than all other phases. Similarly, women reported significantly greater verbal aggression during menses than all other phases. Again, higher BMI was associated with increased verbal aggression ($F(1,68)=4.57$, $p=0.036$, $d=0.52$). For physical aggression, there was a trend for NC women to be more physically aggressive than OC users.

State-Trait Anger Expression Inventory (STAXI): Logit transformation was applied to the subscales T-Anger/R, AX/In and AX/Out, and box-cox transformation was applied to the subscales T-Anger (Lambda=-0.6), S-Anger (Lambda=-2) and T-Anger/T (Lambda=-1.4). There was a trend for NC women to rate their state anger (S-Anger) and trait anger (T-Anger) as higher than OC users, as well as a significant main effect of phase on T-Anger with higher T-Anger during menses than the follicular and ovulatory phases. For reactive anger (T-Anger/R) there was a trend for NC women to have more angry reactions than OC users, as well as a main effect of phase with higher T/Anger-R ratings in menses than all other phases. There was a trend for NC women to score higher on the anger in (AX/In) scale than OC users, as well as a significant negative association between age and AX/In ($F(1,67)=4.30$, $p=0.042$, $d=0.51$). A trend was also found for NC women to have higher anger-out (AX/Out) scores than OC users. OC users had significantly higher anger control (AX/Con) scores ($d=0.71$) and significantly lower overall anger expression (AX/EX) scores ($d=0.71$) than NC women.

Table 7.6 *Means and standard deviations for subjective and objective measures of aggression during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction*.

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory[+]	Luteal	Cycle Phase (P); df=3,178	Treatment Group (T); df=1,68	P x T interaction; df=3,178
BPAQ: *Total*	OC	114.19 (15.50)	119.20 (16.41)	118.34 (15.21)	120.53 (15.10)	8.15**	3.98^	NS
	NC	104.40 (21.59)	111.47 (22.86)	115.76 (22.63)	113.28 (23.41)			
Hostility	OC	31.39 (6.39)	33.95 (6.15)	32.15 (7.39)	33.20 (5.97)	8.66**	NS	NS
	NC	28.13 (8.23)	30.98 (7.99)	32.08 (8.53)	32.18 (7.06)			
Anger	OC	25.67 (5.46)	27.25 (5.36)	27.49 (4.82)	27.10 (5.35)	5.54**	NS	NS
	NC	23.56 (7.49)	25.36 (6.61)	26.93 (6.01)	26.54 (6.85)			
Verbal	OC	17.57 (3.65)	18.10 (4.56)	18.75 (3.85)	19.73 (3.69)	9.69**	NS	NS
	NC	16.20 (5.20)	18.27 (4.73)	18.72 (4.97)	17.38 (5.35)			
Physical	OC	39.57 (4.41)	39.90 (4.14)	39.95 (4.10)	40.50 (3.72)	NS	2.90^	NS
	NC	36.51 (5.83)	36.87 (7.51)	38.02 (7.09)	37.18 (6.92)			
STAXI: *S-Anger*	OC	10.43 (0.95)	10.61 (1.32)	10.70 (1.34)	11.45 (2.91)	NS	3.53^	NS
	NC	12.25 (5.04)	11.46 (2.77)	12.30 (5.12)	12.18 (3.46)			
T-Anger	OC	17.03 (5.64)	15.73 (4.84)	15.65 (5.62)	16.42 (6.06)	3.63*	3.38^	NS
	NC	19.07 (6.22)	18.98 (5.64)	18.09 (6.20)	18.77 (7.51)			
T-Anger/T	OC	5.61 (2.46)	5.55 (2.09)	5.45 (2.26)	5.90 (2.55)	NS	NS	NS
	NC	6.69 (3.00)	6.78 (2.68)	6.59 (2.80)	6.76 (3.30)			
T-Anger/R	OC	8.39 (3.26)	7.55 (2.82)	7.35 (2.92)	7.60 (2.68)	4.08**	3.99^	NS
	NC	9.09 (2.95)	8.95 (2.75)	8.24 (2.94)	8.69 (3.25)			
AX/In	OC	15.96 (3.66)	15.45 (4.35)	15.02 (3.94)	15.15 (4.40)	NS	2.88^	NS
	NC	17.51 (5.60)	16.66 (5.01)	16.50 (4.58)	16.67 (4.99)			
AX/Out	OC	13.26 (3.43)	14.20 (4.72)	13.55 (4.41)	13.05 (4.01)	NS	3.60^	NS
	NC	15.71 (5.16)	15.66 (4.64)	14.65 (4.05)	15.79 (4.98)			
AX/Con	OC	24.96 (4.07)	25.40 (5.07)	25.63 (4.24)	25.45 (4.47)	NS	7.57**	NS

	NC	22.36 (5.36)	21.95 (4.43)	22.35 (5.42)	22.22 (5.82)			
AX/EX	OC	20.26 (7.82)	20.25 (10.93)	18.94 (9.25)	18.75 (9.42)	NS	7.76**	NS
	NC	26.86 (11.95)	26.37 (10.06)	24.80 (10.40)	26.24 (12.04)			
DSR*: Anger*	OC	0.43 (0.68)	0.32 (0.57)	0.22 (0.42)	0.20 (0.31)	NS	4.23*	NS
	NC	0.69 (0.84)	0.36 (0.41)	0.32 (0.39)	0.53 (0.55)			
Aggression	OC	0.36 (0.65)	0.17 (0.33)	0.16 (0.36)	0.16 (0.34)	2.57^	6.14*	NS
	NC	0.58 (0.68)	0.32 (0.44)	0.32 (0.42)	0.40 (0.52)			
Impulsiveness	OC	0.48 (0.84)	0.27 (0.45)	0.26 (0.37)	0.22 (0.53)	NS	3.24^	NS
	NC	0.72 (0.89)	0.50 (0.63)	0.40 (0.53)	0.50 (0.56)			
Emotional Stroop: *colour*	OC	741.16 (75.59)	686.74 (78.23)	639.04 (82.57)	645.18 (85.54)	25.67**	NS	NS
	NC	752.48 (88.19)	689.06 (89.53)	670.99 (100.57)	654.19 (104.72)			
Direct aggressive	OC	627.51 (81.98)	606.39 (93.91)	588.01 (86.15)	591.14 (83.00)	12.64**	NS	NS
	NC	629.64 (78.68)	600.43 (76.75)	603.04 (88.22)	597.45 (84.18)			
Indirect aggressive	OC	622.73 (81.39)	612.04 (94.30)	585.07 (82.69)	602.32 (94.71)	7.85**	NS	NS
	NC	625.00 (76.54)	606.11 (73.12)	606.00 (87.35)	601.21 (80.25)			
Positive affect	OC	620.26 (81.53)	603.03 (89.31)	580.69 (81.11)	594.25 (88.31)	11.90**	NS	NS
	NC	627.55 (75.27)	602.11 (78.04)	600.78 (78.34)	598.88 (81.12)			
Negative affect	OC	623.98 (80.78)	618.17 (99.72)	587.14 (80.88)	598.92 (88.40)	11.15**	NS	NS
	NC	627.70 (73.95)	600.06 (79.88)	604.64 (84.49)	599.16 (76.53)			
Neutral	OC	632.06 (85.65)	611.16 (94.82)	579.61 (72.90)	596.04 (83.05)	17.41**	NS	NS
	NC	632.29 (78.72)	604.47 (79.67)	603.43 (84.60)	594.90 (78.97)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant; $^{+}$, For DSR value is for the "rest" phase

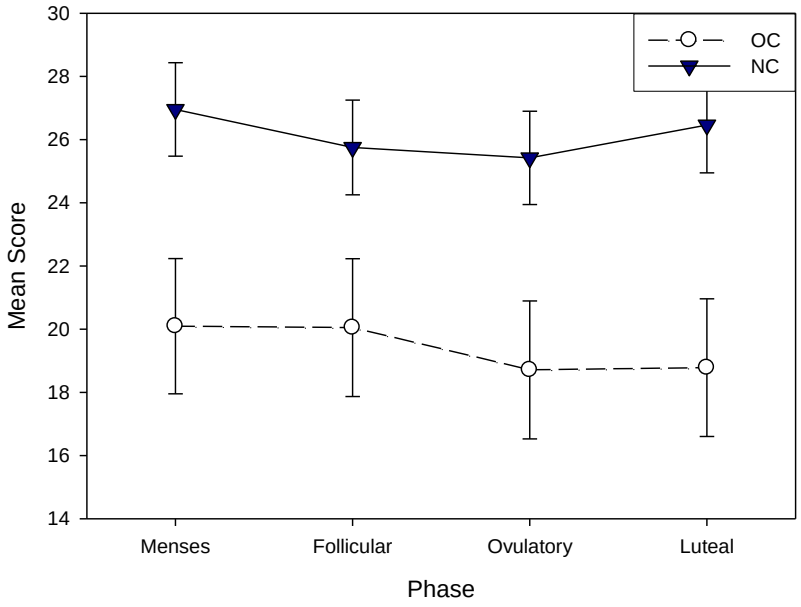

Figure 7.4 Mean total anger expression scores measured using the STAXI for OC users and NC women during different phases of the baseline menstrual cycle.

Modified Daily Symptom Report (DSR-20): For the individual items of interest on the DSR-20, square root transformation was applied to anger and aggression scores. NC women reported significantly more severe anger (d=0.60) and aggression (d=0.73) than OC users, and there was also a significant main effect of phase for aggression, with more severe aggression during menses than all other phases. There was a trend for NC women to report being more impulsive than OC users.

Emotional stroop task: Box-cox transformation was applied to response times to negative affect and positive affect stimuli (Lambda=-0.9). There was a significant main effect of phase on response times to all stimulus categories. For colour words response times were slower during menses compared with all other phases, and slower in the follicular than the ovulatory and luteal phases. For direct aggressive stimuli response times were slower during menses than all other phases, and slower in the follicular phase than the luteal phase. For indirect aggressive stimuli response times were slower in menses than all other phases, and slower in the follicular phase than the ovulatory phase. For positive affect, response times were slower in menses than all other phases.

For negative affect stimuli, response times were slower in menses than all other phases, as well as being slower in the follicular phase than the luteal phase. For neutral stimuli response times were slower during menses than all other phases, and slower during the follicular phase than the ovulatory and luteal phases.

Table 7.7 *Results of post hoc comparisons of estimated marginal means where significant main effects of phase and significant interactions were found on aggression outcomes.*

Outcome	Main effect of Phase	Treatment x Phase Interaction
Buss-Perry Aggression Questionnaire (BPAQ) Total Scores	M < F (t=3.97, p<0.001, d=1.06) M < O (t=4.08, p<0.001, d=1.05) M < L (t=3.85, p<0.001, d=1.06)	Menses only: NC < OC (t=2.66, p=0.009, d=0.69) NC only: M < F (t=4.61, p<0.001, d=1.54); M < O (t=5.42, p<0.001, d=1.69); M < L (t=4.25, p<0.001, d=1.48)
BPAQ Anger	M < F (t=2.99, p=0.003, d=0.80) M < O (t=3.67, p<0.001, d=0.94) M < L (t=2.99, p=0.003, d=0.82)	NS
BPAQ Hostility	M < F (t=4.35, p<0.001, d=1.16) M < O (t=3.68, p<0.001, d=0.94) M < L (t=4.12, p<0.001, d=1.13)	NS
BPAQ Verbal Aggression	M < F (t=3.85, p<0.001, d=1.03) M < O (t=4.57, p<0.001, d=1.17) M < L (t=4.43, p<0.001, d=1.22)	NS
State-Trait Anger Expression Inventory (STAXI) Trait Anger	M > F (t=2.00, p=0.016, d=0.54) M > O (t=2.67, p=0.002, d=0.68)	NS
STAXI Reactive Anger	M > F (t=2.21, p=0.028, d=0.59) M > O (t=3.48, p=0.001, d=0.89) M > L (t=2.04, p=0.044, d=0.56)	NS
Modified Daily Symptom Report (DSR-20) Aggression	M > F (t=2.47, p=0.014, d=0.67) M > R (t=2.09, p=0.037, d=0.57) M > L (t=2.13, p=0.034, d=0.58)	NS
Stroop Task Colour	M > F (t=4.42, p<0.001, d=1.18) M > O (t=7.09, p<0.001, d=1.82) M > L (t=7.86, p<0.001, d=2.16) F > O (t=2.56, p=0.011, d=0.67) F > L (t=3.28, p=0.001, d=0.97)	NS
Stroop Task Direct Aggressive	M > F (t=3.54, p=0.001, d=0.95) M > O (t=4.55, p<0.001, d=1.17) M > L (t=5.76, p<0.001, d=1.58) F > L (t=2.11, p=0.036, d=0.62)	NS
Stroop Task	M > F (t=2.11, p=0.036, d=0.56)	NS

Indirect Aggressive	M > O (t=4.23, p<0.001, d=1.08) M > L (t=4.01, p<0.001, d=1.10) F > O (t=2.03, p=0.044, d=0.53)	
Stroop Task Positive Affect	M > F (t=3.43, p=0.001, d=0.92) M > O (t=5.22, p<0.001, d=1.34) M > L (t=5.15, p<0.001, d=1.42)	NS
Stroop Task Negative Affect	M > F (t=2.93, p=0.004, d=0.78) M > O (t=4.74, p<0.001, d=1.21) M > L (t=5.11, p<0.001, d=1.40) F > L (t=2.11, p=0.039, d=0.62)	NS
Stroop Task Neutral	M > F (t=3.54, p=0.001, d=0.95) M > O (t=6.05, p<0.001, d=1.55) M > L (t=6.28, p<0.001, d=1.73) F > O (t=2.41, p=0.017, d=0.63) F > L (t=2.60, p=0.01, d=0.77)	NS

Note: Lower scores on the BPAQ indicate higher aggression

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; R, Rest of the cycle; NS, Non-significant; OC, Oral Contraceptive Users; NC, Normally Cycling Women

7.7. Cognitive outcomes

Tables 7.8, 7.9, 7.10 and 7.12 show means, standard deviations and statistical values for tasks of attention and reaction time, executive function, secondary memory and working memory, respectively at baseline. Findings from post hoc comparisons of estimated marginal means of significant main effects of phase and significant interactions are detailed in Table 7.11.

Reaction time: Box-cox transformation was applied to simple reaction time, choice reaction time and four choice reaction time responses (Lambda=-1). OC users had significantly faster responses on the simple reaction time (d=0.70) and four choice reaction time (d=0.56) tasks than NC women. For choice reaction time there was a trend for OC users to have faster reaction times than NC women.

Rapid Visual Information Processing (RVIP): Box-cox transformation was applied to reaction times on the RVIP task (Lambda=-0.6) and logit transformation was applied to false alarm scores. OC users had significantly higher accuracy than NC women (d=0.77), and there was also a significant main effect of phase with higher accuracy in the ovulatory and luteal phases than menses, and higher accuracy in the luteal phase than the follicular phase. There were no effects of treatment or phase on the number of false alarms, however there was a significant effect of years of education with fewer false alarms as years of education increased ($F(1,52)=7.25$, $p=0.01$, $d=0.75$).

Card sorting task: Box-cox transformation was applied to reaction times (Lambda=-0.9). There was a trend towards a main effect of phase on accuracy, with pairwise comparisons revealing significantly reduced accuracy in menses compared with the follicular and ovulatory phases. There was also a significant main effect of phase on reaction times, with slower reaction times in menses compared to the ovulatory and luteal phases. Whilst a phase x treatment interaction was not found, pairwise comparisons revealed that this phase effect was only significant for NC women, whereas no cyclical variation was found for OC users.

Peg and ball task: Box-cox transformation was applied to thinking time (Lambda=-0.7), completion time (Lambda=-0.4) and number of extra moves (Lambda=-0.1), and logit transformation was applied to overall accuracy scores. There was a significant main effect of phase on thinking time with longer thinking times during menses than all other phases, and longer thinking times in the follicular phase than the ovulatory and luteal phases. There was a similar finding for completion times, with slower completion times during menses than all other phases, and slower completion times in the follicular phase than the ovulatory and luteal phases.

Table 7.8 *Means and standard deviations for tasks of reaction time and attention during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction on each outcome.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,177	Treatment Group (T); df=1,68	P x T interaction; df=3,177
Simple reaction time	OC	291.23 (111.16)	260.05 (44.41)	274.08 (38.78)	301.67 (78.64)	NS	7.44**	NS
	NC	348.90 (173.58)	381.31 (213.61)	356.23 (119.33)	351.95 (155.50)			
Choice reaction time	OC	391.23 (112.43)	368.65 (49.25)	373.02 (32.60)	457.18 (273.82)	NS	3.27^	NS
	NC	411.14 (68.82)	400.30 (56.93)	415.31 (78.69)	406.93 (53.91)			
Four choice reaction time	OC	455.12 (105.11)	421.22 (63.14)	430.63 (50.21)	439.78 (70.32)	NS	4.83*	NS
	NC	484.34 (132.63)	453.92 (69.55)	457.56 (64.38)	473.62 (96.19)			
RVIP: *Accuracy*	OC	47.38 (20.76)	50.13 (23.79)	55.63 (22.77)	55.14 (20.73)	3.12*	8.97**	NS
	NC	36.31 (20.93)	36.80 (22.58)	36.49 (20.30)	40.17 (19.07)			
False alarms	OC	10.30 (13.55)	8.95 (10.89)	6.35 (6.38)	7.16 (8.09)	NS	NS	NS
	NC	9.58 (8.19)	7.56 (9.15)	8.08 (9.98)	6.36 (6.96)			
Reaction time	OC	490.93 (59.70)	476.54 (50.30)	485.10 (55.18)	501.15 (81.93)	NS	NS	NS
	NC	484.60 (71.57)	495.37 (79.59)	497.45 (77.68)	487.88 (72.73)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Table 7.9 *Means and standard deviations for tasks of executive function during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,174	Treatment Group (T); df=1,67	P x T interaction; 3,174
Card sort: *Accuracy*	OC	85.55 (3.40)	87.20 (2.46)	87.65 (2.81)	85.60 (5.59)	2.15^	NS	NS
	NC	84.91 (4.11)	85.68 (4.75)	85.61 (4.61)	86.37 (3.17)			
Reaction time	OC	1146.57 (245.83)	1129.40 (209.04)	1067.85 (169.17)	1146.90 (281.55)	3.64*	NS	NS
	NC	1230.44 (203.73)	1184.75 (248.30)	1159.44 (205.92)	1186.23 (257.14)			
Peg and ball: *Accuracy*	OC	90.87 (9.00)	90.50 (8.72)	91.75 (8.32)	91.75 (8.16)	NS	NS	NS
	NC	89.44 (10.24)	91.13 (7.29)	90.00 (10.28)	90.51 (8.49)			
Extra moves	OC	3.68 (4.35)	4.15 (4.49)	3.25 (3.86)	3.45 (3.47)	NS	NS	NS
	NC	4.66 (4.73)	3.53 (3.48)	2.95 (3.53)	3.71 (4.16)			
Thinking time	OC	2472.17 (828.23)	2196.75 (530.06)	1919.74 (435.35)	2024.90 (679.87)	13.18**	NS	NS
	NC	2195.88 (571.24)	2049.47 (620.91)	1874.20 (412.32)	1913.26 (511.48)			
Completion time	OC	6814.27 (1222.76)	6456.40 (1073.57)	6015.45 (1349.01)	5811.40 (883.99)	26.11**	NS	NS
	NC	6804.73 (1144.41)	6409.23 (1419.54)	5903.62 (1064.96)	5961.51 (1116.09)			
Serial 3s: *Total*	OC	36.55 (16.34)	37.80 (16.64)	41.20 (15.49)	41.85 (14.73)	5.05**	3.24^	NS
	NC	32.60 (14.38)	34.65 (13.40)	35.88 (15.04)	36.23 (11.45)			
Accuracy	OC	92.74 (7.45)	91.55 (8.46)	91.55 (6.34)	94.30 (5.51)	NS	NS	NS
	NC	92.31 (8.26)	92.70 (7.01)	89.40 (9.51)	91.29 (7.82)			
Reaction time	OC	3332.00 (1012.16)	3692.55 (1524.10)	3344.80 (1413.33)	3273.70 (1363.42)	4.50**	NS	NS
	NC	4065.65 (1557.43)	3862.79 (1472.97)	3732.53 (1530.48)	3526.13 (1164.46)			
Serial 7s: *Total*	OC	21.23 (8.46)	21.95 (10.14)	24.25 (11.25)	23.55 (10.90)	2.38^	NS	NS
	NC	19.76 (9.49)	21.25 (8.17)	20.77 (8.80)	21.77 (8.07)			
Accuracy	OC	84.92 (12.23)	82.36 (16.94)	89.37 (9.22)	87.42 (10.44)	NS	NS	NS
	NC	85.37 (15.02)	82.45 (16.00)	85.27 (13.67)	87.60 (11.47)			
Reaction time	OC	6281.36 (2649.96)	6193.60 (2348.24)	5530.30 (2088.40)	5864.65 (2570.62)	2.38^	NS	NS
	NC	6446.21 (2620.24)	5904.86 (1939.60)	6075.72 (2640.20)	5967.29 (2674.24)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Serial 3 subtractions: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-0.2). There was a trend for OC users to complete more subtractions than NC women, as well as a significant effect of phase with fewer subtractions during menses than the ovulatory and luteal phases, and fewer subtractions during the follicular phase than the luteal phase. There was no treatment or phase effect on accuracy, however there was a significant positive association between years of education and accuracy ($F(1,62)=4.00$, $p=0.05$, $d=0.51$). For reaction time, there was a significant main effect of phase, with slower reaction times in menses than the ovulatory and luteal phases, and slower reaction times in the follicular phase than the ovulatory and luteal phases. Although no significant interaction was found, pairwise comparisons revealed that these phase effects were only significant for NC women.

Serial 7 subtractions: Box-cox transformation was applied to reaction times (Lambda=-0.06) and logit transformation was applied to accuracy scores. There was a trend towards a main effect of phase on the total number of subtractions and reaction times, with fewer subtractions and slower reaction times during menses than the ovulatory and luteal phases.

Word recall: There was a trend for OC users to correctly recall more words after a delay than NC women.

Delayed word recognition: Box-cox transformation was applied to reaction time data (Lambda=-1.07). OC users were significantly faster to respond than NC women ($d=0.59$).

Delayed picture recognition: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-1.07). There was a trend towards a main effect of phase on picture recognition accuracy, with pairwise comparisons revealing significantly higher accuracy during menses compared with the

luteal phase. There was also a significant main effect of phase on reaction times, with significantly faster reaction times during the luteal phase than menses and the follicular phase.

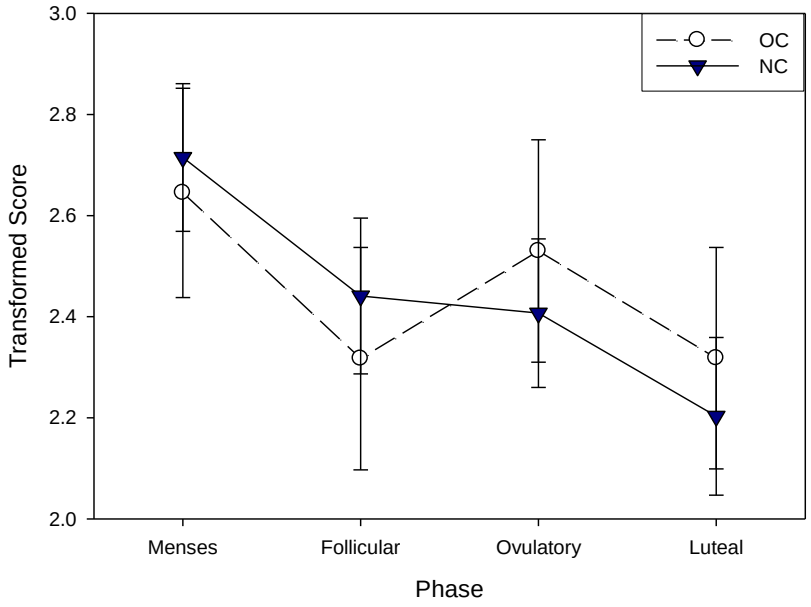

Figure 7.5 Mean transformed accuracy scores on the delayed picture recognition task for OC users and NC women across the baseline menstrual cycle.

Sentence verification: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-0.09). OC users were significantly faster than NC women to respond (d=0.52), and reaction times were significantly slower during menses than all other phases.

Table 7.10 *Means and standard deviations for tasks measuring secondary memory during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,179	Treatment Group (T); df=1,71	P x T interaction; df=3,179
Immediate word recall	OC	8.70 (1.82)	8.30 (2.42)	8.65 (2.07)	8.48 (2.39)	NS	NS	NS
	NC	8.12 (2.27)	8.00 (2.69)	8.13 (2.42)	8.67 (2.65)			
Delayed word recall	OC	6.54 (2.17)	6.18 (2.03)	6.95 (2.13)	6.65 (2.81)	NS	3.38^	NS
	NC	5.41 (2.58)	5.74 (3.19)	5.54 (2.63)	5.81 (2.83)			
Word recognition: *Accuracy*	OC	81.60 (8.81)	82.00 (8.68)	85.50 (7.28)	80.50 (11.51)	NS	NS	NS
	NC	81.19 (10.25)	83.17 (7.88)	82.66 (9.35)	83.08 (11.95)			
Reaction time	OC	797.09 (161.54)	796.10 (168.93)	781.80 (129.37)	766.95 (145.34)	NS	5.35*	NS
	NC	898.77 (196.02)	880.30 (240.41)	894.68 (256.88)	877.58 (254.67)			
Picture recognition: *Accuracy*	OC	93.84 (4.33)	90.62 (7.63)	92.71 (6.32)	89.58 (9.02)	2.50^	NS	NS
	NC	92.68 (8.93)	91.56 (7.22)	90.57 (9.75)	89.00 (10.00)			
Reaction time	OC	727.04 (115.00)	759.15 (146.22)	717.85 (85.19)	713.90 (125.30)	2.69*	NS	NS
	NC	766.49 (148.06)	743.97 (128.75)	758.80 (136.04)	727.58 (138.53)			
Sentence verification: *Accuracy*	OC	96.52 (6.65)	94.25 (7.30)	95.25 (4.72)	94.50 (8.09)	NS	NS	NS
	NC	93.11 (7.78)	93.33 (8.06)	93.70 (7.03)	93.08 (8.16)			
Reaction time	OC	1431.74 (362.88)	1307 (311.50)	1213.95 (241.15)	1222.45 (258.32)	14.39**	4.11*	NS
	NC	1593.45 (427.93)	1480.83 (415.33)	1420.63 (398.37)	1465.22 (502.05)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Alphabetic working memory: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-0.7). There was a trend for OC users to respond faster than NC women, as well as a significant main effect of phase with slower reaction times during menses than all other phases, and slower reaction times in the follicular phase than the ovulatory and luteal phases.

Numeric working memory: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-0.7). There was a significant main effect of phase on reaction times with faster responses in the luteal phase than all other phases, and faster responses in the ovulatory phase than menses.

N-back: Logit transformation was applied to accuracy scores and box-cox transformation was applied to reaction times (Lambda=-0.06). There was a trend for OC users to have higher accuracy than NC women, as well as a significant main effect of phase on reaction times, with slower responses during menses than the ovulatory and luteal phases, and slower responses during the follicular phase than the luteal phase.

Table 7.11 *Results of post hoc comparisons of estimated marginal means where significant main effects of phase were found on cognitive outcomes.*

Outcome	Main effect of Phase
Rapid Visual Information Processing (RVIP) Accuracy	O > M (t=2.23, p=0.027, d=0.57) L > M (t=2.50, p=0.014, d=0.69) L > F (t=2.05, p=0.042, d=0.61)
Card Sorting Task Accuracy	F > M (t=1.99, p=0.049, d=0.53) O > M (t=2.33, p=0.021, d=0.60)
Card Sorting Task Reaction Time	M > O (t=3.17, p=0.002, d=0.81) M > L (t=2.03, p=0.046, d=0.56)
Peg and Ball Task Thinking Time	M > F (t=2.03, p=0.044, d=0.54) M > O (t=5.13, p<0.001, d=1.31) M > L (t=5.32, p<0.001, d=1.46) F > O (t=3.02, p=0.003, d=0.79) F > L (t=3.15, p=0.002, d=0.93)
Peg and Ball Task Completion Time	M > F (t=3.35, p<0.001, d=0.90) M > O (t=7.23, p<0.001, d=1.85) M > L (t=7.68, p<0.001, d=2.11) F > O (t=3.74, p<0.001, d=0.98)

	F > L (t=4.20, p<0.001, d=1.24)
Serial 3 Subtractions Total	M < O (t=2.72, p=0.007, d=0.70) M < L (t=3.57, p<0.001, d=0.98) F < L (t=2.38, p=0.018, d=0.70)
Serial 3 Subtractions Reaction Time	M > O (t=2.24, p=0.027, d=0.57) M > L (t=3.10, p=0.002, d=0.85) F > O (t=1.98, p=0.049, d=0.52) F > L (t=2.80, p=0.006, d=0.83)
Serial 7 Subtractions Total	M < O (t=2.34, p=0.02, d=0.60) M < L (t=2.23, p=0.027, d=0.61)
Serial 7 Subtractions Reaction Time	M > O (t=2.46, p=0.015, d=0.63) M > L (t=2.03, p=0.044, d=0.56)
Picture Recognition Accuracy	M > L (t=2.62, p=0.009, d=0.72)
Picture Recognition Reaction Time	M > L (t=2.50, p=0.015, d=0.69) F > L (t=2.53, p=0.013, d=0.75)
Sentence Verification Reaction Time	M > F (t=3.62, p<0.001, d=0.97) M > O (t=5.68, p<0.001, d=1.45) M > L (t=5.57, p<0.001, d=1.53)
Alphabetic Working Memory Reaction Time	M > F (t=2.66, p=0.009, d=0.71) M > O (t=5.10, p<0.001, d=1.31) M > L (t=5.88, p<0.001, d=1.62) F > O (t=2.33, p=0.021, d=0.61) F > L (t=3.09, p=0.002, d=0.91)
Numeric Working Memory Reaction Time	M > O (t=2.83, p=0.005, d=0.73) M > L (t=5.26, p<0.001, d=1.45) F > L (t=4.11, p<0.001, d=1.21) O > L (t=2.44, p=0.016, d=0.66)
N-Back Reaction Time	M > O (t=3.21, p=0.002, d=0.82) M > L (t=3.92, p<0.001, d=1.08) F > L (t=2.19, p=0.03, d=0.65)
Reversed Corsi Blocks Span Score	M < F (t=2.54, p=0.012, d=0.68) M < O (t=3.12, p=0.002, d=0.80) M < L (t=2.94, p=0.004, d=0.81)

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; NS, Non-significant; OC, Oral Contraceptive Users; NC, Normally Cycling Women

Table 7.12 *Means and standard deviations for tasks of working memory during the baseline menstrual cycle, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Group	Mean score (SD)				F values		
		Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,177	Treatment Group (T); df=1,70	P x T interaction; df=3,177
Alphabetic working memory: *Accuracy*	OC	94.93 (4.11)	94.89 (3.97)	94.72 (3.32)	93.51 (3.93)	NS	NS	NS
	NC	93.71 (5.02)	92.97 (4.12)	93.24 (4.78)	92.93 (4.24)			
Reaction time	OC	460.10 (126.15)	441.83 (126.56)	409.20 (84.63)	409.82 (88.35)	14.17**	3.67^	NS
	NC	496.56 (107.07)	467.59 (117.11)	444.82 (98.18)	443.44 (128.39)			
Numeric working memory: *Accuracy*	OC	94.95 (4.56)	94.56 (4.05)	95.56 (2.87)	94.62 (4.92)	NS	NS	NS
	NC	94.03 (4.86)	94.17 (4.06)	93.89 (5.00)	92.88 (4.44)			
Reaction time	OC	455.77 (117.15)	447.35 (108.75)	434.62 (91.25)	403.75 (102.14)	10.42**	NS	NS
	NC	461.95 (98.18)	462.48 (109.03)	437.40 (96.04)	436.57 (107.95)			
N-back: *Accuracy*	OC	83.04 (14.87)	84.67 (15.00)	88.00 (11.77)	89.00 (9.37)	NS	3.22^	NS
	NC	77.11 (18.69)	77.58 (20.58)	79.25 (20.08)	80.00 (18.35)			
Reaction time	OC	1036.49 (353.41)	982.85 (297.58)	885.82 (217.15)	882.98 (337.80)	6.06**	NS	NS
	NC	1103.00 (345.62)	1044.68 (394.71)	966.12 (311.80)	981.61 (373.97)			
Corsi blocks: *Span score*	OC	6.15 (1.33)	6.15 (1.14)	6.07 (1.75)	6.18 (1.11)	NS	NS	NS
	NC	5.99 (1.08)	5.90 (1.21)	6.12 (1.01)	5.82 (1.07)			
Reaction time	OC	5023.48 (1846.87)	5252.20 (1438.37)	4867.05 (1587.77)	5245.60 (1355.74)	NS	NS	NS
	NC	5092.70 (1260.55)	5340.84 (1575.27)	5261.17 (1911.07)	4794.97 (1422.51)			

Corsi blocks reversed: *Span score*	OC	2.83 (2.90)	4.38 (2.72)	4.40 (2.42)	4.27 (2.55)	4.37**	NS	4.32**
	NC	3.25 (2.75)	2.85 (2.78)	3.28 (2.86)	3.36 (2.81)			
Reaction time	OC	3754.09 (1522.81)	4330.65 (1247.27)	3939.10 (1326.69)	4451.50 (1690.08)	NS	NS	NS
	NC	4096.98 (1627.40)	4105.98 (1796.24)	3959.80 (1733.08)	3934.05 (1848.87)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Corsi blocks forward and reversed: Box-cox transformation was applied to reaction times for the standard (Lambda=-0.6) and reversed (Lambda=0.5) tasks. For the reversed corsi blocks task, there was a significant main effect of phase on span scores with fewer correct pattern replications in menses compared with all other phases. There was also a significant phase x treatment interaction for span scores on the reversed task with pairwise comparisons revealing that these phase effects were only significant for the OC users.

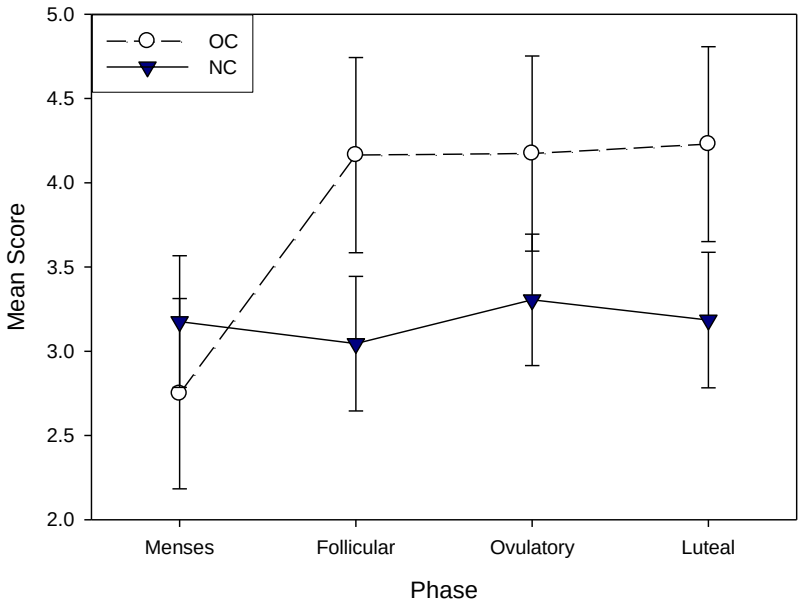

Figure 7.6 Mean span scores on the reversed corsi blocks task for OC users and NC women during different phases of the baseline menstrual cycle.

7.8. EEG measures: Behavioural results

7.8.1. Standard go/nogo task

25% Nogo condition

Box-cox transformation was applied to correct responses to go stimuli (Lambda=1.96) and mean reaction times to go stimuli (Lambda=-1.11) in order to normalise the data prior to analysis. There was a significant main effect of phase on the number of errors of commission (failures to inhibit responses to up arrows) with significantly more errors

during menses than the luteal phase (F(1,32)=6.46, p=0.016, d=0.86). Whilst there was no significant phase x treatment interaction, pairwise comparisons of estimated marginal means revealed that this effect was significant only for OC users (t=2.27, p=0.03, d=0.80) whereas no significant phase effects were found for NC women. For up arrow response (error) reaction times, there was a significant main effect of treatment with faster responses by OC users compared with NC women (F(1,29)=7.14, p=0.012, d=0.97) as well as a significant main effect of phase with faster responses in the luteal phase compared with during menses (F(1,28)=6.174, p=0.019, d=0.84).

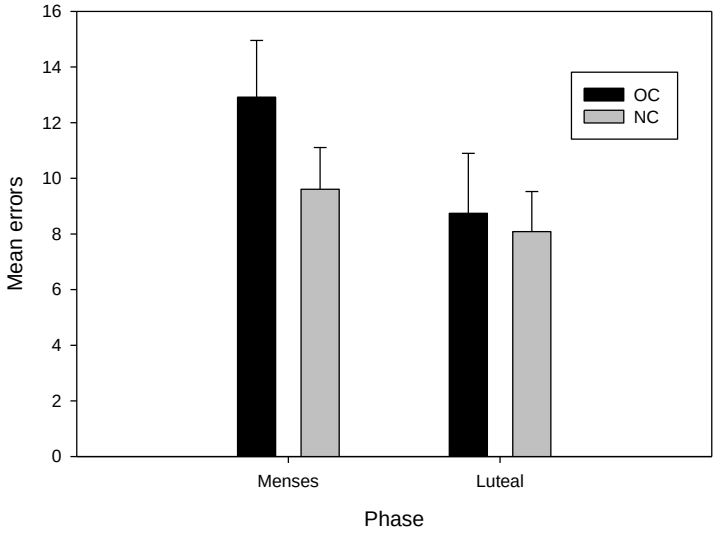

Figure 7.7 Mean number of errors of commission to nogo stimuli in the standard go/nogo task with abstract stimuli for OC users and NC women during the menses and luteal phases of the baseline cycle.

There was a trend towards a treatment x phase interaction for the number of correct responses to go stimuli (F(1,32)=3.87, p=0.058) with pairwise comparisons revealing a trend for OC users to have more correct responses during menses compared with NC women (t=1.87, p=0.068) and no difference between groups during the luteal phase, as well as a trend towards an effect of phase for OC users only who had more correct responses during menses than the luteal phase (t=1.73, p=0.093). There was a significant main effect of treatment on reaction times to go stimuli with NC women responding slower than OC users (F(1,52)=6.06, p=0.015, d=0.90) as well as a

significant treatment x phase interaction (F(1,42)=4.30, p=0.044) with pairwise comparisons revealing that OC users responded faster than NC women in the luteal phase only (t=2.90, p=0.005, d=1.06).

25% Go condition

Box-cox transformation was applied to correct responses to go stimuli (Lambda=2) and mean reaction times to go stimuli (Lambda=-1.07) in order to normalise the data prior to analysis. There were no significant effects of treatment or phase on the number of go stimuli correctly responded to, however there was a significant main effect of treatment on reaction times to go stimuli with OC users having faster response times than NC women (F(1,52)=7.52, p=0.008, d=0.81).

7.8.2. Go/nogo task with face emotion stimuli

Neutral faces as the 25% nogo stimulus

There was a significant main effect of phase on the number of correct responses to happy go stimuli, with more responses during menses than the luteal phase (F(1,25)=6.53, p=0.017, d=0.86). There was also a significant main effect of phase on the number of errors of commission (failure to inhibit the response to neutral stimuli) with more errors during menses than the luteal phase (F(1,29)=5.21, p=0.03, d=0.85). Years of education also contributed significantly to the model with more years of education being associated with an increased number of errors (F(1,32)=4.84, p=0.035, d=0.79).

Neutral faces as the 25% go stimulus

There was a trend towards a main effect of treatment on reaction times to neutral faces with OC users responding faster than NC women (F(1,33)=3.20, p=0.083). There was also a significant contribution of age to the model for number of correct response

inhibits to angry faces, with increased age being associated with an increased number of correct inhibits (F(1,39)=6.68, p=0.014, d=0.83).

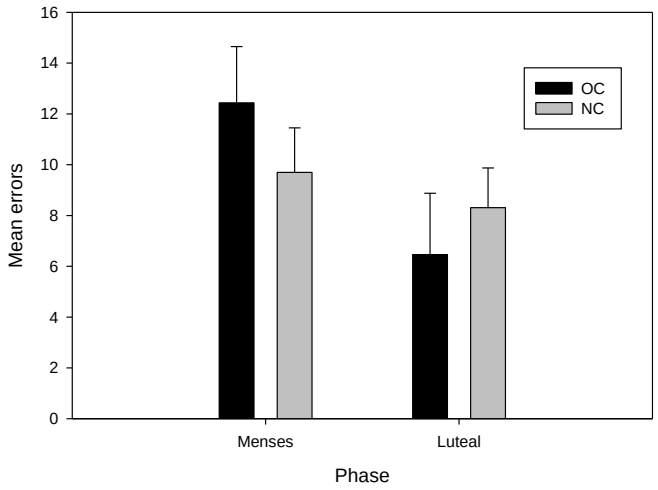

Figure 7.8 Mean number of errors of commission in the go/nogo task with face emotion stimuli, where neutral faces were the 25% nogo stimulus type. Graph depicts mean number of errors during menses and the luteal phase of the baseline cycle for OC users and NC women.

Angry faces as the 25% nogo stimulus

Box-cox transformation was applied to error of commission scores (failure to inhibit response to angry faces; Lambda=0.2) in order to normalise the data prior to analysis. There were no treatment or phase differences in the number of correct responses to happy or neutral faces, nor were there any differences in error responses to angry faces. For reaction times to neutral faces there was a trend for OC users to respond faster than NC women (F(1,34)=3.29, p=0.078). No differences between groups or phases were found in reaction times to happy faces.

Angry faces as the 25% go stimulus

Box-cox transformation was applied to correct responses to angry faces (Lambda=2) as well as to the number of correct response inhibitions to neutral faces (Lambda=2) in order to normalise the data prior to analysis. There was a significant positive association between BMI and number of correct inhibits to neutral faces ($F(1,29)=4.29$, $p=0.047$, $d=0.77$). No effects of treatment or phase were found on the number of correct responses to angry faces, or for the number or correct inhibitions to neutral or happy faces. There was a trend towards a main effect of treatment on reaction times to angry faces, with OC users responding faster than NC women ($F(1,34)=3.26$, $p=0.08$).

Table 7.13 *Mean (SD) number of responses/inhibits and reaction times to each stimulus type for OC users and NC women during each phase of the baseline cycle.*

Task	OC		NC	
	Menses	Luteal	Menses	Luteal
Up arrow 25% nogo				
Correct go response	208.08 (37.53)	175.40 (75.53)	175.10 (47.21)	184.04 (54.20)
Go reaction time	292.75 (43.25)	283.60 (44.12)	324.76 (77.67)	330.04 (56.76)
Errors of commission	12.92 (8.32)	8.60 (7.28)	9.43 (5.91)	8.08 (7.49)
Up arrow 25% go				
Correct go response	69.08 (12.00)	63.60 (20.31)	64.24 (11.77)	64.83 (14.82)
Go reaction time	370.33 (52.40)	356.70 (46.23)	405.43 (67.47)	402.79 (59.37)
Neutral 25% nogo				
Angry response	94.00 (33.03)	79.78 (32.55)	80.12 (32.97)	80.91 (31.56)
Angry reaction time	400.55 (78.44)	397.22 (74.72)	438.24 (105.73)	429.55 (81.88)
Happy response	95.27 (33.47)	77.11 (33.31)	82.65 (32.73)	79.36 (31.38)
Happy reaction time	394.55 (72.01)	409.56 (99.35)	426.88 (94.71)	425.00 (90.63)
Errors of commission	11.73 (11.65)	6.11 (5.75)	9.82 (7.90)	8.50 (5.95)
Neutral 25% go				
Angry inhibit	111.36 (5.32)	113.00 (4.82)	112.38 (3.84)	111.77 (5.60)
Happy inhibit	112.91 (4.95)	112.89 (3.44)	113.31 (4.66)	110.82 (6.28)
Neutral response	63.18 (15.76)	61.00 (15.47)	55.06 (19.05)	55.50 (15.06)
Neutral reaction time	474.73 (72.32)	478.56 (69.73)	514.88 (73.84)	520.95 (68.10)
Angry 25% nogo				
Happy response	99.82 (28.45)	87.00 (29.80)	87.75 (27.69)	90.83 (27.96)
Happy reaction time	392.91 (70.62)	407.30 (69.84)	433.45 (81.66)	438.39 (81.52)
Neutral response	98.73 (27.97)	88.90 (29.81)	86.35 (30.83)	86.91 (29.70)
Neutral reaction time	384.36 (75.25)	400.60 (66.22)	442.50 (94.71)	441.61 (94.28)
Errors of commission	17.09 (11.23)	14.30 (12.59)	10.90 (8.53)	11.52 (6.85)
Angry 25% go				
Happy inhibit	112.10 (4.68)	112.44 (4.64)	111.80 (7.47)	111.22 (6.20)
Neutral inhibit	113.80 (6.63)	112.67 (5.50)	111.35 (7.46)	112.22 (6.49)
Angry response	72.40 (4.60)	69.89 (6.03)	59.10 (17.67)	63.00 (12.26)
Angry reaction time	453.30 (68.73)	454.11 (66.22)	490.50 (68.07)	491.22 (62.99)

OC, Oral Contraceptive; NC, Normally Cycling

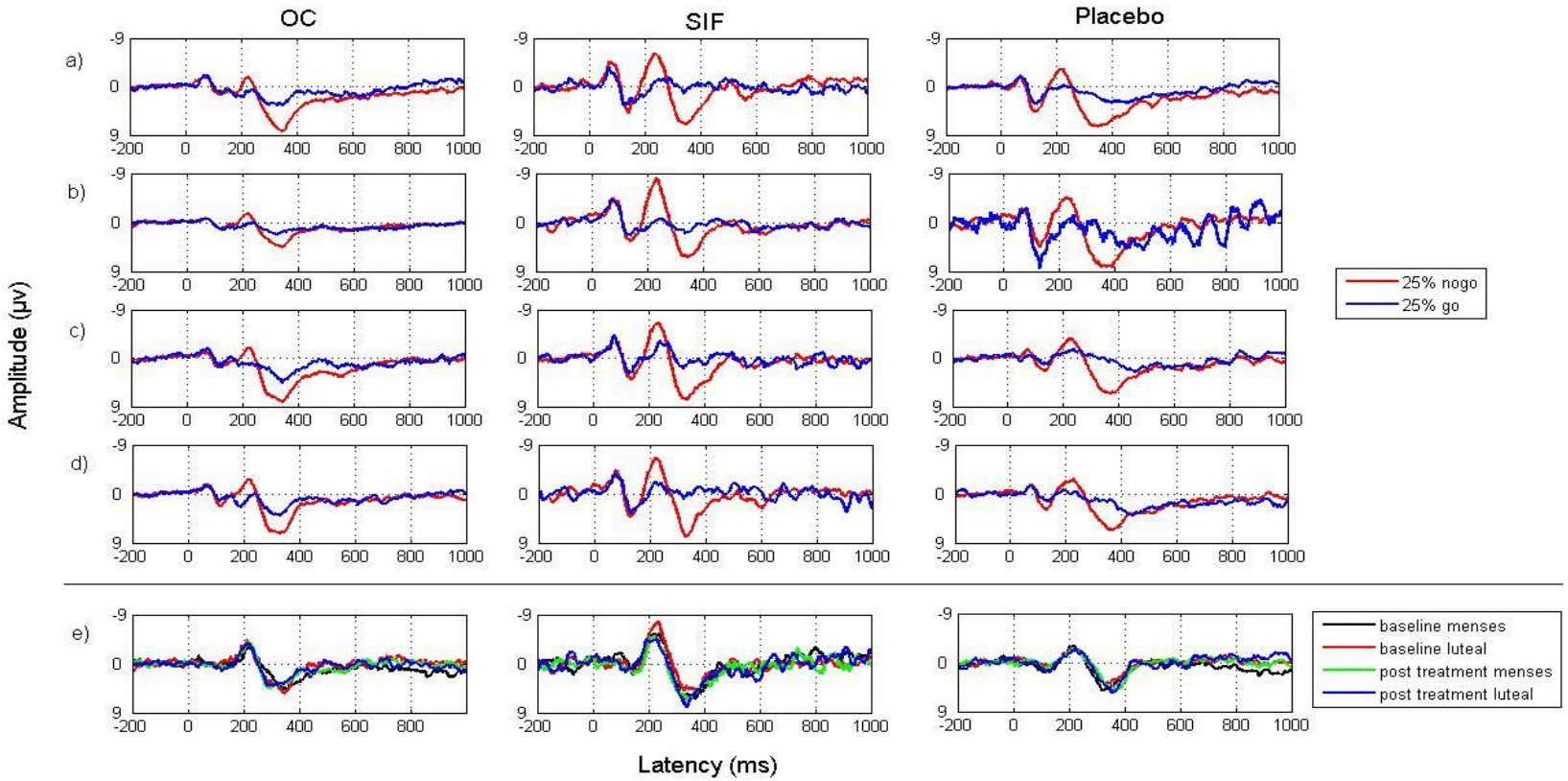

Figure 7.9. ERP waveforms to 25% nogo and 25% go abstract stimuli for OC users (left), women treated with SIF (middle) and women treated with placebo (right). Graphs depict waveforms following nogo (red) and go (blue) stimuli during **a.** menses of the baseline cycle, **b.** the luteal phase of the baseline cycle, **c.** menses of the post treatment cycle, and **d.** the luteal phase of the post treatment cycle. In addition, **e.** depicts nogo minus go difference waves for each cycle phase.

7.9. EEG Measures: Electrophysiological results

7.9.1 Standard go/nogo task

For the sake of brevity, only the nogo minus go differences are reported in this thesis since they were the primary outcomes of interest and provide more information regarding response inhibition than the go or nogo waveforms alone. There was a significant main effect of cycle phase on N2d peak amplitudes, with enhanced N2d peaks in the luteal phase compared with during menses ($F(1,34)=5.20$, $p=0.029$, $d=0.78$). P3d mean amplitudes showed a trend towards a main effect of phase with greater P3d mean amplitudes during menses than the luteal phase ($F(1,34)=3.24$, $p=0.081$). A significant treatment x phase interaction was found for P3d latency ($F(1,33)=4.76$, $p=0.036$), with pairwise comparisons revealing that NC women had longer P3d latency than OC users during the luteal phase ($t=2.70$, $p=0.009$, $d=1.05$). There was also a trend towards an overall main effect of treatment on P3d latency, with NC women having longer P3d latency than OC users ($F(1,36)=3.52$, $p=0.069$).

7.9.2. Go/nogo task with face emotion stimuli

Neutral faces as the 25% stimulus

There was a significant main effect of treatment on N2d peak amplitude with OC users having larger N2d than NC women ($F(1,36)=4.50$, $p=0.041$, $d=0.77$). The linear mixed model with random intercept for N2d mean amplitude did not converge, therefore a mixed marginal model was run on this data which revealed a trend towards a main effect of treatment with OC users having larger N2d mean amplitude than NC women ($F(1,25)=3.44$, $p=0.075$). There was also a significant main effect of treatment on P3d mean amplitude, with NC women having larger P3d mean amplitude than OC users ($F(1,29)=4.33$, $p=0.047$, $d=0.76$).

Angry faces as the 25% stimulus

There was a significant treatment x phase interaction for N2d mean amplitude ($F(1,30)=4.89$, $p=0.035$), although pairwise comparisons of estimated marginal means revealed only a trend for NC women to have larger N2d mean amplitude than OC users in the luteal phase ($t=1.77$, $p=0.083$) as well as a trend towards an effect of phase for only the OC group where N2d mean amplitude was larger during menses than the luteal phase ($t=1.72$, $p=0.096$). For N2d latency there was a trend towards a main effect of treatment with NC women having longer N2d latency than OC users ($F(1,30)=3.44$, $p=0.073$).

There was also a significant treatment x phase interaction for P3d peak amplitude ($F(1,31)=5.41$, $p=0.027$) with pairwise comparisons revealing larger P3d peak amplitude for OC users than NC women in the luteal phase ($t=2.07$, $p=0.043$, $d=0.85$) as well as significant phase effects for only the OC group where differences were larger during the luteal phase than menses ($t=2.12$, $p=0.042$, $d=1.28$). Years of education also contributed significantly to the model with more years of education being associated with reduced P3d peak amplitude ($F(1,31)=11.91$, $p=0.002$, $d=1.24$). For P3d latency there was a trend towards a main effect of treatment ($F(1,34)=4.04$, $p=0.052$) with NC women having longer P3d latency than OC users.

Table 7.14 *Mean (SD) ERP values for N2 and P3 nogo minus go difference wave peak amplitudes, mean amplitudes and peak latencies for OC users and NC women during each phase of the baseline cycle.*

Task	OC		NC	
	Menses	Luteal	Menses	Luteal
Standard go/nogo				
N2 peak amplitude	-4.40 (3.07)	-6.16 (2.24)	-4.42 (1.90)	-4.87 (1.50)
N2 mean amplitude	0.47 (1.98)	0.63 (2.67)	0.81 (2.19)	-0.50 (1.92)
N2 peak latency	226.45 (23.23)	207.30 (29.68)	217.95 (29.00)	222.33 (31.58)
P3 peak amplitude	7.75 (2.41)	8.96 (3.21)	7.26 (4.30)	6.32 (3.44)
P3 mean amplitude	2.28 (2.38)	1.75 (2.36)	1.76 (2.35)	0.78 (1.83)
P3 peak latency	362.55 (57.34)	336.10 (43.74)	384.81 (82.34)	425.71 (98.82)
Neutral go/nogo				
N2 peak amplitude	-4.41 (2.71)	-5.46 (3.74)	-3.51 (2.57)	-2.72 (1.69)
N2 mean amplitude	0.02 (2.22)	-1.75 (2.48)	-0.04 (1.95)	0.05 (1.26)
N2 peak latency	243.18 (69.72)	231.75 (43.50)	237.24 (233.36)	233.36 (56.97)
P3 peak amplitude	6.38 (4.37)	6.94 (4.23)	5.82 (3.08)	5.44 (2.25)
P3 mean amplitude	0.47 (2.73)	-0.16 (2.32)	1.47 (2.35)	1.39 (1.50)
P3 peak latency	415.45 (120.04)	415.75 (109.97)	424.47 (86.18)	444.64 (86.75)
Angry go/nogo				
N2 peak amplitude	-3.70 (1.88)	-3.89 (2.25)	-3.88 (2.06)	-4.05 (2.44)
N2 mean amplitude	-0.74 (2.93)	0.33 (1.38)	-0.21 (1.64)	0.82 (1.39)
N2 peak latency	231.00 (42.90)	218.22 (38.99)	258.70 (48.84)	239.48 (56.85)
P3 peak amplitude	6.10 (2.79)	8.52 (3.85)	6.68 (4.15)	5.96 (3.05)
P3 mean amplitude	1.21 (2.78)	2.51 (2.08)	1.59 (2.65)	1.36 (1.64)
P3 peak latency	421.10 (77.20)	442.78 (78.59)	476.50 (83.85)	473.96 (61.39)

OC, Oral Contraceptive; NC, Normally Cycling

Chapter 8

Discussion of Baseline Effects of Menstrual Cycle and Oral Contraceptive use

In the current study a variety of measures were used to assess the effects of menstrual cycle phase on mood, aggression, cognitive function and brain electrical activity, as well as to explore the relationship between oral contraceptive (OC) use and these outcomes. Whilst some of the findings complement the existing literature, others are divergent from the broader literature as discussed in sections 8.1 and 8.2. Importantly, as hypothesised ratings of mood and premenstrual symptoms were higher in normally cycling (NC) women than OC users at baseline, and were more severe during menses than any other phase. Menses was also characterised by more impulsive responding in the go/nogo task than the luteal phase, although this effect was found only for OC users. NC women reported higher levels of aggression than OC users on all self-report measures, and OC users demonstrated enhanced performance on several cognitive tasks compared with NC women, as well as responding faster in the go/nogo task during the luteal phase.

The findings regarding the electrophysiological effects of steroid hormones on the N2 and P3 components were somewhat surprising. Whilst these components are both thought to reflect response inhibition processes (e.g. Pfefferbaum et al., 1985; Smith et al., 2006) they were differentially affected by menstrual cycle phase. In addition, the effects of menstrual cycle phase on these components depended on stimulus type, with no effects observed for neutral faces, whilst the N2 and P3 to angry faces showed the opposite effects of hormonal status than the N2 and P3 to abstract stimuli. The effects of OC use on these components were largely constrained to the luteal phase, and were also dependent on the emotional valence of the stimulus.

8.1. Baseline menstrual cycle effects

8.1.1. Changes in mood across the menstrual cycle

In the current study mood was found to be significantly poorer during menses than all other phases of the cycle, including the luteal phase for most outcomes. This was true for measures of depression, impulsivity, global mood and mental fatigue as well as the experience of traits related to schizotypal personality disorder. Interestingly, for some of the measures such as non-planning impulsiveness these phase effects were significant only for OC users, a finding which complements previous research suggesting negative

mood during the hormone free interval (Walker & Bancroft, 1990). Only the tension-anxiety subscale of the POMS showed the expected phase effects of higher scores in both the luteal phase and menses in NC women, suggesting that for women who are not seeking help for premenstrual syndrome most aspects of mood are poorer during menses and are not worsened in the luteal phase as is the case in women suffering from PMS/PMDD. This supports the findings of Meaden, Hartlage and Cook-Carr (2005) who found that symptom severity peaks during menses, as well as Romans et al. (2012) who reported that there is little evidence for premenstrual worsening of mood in non-help-seeking women.

The finding of poorer mood during menses supports previous research showing that reduced levels of female sex hormones are associated with more negative mood (e.g. Young, Midgley, Carlson, & Brown, 2000). Moreover, since these effects were only seen when both estrogen and progesterone levels were low, and not during the late follicular and ovulatory phases when estrogen levels were high but progesterone was low this suggests a more central role of estrogen in the promotion of positive mood than progesterone. An alternative explanation is that the poorer mood during menses reflects a delayed effect of the increased progesterone levels during the luteal phase, as has been previously suggested (Redei, 1995; Schmidt, Nieman, Danaceau, Adams, & Rubinow, 1998).

On the other hand, total ratings of premenstrual symptoms assessed using the DSR were most severe during menses but were also more severe during the luteal phase than the "rest" phase. This appeared to be driven by ratings of physical rather than psychological symptoms since only scores on the physical factor were significantly higher during the luteal phase than the "rest" phase, although scores on the psychological factor were also higher during the luteal phase but this effect did not reach statistical significance. This may be due to the higher scores in the luteal phase being only apparent for NC women and not OC users, whereas scores for both groups were higher during menses. The finding of poorer mood in the luteal phase on this measure, although not significant, may differ from the lack of this finding on other measures in this study due to the slightly different definition of phases for this measure, which included the late luteal phase, compared to the other measures which included the midluteal phase. Taken together these findings not only suggest that in non-help-seeking women mood and

psychological symptoms are not significantly more severe during the luteal phase, but also refutes the "physical distress" hypothesis (Kiesner & Pastore, 2010) as mood was not affected by increased physical symptoms during the luteal phase.

The mechanisms underlying these effects can only be speculated upon, particularly since no reliable measures of circulating steroid hormones were available from this study. Although saliva samples were collected, analysis revealed very large variability in estradiol concentration, with many samples falling outside the normal range (see section 5.3.5.1). However, based on previous literature it could be suggested that the interaction between estrogen and the serotonergic system may explain these findings. Estrogen is known to increase serotonergic function (Bethea, Lu, Gundlah, & Streicher, 2002; Rubinow, Schmidt, & Roca, 1998), and since increased serotonin is associated with elevated mood (e.g. Hamon & Blier, 2013) it follows that during periods of low estrogen the reduction in serotonin would result in poorer mood. This is in line with several studies that have shown lowered serotonin and poorer mood when estrogen is reduced (Amin, Canli, & Epperson, 2005).

8.1.2 Changes in aggression across the menstrual cycle

In accordance with the findings of poorer mood during menses, aggression was also found to be increased during menses compared with the other phases of the cycle. For self-ratings on the BPAQ only physical aggression did not show any cyclical variation. Interestingly, verbal aggression did not show the expected increase during the luteal phase but was actually also highest during menses, when levels of estrogen and progesterone are at their nadir. However, the lack of significant increase during the luteal phase is likely due to the reduction in aggression in OC users during this phase, whereas a slight increase was seen in NC women although this was not significant. The increased ratings by NC women in the luteal phase were not seen for any other subscale, suggesting that only verbal aggression was increased during this phase. Some aspects of anger such as trait anger and angry reactions measured using the STAXI were also rated as more severe during menses, although overall anger expression did not show variations with phase.

These findings are partially in accordance with those of Ritter (2003) who found a trend for verbal aggression to be increased during menses compared with the midluteal phase. However, in that study, a significant increase in physical aggression was also observed during menses, an effect that was not replicated in the current study. The findings are also in contrast to those of Brambilla, Speca, Pacchiarotti, and Biondi (2010) who reported a positive association between estrogen and verbal aggression, which suggests that verbal aggression should be highest when estrogen levels are highest, rather than during menses when estrogen levels are lowest.

For the objective measure of aggression using the Stroop task responses were slowest during menses compared with all other phases. Whilst this could reflect an increased salience of the stimuli during this phase it is unlikely that this is the case. Firstly, response times to control stimuli were also slowest during this phase, suggesting an overall slowing of responses during menses rather than an effect of the salience of the stimuli. Secondly, since the order of testing across phases was not counterbalanced and all participants were first tested during menses it is more likely that this finding reflects a practice effect.

As with the cyclical variations in mood and other premenstrual symptoms, the most likely explanation for the finding of increased aggression during menses may be modulation of the serotonergic system by steroid hormones. Reduced serotonergic function has been shown to increase aggression in both men and women (Bond, Wingrove, & Critchlow, 2001; Cleare & Bond, 1995). However, it has been suggested that this relationship is only evident for impulsive aggression, whereas traits such as hostility and anger may actually show the opposite relationship with serotonin (Suarez & Krishnan, 2006). This would suggest that hostility, anger and possibly verbal aggression should be higher during the luteal phase when steroid hormones, and therefore serotonin, are increased.

An alternative explanation is that oxytocin is reduced in times of lowered estrogen. ERβ are expressed in oxytocinergic cells, and projections to the amygdala from these cells are known to be important for social interactions, including reduced aggression (Mong et al., 2003). Therefore when estrogen levels are low and activation of ERβ is reduced, oxytocinergic activity is also reduced, suggesting a possible mechanism by which aggression is increased during periods of low circulating estradiol.

8.1.3 Changes in cognitive function across the menstrual cycle

The menses phase was also characterised by poorer cognitive performance on several of the tasks used in this study. Reaction times in the majority of tasks were slower during menses which may again reflect practice effects rather than a genuine effect of hormone status. However, reaction time as measured using the simple reaction time, choice reaction time and four choice reaction time tasks was not affected by menstrual cycle phase. In addition, for some of the tasks such as card sorting and serial 3 subtractions these phase effects were evident only in NC women, suggesting that for these tasks at least an effect of hormone status on reaction time cannot be ruled out. It is therefore possible that more effort was required during menses when levels of both estrogen and progesterone were low as reflected by increased reaction times. These findings are in accordance with those of Wuttke et al. (1975) and Simic and Ravlic (2013) who found that reaction times were faster during periods of higher estrogen and progesterone levels.

Performance in terms of accuracy or the number of attempted items in tasks relying on frontal lobe function was poorer during menses and the follicular phase, periods characterised by lower levels of circulating steroid hormones. Attention measured by the RVIP task was also improved during the ovulatory and luteal phase, which is in line with previous evidence showing improved sustained attention during the luteal phase (Solis-Ortiz & Corsi-Cabrera, 2008). However, delayed picture recognition was enhanced during menses compared with the luteal phase, a finding that contrasts with Phillips and Sherwin (1992) who found picture recall to be more accurate during the luteal phase. This finding demonstrates that not all outcomes were improved in the later phases of the cycle, suggesting that positive effects on other outcomes in the luteal phase may be genuine effects of hormonal status rather than practice effects.

Interestingly, performance on the reversed corsi blocks task was found to be poorer during menses for only the OC users, suggesting a detriment to spatial working memory during the hormone free interval. This may be due to the slight increase in endogenous steroid hormones during the inactive pill phase, as spatial ability has been shown to be poorer during times of increased female sex hormones (Hampson, 1990a; Hausmann et al., 2000). It is surprising that no phase effects were found for NC women given that previous studies have demonstrated better performance on tasks of spatial abilities

during menses (Hampson, 1990a; Hausmann et al., 2000). However, this may be due to practice effects, and significant findings may have been observed had the order of testing been counterbalanced. Alternatively, since several other studies have also demonstrated no cyclical variation in spatial abilities in healthy young women (Gordon & Lee, 2003; Hampson, 1990b; Rosenberg & Park, 2002) it is possible that the lack of significant findings is due to ceiling effects since the participants in this study were cognitively healthy.

The improved performance on tasks of attention during phases characterised by higher estrogen levels may be due to increased activity of the cholinergic system. This system is thought to be of particular importance for attention (Gais & Born, 2004), and estrogen has been shown to increase cholinergic activity (Kaufman, Vadasz, & Lajtha, 1988). Indeed, administration of estrogen has been shown to protect against drug-induced anti-cholinergic impairments in tasks of attention (Dumas et al., 2006).

The neuroprotective and antioxidant effects of estrogen may also help to explain the finding of improved cognition during the luteal phase. Estrogen has been shown to protect hippocampal neurons from the damage associated with oxidative stress (Behl, Widmann, Trapp, & Holsboer, 1995) as well as increasing dendritic spine density in hippocampal CA1 pyramidal cells (e.g. Woolley & McEwen, 1993). Working memory functions are particularly dependent on the hippocampus, especially spatial working memory. Although no effects of menstrual cycle phase were found for accuracy on many of the working memory tasks in this study, more cognitively demanding measures of working memory such as serial subtractions did show cyclical variation in the number of subtractions attempted. It has previously been suggested that the effects of estrogen may only be seen in more cognitively demanding tasks that rely on frontal functions (Dumas, Kutz, Naylor, Johnson, & Newhouse, 2010) which may explain why significant improvements in the luteal phase were found only for the more difficult tasks in this study.

8.1.4 Changes in inhibition and associated brain electrical activity across the menstrual cycle

8.1.4.1 Behavioural findings

The behavioural data from the current study indicate that more errors of commission to infrequent stimuli were made during menses compared with the luteal phase for both the standard go/nogo task using abstract stimuli, as well as the go/nogo task using neutral face stimuli. This may reflect poorer response inhibition during menses when steroid hormone levels are low, however there were also more correct responses to go stimuli during this phase, suggesting that perhaps this effect was due to an greater response prepotency during menses. It should also be noted that for the standard go/nogo task this effect was evident only for OC users, whereas when neutral faces were the infrequent stimulus type the phase effect was also apparent in NC women. Interestingly, no significant phase effects were found for angry faces in either group.

In contrast to reports from several other authors who have found increased impulsivity during the luteal phase (e.g. Canning et al., 2012; Hsu et al., 2007), women in the current study appear to be more impulsive in their responding during menses compared with the luteal phase. Nevertheless, the greater response prepotency and increased number of errors of commission during menses compared with the luteal phase is consistent with findings of higher self-ratings of both impulsivity and aggression during menses than any other phase.

The findings from this experiment suggest that low levels of circulating steroid hormones may be associated with an increased response prepotency to certain stimuli, and in particular the hormone-free interval in OC users may be characterised by increased responding. In addition, the finding that these phase effects differed according to stimulus type provides support for the notion that cyclical variations in inhibition performance depend on the emotional salience of the stimulus (Aron & Poldrack, 2006). However, contrary to the hypothesis that response inhibition would only be affected by cycle phase when discrimination between emotional stimuli was necessary, inhibition of responses to angry faces did not vary according to hormonal status. Previous studies have shown that perception of negative emotions is more accurate during the follicular phase compared with the luteal phase (Conway et al., 2007; Derntl, Kryspin-Exner, et al., 2008; Guapo et al., 2009), which would suggest that performance should be poorer

during the luteal phase when angry faces were the target stimulus. However, since women in the current study did not exhibit greater aggression or impulsivity during the luteal phase this may explain the lack of impairment in processing negative emotions during this phase.

8.1.4.2 Effects on ERP components

It is important to note that the inhibition-related ERP components peaked at approximately the same time as has been found in other studies (N2: 200-300ms, P3: 250-500ms), and that nogo stimuli consistently elicited larger N2s and P3s than go stimuli. Regarding the effects of menstrual cycle phase on inhibition-related ERP components, whilst the difference between N2 peak amplitudes to successful nogo and go trials was larger during the luteal phase, P3 mean amplitude differences were larger during menses. These effects were seen only for the standard go/nogo task. No significant effects of phase were found when neutral faces were the target stimulus, however for angry faces the opposite pattern to the standard go/nogo task was found with larger N2 mean amplitude differences between nogo and go conditions during menses and larger P3 peak amplitude differences during the luteal phase. These effects when angry faces were the target stimuli were seen only in OC users, with NC women showing no phase effects in electrophysiological responses to either neutral or angry face stimuli. These findings do not support the hypothesis that N2 and P3 would both be reduced during the luteal phase and would only vary with phase for emotional face stimuli.

The finding that the N2 and P3 components were differentially affected by menstrual cycle phase provides support for previous theories that have suggested that these components may reflect processes related to response inhibition, but do not reflect response inhibition *per se* (e.g. Botvinick et al., 2004; Bruin, Wijers, & Van Staveren, 2001). The finding that the N2 to abstract nogo stimuli was attenuated during menses is consistent with the behavioural findings of increased errors of commission during this phase, suggesting that this component may indeed reflect response inhibition processes (Pfefferbaum et al., 1985). However, the lack of effects of menstrual cycle phase on N2 amplitude to neutral faces, even though more errors were made during menses, calls this

interpretation into question. Since nogo stimuli consistently elicited more negative N2 peak amplitudes than go stimuli, and since these stimuli were presented with equal probability, this refutes the theory that the enhanced N2 to nogo stimuli represents a response to novel stimuli (Dimoska & Johnston, 2008; Folstein et al., 2008). The theory that the N2 in standard go/nogo tasks reflects a response conflict monitoring mechanism is therefore more plausible in the context of the current findings (Botvinick et al., 2004).

Due to the lack of effect of menstrual cycle phase on either of the ERP components in NC women, this suggests that the behavioural findings of increased errors of commission during menses in this group may have been due to an overall increase in responding during this phase rather than impairment in response inhibition. In OC users, the findings suggest that during the pill withdrawal phase when levels of endogenous hormones are rising but synthetic hormone levels are reduced, N2 is attenuated in the standard go/nogo task which is in line with the findings of poorer performance during this phase. However, as with the NC group this may be due to an overall increase in responding. The reduced N2 may therefore reflect a greater effort in withholding a response rather than impaired inhibition performance. Previous studies have not investigated cyclical changes in the N2 in OC users, however as in the current study, Walpurger et al. (2004) reported no changes in N2 amplitude across the cycle in NC women. In that study N2 latency did vary with cycle phase in NC women, a finding which was not replicated in the current study.

Conversely, P3 mean amplitude in the standard go/nogo task was attenuated during the luteal phase in OC users only. This contrasts with the findings of Kluck et al. (1992) and Sun et al. (2012) who both reported increased P3 amplitude during the luteal phase, however those studies examined the effects in NC women and not OC users. Since OCs are known to lower levels of endogenous hormones (Griksiene & Ruksenas, 2011) this may explain the discrepant findings. On the other hand, since mean amplitude but not peak amplitude was reduced in the luteal phase this finding may rather be due to the time window chosen for computing mean amplitude. The findings of the current study are consistent with other studies which have also reported no effects of menstrual cycle phase on the P3 to non-emotional stimuli in NC women (Fleck & Polich, 1988; Johnston & Wang, 1991).

Both the N2 and P3 are thought to be modulated by dopamine levels, with lowered dopamine levels being associated with reduced N2 and P3 amplitudes (Beste et al., 2010; Polich & Criado, 2006). However, OCs are known to reduce dopaminergic activity (Arangino et al., 1998; Moeller, 1981), which may explain the reduced P3 during the luteal phase but also suggests that other mechanisms may underlie the enhanced N2 during the active pill phase in the standard go/nogo task.

On the other hand, the N2 and P3 components were affected in the opposite direction when angry faces were the infrequent stimulus type, despite no behavioural differences in either accuracy or reaction time between phases. The enhanced N2 to nogo angry faces during menses suggests an improvement in the processing of these stimuli, whereas the enhanced P3 during the luteal phase may reflect improved attention during this phase as the P3 has been suggested as an index of attention shifting (Friedman, Cycowicz, & Gaeta, 2001; Polich, 2003). The different effects of menstrual cycle phase on these components to different stimulus type are consistent with the notion that these effects depend on the emotional salience of the stimulus (Johnston & Wang, 1991). Since aggressive individuals show an attentional bias towards angry faces (van Honk et al., 2001), this suggests that the enhanced P3 during the luteal phase may reflect increased aggression during this phase. The reduced N2 during the luteal phase compared with menses provides further support for this idea, as aggression is associated with reduced N2 amplitude (Albrecht et al., 2005; Chen et al., 2005). However, the findings from this study showed that self-reported aggression in this sample was actually higher during menses, suggesting that factors other than aggression may be involved in the increased salience of angry stimuli during the active pill phase in OC users.

Whilst the nogo N2 in standard go/nogo tasks is thought to be generated by the anterior mid-cingulate cortex (MCC; Huster et al., 2010; Nieuwenhuis et al., 2003), the fronto-central N2 to facial emotions is thought to reflect emotion processing through projections from the amygdala (Kiss & Eimer, 2008). These different neural underpinnings provide a potential explanation for the divergent effects of menstrual cycle phase on the N2 between abstract and angry stimuli. It is plausible that the P3 to emotional stimuli may similarly be generated by regions other than the MCC, although the low spatial resolution of ERPs means that these neural correlates cannot be

examined in the current study. Future research could utilise techniques such as fMRI to investigate this further.

8.2 Baseline effects of oral contraceptive use

8.2.1 Effects of OC use on mood and premenstrual symptoms

As expected, NC women had significantly poorer mood than OC users as indexed by higher depression, impulsivity, total mood disturbance, trait anxiety and stress ratings as well as higher scores on many of the subscales of the questionnaires. In addition, NC women had higher ratings on the schizotypal personality questionnaire than OC users, including most of the subscales. These findings differ from those of other studies that have reported increased depression and irritability with OC use (Grinspoon et al., 2003; Kulkarni, Liew, & Garland, 2005; Oddens, 1999), however they do support the findings of several other trials that have found improved mood with the use of monophasic OCs (Greco et al., 2007; Mordecai, Rubin, & Maki, 2008; Ott, Shew, Ofner, Tu, & Fortenberry, 2008; Toffol, Heikinheimo, Koponen, Luoto, & Partonen, 2012). The strength of the current study lies in the use of prospective rather than retrospective measurements of mood across all phases of the menstrual cycle, as well as the inclusion of only women using monophasic preparations. Many of the previous studies showing negative effects of OC use on mood included triphasic preparations, as well as older generation, more androgenic OCs. Although OCs of different generations were used in the current study, only combined, monophasic preparations were included which may explain the positive effects. Several of the previous trials mentioned also used retrospective measures of mood, which are known to be far less accurate than prospective measures (Bryant et al., 2005) and are subject to recall bias, which is another possible reason for the discrepancies in findings.

Interestingly, few interactions with menstrual cycle phase were found and where there were interactions these were not due to group differences in the luteal phase as was expected. This is due to the finding that NC women did not experience more severe negative mood during the luteal phase as has been found in other studies (e.g. Reed, Levin, & Evans, 2008; Seippel & Backstrom, 1998) but rather had poorer mood during menses. OC users also demonstrated this pattern of more severe mood during menses

(the inactive pill phase) which was expected based on the findings of previous studies (Freeman et al., 2012; Sulak, Willis, Kuehl, Coffee, & Clark, 2007) and supports the finding that monophasic OCs stabilise mood during the active pill phase but may result in poorer mood outcomes during the pill withdrawal phase (Walker & Bancroft, 1990).

Daily ratings of premenstrual symptoms on the DSR were also found to be higher in NC women than OC users. This supports the findings of our previous research (Perry, Canning, Scholey, & Dye, under review), although no significant interaction with menstrual cycle phase was found for psychological symptoms in the current study, whereas in the previous study there was a larger difference between groups during the luteal phase than any other phase. However, total DSR scores in the current study differed significantly between groups during menses and the luteal phase where NC women had more severe symptoms, which is in line with the notion that naturally cycling women experience the most severe symptoms during both the luteal phase and menses (Meaden, Hartlage, & Cook-Carr, 2005; Romans, 2012). In fact, whereas both groups in the current study showed an increase in symptoms during menses, in the luteal phase when symptom severity increased for NC women, for OC users symptom severity was actually reduced. This may be due to a steady decline in symptoms with the increased number of days of OC use, and is in line with research suggesting that continuous OC use results in more positive outcomes (Freeman et al., 2012).

As discussed in chapter 3.5.1 it is unlikely that the positive effects of OCs on mood and premenstrual symptoms reflect the suppression of endogenous hormones as levels of endogenous hormones including testosterone are positively correlated with wellbeing in women (Bell, Donath, Davison, & Davis, 2006; Cawood & Bancroft, 1996). The effects on neurotransmitters provide a more plausible explanation. Synthetic estrogens such as EE which is the estrogen used in OCs have been shown to increase serotonergic function by increasing the number of serotonin receptors, increasing serotonin synthesis and reducing monoamine oxidase (MAO) activity (Halbreich & Kahn, 2001). As already discussed, increased serotonergic function is associated with improved mood. Effects of OC use on GABA may also explain the reduction in premenstrual symptoms in this group, since women suffering from PMS/PMDD have reduced GABA during the luteal phase suggesting a role of this neurotransmitter in the symptoms of PMS (Rapkin & Akopians, 2012). EE has been shown to upregulate GABA receptors in the cerebral

cortex (Follesa et al., 2002), and the metabolites of progestins used in OCs also have GABA receptor effects (Andreen et al., 2009).

8.2.2 Effects of OC use on aggression

OC users reported being less aggressive than NC women as demonstrated by their higher total scores on the BPAQ, although this effect just missed statistical significance. In contrast to expectations, only the subscale of physical aggression showed a trend towards a treatment effect with NC women being more physically aggressive than OC users. However, several measures of anger on the STAXI were also found to be higher in NC women than OC users, suggesting that this component of aggression may also be affected by OC status. Similarly, both anger and aggression items on the DSR showed treatment effects with higher ratings by NC women than OC users.

Also in contrast to our previous research no difference was found between OC users and NC women in response times to aggressive stimuli in the emotional stroop task. Several explanations for this lack of group difference are plausible. Firstly, in the previous study NC women were most aggressive during the luteal (premenstrual) phase and group differences were found only during this phase rather than an overall slowing of responses across phases. In the current study, since NC women were more aggressive during menses than any other phase, and OC users were also more aggressive during this phase, this could explain the lack of group differences. Secondly, in the previous study the premenstrual phase was defined as the six days prior to menstruation whereas in the current study a slightly different window of days 20-26 was used to define the luteal phase in order to capture maximal hormone differences across the cycle. Finally, the stroop task used in the current study relied on button-press responses to stimuli rather than a voice-key activated response system as was used in the previous study, which introduces more motor response interference and may not be as sensitive to small changes in reaction time as the voice-key system.

It is unlikely that the reduced aggression in OC users on self-report measures is due to reductions in androgens since the most commonly used OCs by women in this study were second generation OCs, which contain progestins that are derived from testosterone and therefore may have androgenic effects (McFadden, 2000). As with the

effects on mood, the modulation of neurotransmitters by EE is a more compelling explanation. In particular the upregulation of serotonin by OCs may explain the lower aggression ratings in this group, since increased serotonin is associated with lower aggression levels (Bond, Wingrove, & Critchlow, 2001; Cleare & Bond, 1995).

8.2.3 Effects of OC use on cognition

OC use was found to be associated with improved performance of several cognitive tasks. In particular, reaction times in many of the tasks, accuracy on the RVIP task, number of serial 3 subtractions attempted, accuracy of delayed word recall and accuracy on the Nback task were all improved in OC users compared with NC women, whereas NC women did not outperform OC users on any of the tasks. In addition, reaction times on the card sorting task and serial 3 subtractions were found to be slower during menses for NC women only, whereas accuracy on the reversed corsi blocks task was poorer during menses (inactive pill phase) for OC users only.

The slightly superior performance of OC users on verbal memory as assessed with delayed word recall is in line with previous studies which have shown improved performance on tasks of verbal memory during the active pill phase (Gogos, 2013; Mordecai et al., 2008), although in the current study this effect did not reach statistical significance. The lack of effect on immediate word recall may be due to task difficulty, with effects only being evident in more difficult task conditions. Contrary to expectations there was no significant difference in performance between the active and inactive pill phases as has been demonstrated in previous studies (Mordecai et al., 2008). However, that study utilised a different task of verbal memory, the California Verbal Learning Test (CVLT), where auditory rather than visual stimuli are presented. This suggests that the mode of stimulus presentation may affect how well stimuli are recalled.

The enhanced performance on tasks measuring attention, such as the RVIP task and serial 3 subtractions, supports previous findings showing better attention in OC users (Gogos, 2013). The lack of cyclical variation in reaction times in tasks that did show cyclical variations in NC women also supports previous research showing that OC use stabilises cognitive performance (Rosenberg & Park, 2002).

The positive effects of OC use on cognitive performance in this study may be explained by increases in acetylcholine release, which have been found to occur following administration of synthetic estrogens (Gibbs, Hashash, & Johnson, 1997). Muscarinic receptor density was also found to increase with estrogen therapy (Norbury et al., 2007) and administration of synthetic estrogens protected against cognitive impairment following cholinergic challenges (Dumas et al., 2008). The cholinergic system is known to be crucial for cognitive function, particularly learning and attention (Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Gais & Born, 2004; Newhouse, Potter, Kelton, & Corwin, 2001). More specifically, synthetic estrogen administration was found to protect only tasks involving attention, psychomotor function and speed (Dumas et al., 2008), which may explain the improved performance on tasks of attention in the current study as well as the faster reaction times in many of the tasks with OC use.

A second explanation for the superior cognitive function in OC users may be the reduction in cortisol response by OCs (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Cortisol impairs delayed verbal recall in NC women but not OC users (Kuhlmann & Wolf, 2005), which may explain why delayed word recall was enhanced in OC users in the current study.

The findings of differences in mood, premenstrual symptoms, aggression and cognitive function between OC users and NC women, as well as the cyclical variations found in these outcomes in the current study suggest that the measures used are sensitive to the influence of female sex hormones and are appropriate in the investigation of the effects of phytoestrogens such as SIF. The effects of SIF on outcomes in the current study will be explored in the following section.

8.2.4 Effects of OC use on inhibition and associated brain electrical activity

8.2.4.1 Behavioural findings

The findings from this trial suggest an overall reduction in reaction time with OC use, as correct responses to go stimuli and error responses to nogo stimuli on the standard and emotional go/nogo tasks were faster for OC users than NC women. For frequent go stimuli on the standard go/nogo task, this effect was significant only during the luteal

phase and was not at the cost of poorer accuracy with no difference between groups in the number of correct responses or errors of commission during this phase. OC users did make more errors during menses on the standard go/nogo task however they also had more correct responses to go stimuli than NC women.

These findings are consistent with findings from the cognitive aspect of the study where OC users demonstrated faster reaction times on a number of cognitive tasks. The lack of difference between groups in the number of errors of commission does not support the hypothesis that OC users would demonstrate greater response inhibition than NC women. However, although OC users rated themselves as less impulsive overall than NC women, no significant differences between groups were found for motor impulsiveness, which is in line with the lack of group differences in accuracy in the current study. The enhanced performance in terms of reaction time in OC users does not appear to be dependent on stimulus type, since all stimulus categories showed the same effects in the current study. This also refutes the hypothesis that differences would be observed only for emotional stimuli.

Administration of synthetic estrogens has previously been shown to be protective against insult to the cholinergic system in tasks of psychomotor function and speed (Dumas et al., 2008). In addition, acetylcholine release and muscarinic receptor density have been shown to increase following estrogen therapy (Gibbs et al., 1997; Norbury et al., 2007). This suggests that a plausible mechanism through which OC use speeds reaction times may be via the actions of the synthetic estrogens contained in OCs on the cholinergic system.

8.2.4.2 Effects on ERP components

Some interesting findings were observed with regard to the effects of OC use on inhibition-related ERP components. Contrary to the hypothesis that nogo stimuli would elicit enhanced N2 and P3 in OC users compared to NC women, these effects were not found for all stimulus types. For the standard go/nogo task, only the difference in P3 latency between nogo and go conditions was affected by OC use with OC users having shorter P3 latency than NC women, although this effect only reached statistical significance during the luteal phase. For neutral face stimuli this effect on P3 latency

was not evident, however OC users had a larger difference between nogo and go conditions in N2 peak amplitude, as well as a trend towards larger N2 mean amplitude. On the other hand, NC women had a larger difference in P3 mean amplitude to neutral faces. The effects of OC use on ERP responses to angry faces were different than those to neutral faces, with NC women having a larger nogo minus go difference in N2 mean amplitude, although this effect did not reach statistical significance and was only apparent during the luteal phase. NC women also had a longer nogo minus go latency for both the N2 and P3 components to angry faces, whereas OC users had a larger difference in P3 peak amplitude during the luteal phase only.

The finding that OC use was associated with shorter P3 latency during the luteal phase suggests that reductions in female sex hormones may result in shorter P3 latency. OCs are known to reduce levels of endogenous hormones (Griksiene & Ruksenas, 2011), and the lack of difference between groups during menses, when endogenous hormone levels are low in NC women, suggests that it is the increase in circulating hormones during the luteal phase that slows P3 latency to abstract stimuli. P3 latency is thought to reflect processing speed (Polich, 1996), therefore it could be postulated that the longer latency during the luteal phase in NC women reflects a slowing of processing speed when circulating steroid hormone levels are high. These findings are also consistent with the faster reaction times observed in OC users compared with NC women.

The larger N2 to neutral stimuli with OC use suggests enhanced processing and inhibition of neutral face stimuli in this group. Since no behavioural differences were found between groups in their accuracy of response inhibition, the enhanced N2 may reflect an increased ease of inhibition with OC use, which is in line with the findings of faster reaction times in this group. The reduced N2 in NC women compared to OC users supports the findings of previous studies that have shown attenuated N2 amplitude in aggressive individuals (Albrecht et al., 2005; Chen et al., 2005). NC women in the current study had higher ratings of aggression than OC users (see Chapter 5), which is consistent with the finding of attenuated N2 in this group, although the findings of the current study suggest that this effect may depend on stimulus type.

On the other hand, OC use was associated with a reduced P3 to neutral face stimuli. Previous research suggests that the P3 is an index of attention (Friedman et al., 2001; Polich, 2003), and that P3 amplitude is affected by hormonal status only when the

stimulus is salient, such that P3 amplitude is larger to more emotionally or socially salient stimuli (Johnston & Wang, 1991). In the context of these theories, the findings of the current study may therefore represent an increased salience of neutral face stimuli to NC women compared with OC users, and an increase in the direction of attentional resources to these stimuli. Other researchers have postulated that the P3 may represent the evaluation of inhibitory performance (Bruin, Wijers, & Van Staveren, 2001), however if this was the case it would be expected that the effects of hormonal status on P3 amplitude would be the same regardless of stimulus type.

In contrast to the findings for neutral face stimuli, NC women were found to have larger nogo minus go differences to angry face stimuli than OC users, suggesting improved processing of angry faces in this group. This effect was apparent during the luteal phase only, suggesting improved early processing of angry face stimuli during periods of increased steroid hormones. These findings are not consistent with previous research which has found no effect of hormonal status on N2 amplitude (Walpurger et al., 2004) as well as reduced N2 amplitude in more aggressive individuals (Albrecht et al., 2005; Chen et al., 2005) which would suggest that N2 amplitude should be reduced in NC women in the current study. However, the use of stimuli with different emotional valence in the current study may explain these discrepant findings. This highlights the importance of considering stimulus type when investigating the effects of hormonal status on response inhibition.

The finding that both N2 and P3 latency to angry nogo stimuli were longer in NC women than OC users is in line with the longer reaction times taken by NC women. However, the larger P3 difference in OC users in the luteal phase when angry faces were the target stimulus is in contrast to the finding of a larger P3 in NC women when neutral faces were the target stimulus. This may reflect an increased salience of angry faces to OC users during the active pill phase, as well as an increased devotion of attentional resources to these stimuli. Individuals with high trait anger have been shown to demonstrate an attentional bias for angry faces (Van Honk et al., 2001), which suggests that the enhanced P3 in OC users would reflect increased aggression in this group. However, since the findings indicated reduced subjective aggression with OC use, it is unlikely that this is the case. In order to ascertain whether the

electrophysiological responses to these stimuli do reflect stimulus salience, and if so the nature of that salience, future studies should include subjective ratings of each stimulus.

Whilst the reduction in dopamine with OC use may explain the attenuated P3 to neutral faces in OC users compared to NC women, the finding that the effect of OC use was modulated by the emotional valence of the stimulus suggests that other mechanisms may underlie these effects. OCs have been shown to alter brain structure (Pletzer et al., 2010) which could underlie the effects on ERP components reported on here. In particular, the amygdala could be a potential region of interest due to its role in emotion regulation (Davis & Whalen, 2001; Phelps & LeDoux, 2005) as well as the theory that the frontal-midline N2 is elicited through projections from the amygdala (Kiss & Eimer, 2008). Although few studies to date have investigated the effects of OC use on amygdala activity, there is some evidence to suggest that OC users have enhanced activity compared with NC women and men (Merz et al., 2012; Merz et al., 2013). Further research in this area to uncover the effects of OCs on activity in brain regions associated with eliciting inhibition-related ERPs is warranted, particularly with regard to discerning the relationship with emotional valence of stimuli.

8.3 Limitations

Limitations of the study as a whole will be discussed more thoroughly in chapters 10 and 11, therefore only those specific to the baseline analyses will be discussed here. One such limitation was the inclusion of participants using various different brands of OC, containing varying amounts of ethinylestradiol and different progestins. As explained in chapter 3, the different generations of OC may have different effects on mood and cognitive function. Since OCs of various generations were included in the current study the findings regarding the effects of OCs in this study should be interpreted with caution.

Due to ethical issues, current OC users only were recruited for this study rather than new users. This cross-sectional design means that effects of OC use cannot be directly attributed to the OC formulation itself. Furthermore, since OC use had to have commenced at least three months prior to study entry it was likely that those enrolled in

the study did not experience any negative side effects of OC use, creating a "healthy survivor effect" or self-selecting bias (Robinson, Dowell, Pedulla, & McCauley, 2004; Sanders et al., 2001). In addition, women who choose OCs as contraception have been shown to differ from non-pill users in various ways, such as attitudes towards sexuality and previous sexual experience, which may have influenced some of the outcomes of the study independently of OC use (Bancroft, Sherwin, Alexander, Davidson, & Walker, 1991).

8.4 Summary and Conclusions

Despite there being some limitations of the current study, the results do add to the current body of literature on the effects of OC use, suggesting that women who use OCs have better mood outcomes and are less aggressive than NC women, as well as having improved cognitive function at least on some tasks. The current findings also partially support the hypothesis that whilst OC use may stabilise mood and cognitive function during the active pill phase, during the inactive pill phase mood and cognitive performance may worsen. In addition, these baseline results confirm the findings of previous researchers that in non-help-seeking women, peak symptom severity is experienced during menses rather than the luteal phase. Importantly, aggression was shown to vary across the cycle highlighting the importance of including this symptom in studies of PMS. The effects of soy isoflavones across the menstrual cycle are presented in the following chapter.

Chapter 9

Post Treatment Results: Effects of Soy Isoflavones across the Menstrual Cycle

9.1. Compliance

Remaining tablets were counted every four weeks and at the end of the trial. OC use was also monitored through participants in the OC group indicating on the DSR whether or not they had taken their pill that day. Compliance was high, with all participants taking more than 80% of their assigned treatment.

9.2. Treatment side effects

One participant in the SIF group reported suffering from mouth ulcers during the first treatment cycle, which was resolved by the next session. One participant in the placebo group reported dizziness and was referred to her GP, who advised that the symptom was not related to the trial. Neither of these participants discontinued treatment.

9.3. Salivary Estradiol

After controlling for baseline circulating estradiol levels there was no effect of treatment, however age was positively associated with estradiol concentrations in the second treatment cycle ($F(1,58)=6.09$, $p=0.017$, $d=0.65$).

9.4. Mood measures

Means, standard deviations and statistical values associated with all mood outcomes during both treatment cycles are presented in Table 9.1. Where main effects and interactions are significant, results of post hoc comparisons are shown in Table 9.2.

Beck Depression Inventory (BDI): After controlling for baseline scores by including baseline as a covariate in the model, in the first treatment cycle there was a trend towards a treatment x phase interaction with pairwise comparisons revealing that in the

ovulatory phase OC use was associated with lower depression scores than both SIF and placebo, and in the luteal phase SIF treatment was associated with higher depression scores than both OC and placebo. Pairwise comparisons also revealed that phase effects were only significant in the placebo group, where scores were higher in the ovulatory phase than the luteal phase. There was also a trend towards a main effect of treatment with pairwise comparisons revealing that SIF treatment was associated with higher depression than OC use. No significant effects were found in the second treatment cycle.

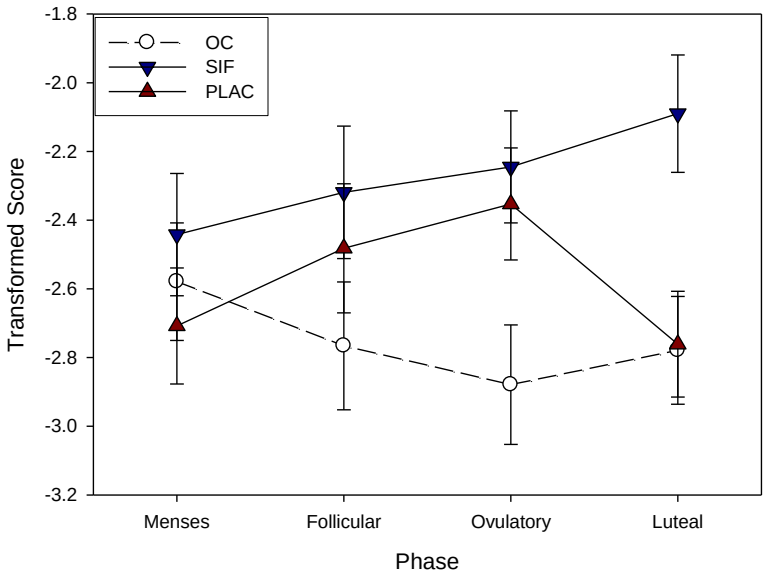

Figure 9.1 Transformed mean depression ratings on the BDI for OC users, women treated with SIF and women treated with placebo in different phase of the first treatment cycle, after controlling for baseline ratings.

Revised Barratt Impulsiveness Scale (BIS-11): After controlling for baseline scores, in the second treatment cycle there was a significant phase x treatment interaction for total BIS scores with SIF treatment being associated with significantly higher impulsivity compared with OC use during menses, and with significant phase effects only apparent for the SIF group where impulsivity was higher during menses than the ovulatory phase. In the first treatment cycle higher BMI scores were significantly associated with higher motor impulsiveness ($F(1,43)=4.32$, $p=0.044$, $d=0.63$). In the second treatment cycle there was a significant phase x treatment interaction with SIF treatment being associated

with significantly higher motor impulsiveness than OC use during menses, and with significant phase effects only apparent for the SIF group where motor impulsiveness was significantly higher during menses than the ovulatory phase. There was a significant negative association of age with non-planning impulsiveness in the first treatment cycle ($F(1,41)=8.90$, $p=0.005$, $d=0.93$).

In the second treatment cycle a significant phase x treatment interaction was found for scores on the attention subscale, with significant phase effects only for OC users where attention was significantly poorer during the ovulatory phase than all other phases. A significant interaction was also found for the motor subscale in the second treatment cycle, with phase effects only evident with SIF treatment where motor impulsiveness was significantly higher in menses than the ovulatory and luteal phases. Age also significantly contributed to the model ($F(1,49)=7.39$, $p=0.009$, $d=0.78$) with older participants having lower motor impulsiveness. Similarly, increased age significantly predicted better self-control ($F(1,47)=8.95$, $p=0.004$, $d=0.87$) and lower scores of cognitive complexity ($F(1,42)=4.77$, $p=0.035$, $d=0.67$) in the first treatment cycle. There was a significant phase x treatment interaction for cognitive complexity scores in the second treatment cycle, with OC use being associated with significantly lower scores than placebo during menses and the follicular phase, and SIF being associated with significantly lower scores than placebo during the follicular and ovulatory phases. There was also a significant main effect of phase with significantly lower scores in the luteal phase than menses and the follicular phase, as well as a trend towards a main effect of treatment with significantly lower scores associated with OC use than placebo. For cognitive instability, BMI significantly contributed to the model in the second treatment cycle ($F(1,49)=4.83$, $p=0.033$, $d=0.63$) with higher BMI being associated with higher cognitive instability. There was a trend towards a phase x treatment interaction for perseverance during the second treatment cycle, with OC use being associated with significantly better perseverance than SIF during menses and the luteal phase and better perseverance than placebo during menses. Furthermore, significant phase effects were only found for the OC group where perseverance was better in menses than the follicular phase. Higher BMI was also significantly associated with poorer perseverance ($F(1,62)=5.39$, $p=0.024$, $d=0.59$).

Table 9.1 *Means and standard deviations for mood outcomes during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction*.

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,53	Treatment Group (T); df=2,50	P x T interaction; df=6,53
BDI	1	OC	7.95 (8.94)	7.57 (10.34)	4.96 (5.50)	6.55 (6.72)	NS	2.92^	2.11^
		SIF	9.76 (9.66)	10.11 (9.75)	12.33 (11.91)	11.09 (10.12)			
		Placebo	7.51 (8.01)	9.44 (10.33)	8.87 (9.25)	4.95 (5.65)			
	2	OC	8.41 (10.22)	8.14 (8.71)	8.45 (11.56)	7.59 (10.00)	NS	NS	NS
		SIF	11.05 (13.28)	10.66 (12.99)	9.11 (13.56)	8.35 (10.62)			
		Placebo	5.68 (7.91)	8.75 (9.49)	6.91 (9.98)	6.63 (10.41)			
BIS: *Total*	1	OC	62.42 (14.41)	60.42 (12.58)	59.50 (12.68)	61.95 (14.54)	NS	NS	NS
		SIF	64.90 (8.03)	63.44 (6.72)	66.30 (9.29)	65.05 (9.25)			
		Placebo	64.29 (11.04)	64.28 (9.48)	61.53 (9.91)	63.07 (11.28)			
	2	OC	60.38 (13.30)	62.70 (15.10)	62.20 (16.76)	60.51 (14.99)	NS	NS	2.45*
		SIF	68.07 (7.32)	65.15 (10.37)	63.05 (11.11)	63.11 (8.40)			
		Placebo	63.50 (11.57)	68.00 (12.36)	65.30 (10.05)	64.27 (12.40)			
Attentional Impulsiveness	1	OC	15.37 (3.95)	15.03 (5.17)	14.50 (4.24)	15.05 (4.29)	NS	NS	NS
		SIF	17.29 (4.23)	16.78 (3.92)	17.76 (4.13)	17.09 (4.06)			
		Placebo	16.83 (5.01)	16.22 (5.13)	15.44 (4.61)	14.79 (4.17)			
	2	OC	15.56 (5.01)	15.36 (4.84)	16.55 (6.01)	15.50 (5.49)	NS	NS	NS
		SIF	17.90 (4.14)	17.65 (5.20)	16.37 (5.01)	16.40 (4.27)			
		Placebo	15.18 (4.64)	16.81 (4.58)	15.52 (4.34)	15.70 (4.91)			
Motor Impulsiveness	1	OC	21.95 (5.46)	21.81 (4.82)	21.20 (4.67)	22.64 (5.73)	NS	NS	NS
		SIF	22.38 (4.02)	21.67 (4.28)	22.97 (4.12)	22.05 (3.66)			
		Placebo	21.21 (2.73)	21.22 (2.58)	21.10 (2.57)	21.65 (3.98)			
	2	OC	21.13 (4.74)	22.65 (6.73)	21.45 (5.02)	22.19 (5.88)	NS	NS	2.31*
		SIF	23.32 (3.76)	22.20 (3.44)	21.84 (4.88)	21.65 (4.07)			
		Placebo	22.23 (3.69)	23.32 (4.64)	22.65 (3.41)	22.27 (4.96)			

Non-planning Impulsiveness	1	OC	25.09 (6.45)	23.59 (5.72)	23.80 (5.67)	24.27 (6.42)	NS	NS	NS
		SIF	25.24 (4.32)	25.00 (4.30)	25.57 (5.64)	25.91 (4.02)			
		Placebo	26.25 (5.67)	26.83 (5.45)	25.00 (5.54)	26.63 (5.93)			
	2	OC	23.69 (5.97)	24.68 (6.49)	24.20 (7.47)	22.82 (6.17)	NS	NS	NS
		SIF	26.85 (4.31)	25.30 (5.31)	24.84 (5.47)	25.06 (4.52)			
		Placebo	26.09 (6.15)	27.87 (5.89)	27.13 (5.58)	26.30 (6.19)			
Attention	1	OC	10.23 (2.86)	10.00 (3.66)	9.95 (3.09)	9.95 (3.03)	NS	NS	NS
		SIF	11.14 (3.00)	11.39 (2.99)	12.00 (3.30)	11.64 (2.92)			
		Placebo	11.08 (3.39)	11.11 (3.88)	10.46 (3.08)	10.20 (3.24)			
	2	OC	10.17 (3.13)	10.05 (3.37)	11.25 (4.14)	10.18 (3.76)	NS	NS	2.36*
		SIF	12.00 (2.75)	11.60 (3.39)	10.63 (3.29)	10.50 (2.96)			
		Placebo	9.86 (3.23)	11.33 (2.97)	10.26 (2.96)	10.50 (3.56)			
Motor	1	OC	15.50 (4.96)	15.47 (4.82)	14.95 (3.66)	16.14 (4.68)	NS	NS	NS
		SIF	15.57 (3.37)	14.72 (2.87)	15.67 (3.44)	15.18 (3.13)			
		Placebo	14.54 (2.11)	14.39 (2.12)	14.60 (2.35)	14.65 (2.68)			
	2	OC	15.05 (4.21)	15.55 (5.44)	14.75 (3.85)	15.48 (4.65)	NS	NS	2.38*
		SIF	16.14 (3.43)	15.45 (3.09)	14.63 (4.15)	14.10 (3.23)			
		Placebo	15.14 (2.88)	16.10 (3.71)	15.43 (2.95)	15.12 (3.53)			
Self-Control	1	OC	13.09 (3.85)	12.33 (3.67)	12.30 (3.56)	12.72 (4.03)	NS	NS	NS
		SIF	13.24 (2.91)	12.78 (2.49)	13.29 (3.10)	13.59 (2.74)			
		Placebo	13.13 (3.59)	13.61 (3.81)	12.70 (3.87)	13.55 (3.83)			
	2	OC	12.46 (3.78)	12.77 (3.96)	12.60 (4.66)	12.09 (4.02)	NS	NS	NS
		SIF	14.00 (2.77)	13.30 (3.42)	13.47 (3.37)	13.25 (2.92)			
		Placebo	13.36 (4.24)	14.57 (4.26)	14.04 (3.71)	13.90 (4.22)			
Cognitive Complexity	1	OC	12.00 (3.13)	11.26 (2.82)	11.50 (2.74)	11.55 (3.08)	NS	NS	NS
		SIF	12.00 (2.61)	12.22 (2.67)	12.29 (3.24)	12.32 (1.86)			
		Placebo	13.13 (2.63)	13.22 (2.13)	12.33 (2.30)	13.10 (2.65)			
	2	OC	11.23 (2.93)	11.91 (3.65)	11.60 (3.78)	10.73 (2.91)	2.92*	3.04^	2.67*
		SIF	12.85 (2.32)	12.00 (2.45)	11.37 (2.45)	11.81 (2.21)			
		Placebo	12.73 (2.29)	13.30 (2.36)	13.09 (2.37)	12.42 (2.41)			
Perseverance	1	OC	6.45 (1.77)	6.38 (1.28)	6.25 (1.41)	6.50 (1.87)	NS	NS	NS
		SIF	6.81 (1.57)	6.94 (1.83)	7.33 (1.80)	6.86 (1.70)			

		Placebo	6.67 (1.61)	6.83 (1.54)	6.50 (1.54)	7.00 (2.03)			
	2	OC	6.06 (1.39)	7.12 (2.30)	6.70 (2.27)	6.70 (2.14)	NS	NS	2.19^
		SIF	7.20 (1.36)	6.75 (1.48)	7.21 (1.40)	7.55 (1.50)			
		Placebo	7.09 (1.69)	7.16 (1.81)	7.22 (1.48)	7.14 (2.06)			
Cognitive Instability	1	OC	5.14 (1.83)	5.05 (2.22)	4.55 (1.67)	5.09 (1.82)	NS	NS	NS
		SIF	6.14 (1.90)	5.39 (1.54)	5.76 (2.02)	5.45 (1.77)			
		Placebo	5.75 (2.38)	5.11 (1.53)	5.00 (2.27)	4.60 (1.43)			
	2	OC	5.36 (2.34)	5.32 (2.21)	5.30 (2.54)	5.32 (2.40)	NS	NS	NS
		SIF	5.90 (1.83)	6.05 (2.26)	5.74 (2.02)	5.90 (2.34)			
		Placebo	5.32 (2.38)	5.48 (2.25)	5.26 (2.28)	5.17 (2.20)			
STAI: *State*	1	OC	33.86 (11.01)	32.48 (11.19)	33.10 (9.15)	35.09 (10.01)	NS	NS	NS
		SIF	37.86 (11.24)	38.89 (13.34)	39.86 (9.22)	39.82 (9.22)			
		Placebo	34.46 (13.73)	36.94 (13.73)	36.00 (14.28)	32.50 (12.44)			
	2	OC	36.05 (13.28)	37.00 (13.19)	37.00 (13.52)	35.82 (14.17)	NS	NS	NS
		SIF	39.73 (12.22)	37.05 (11.95)	39.68 (11.09)	37.80 (9.36)			
		Placebo	32.71 (12.20)	37.29 (12.34)	33.48 (12.42)	32.79 (12.17)			
Trait	1	OC	36.33 (11.43)	37.46 (11.43)	36.10 (10.00)	37.91 (10.91)	NS	NS	NS
		SIF	43.10 (10.18)	42.61 (10.34)	43.45 (10.96)	42.32 (9.39)			
		Placebo	38.91 (11.43)	38.28 (12.20)	38.00 (12.80)	36.14 (10.77)			
	2	OC	38.14 (11.97)	38.62 (12.56)	37.70 (11.70)	38.63 (11.02)	NS	NS	NS
		SIF	43.09 (9.95)	42.50 (10.38)	43.68 (11.09)	41.72 (11.48)			
		Placebo	35.68 (9.47)	39.75 (10.88)	37.00 (11.77)	36.83 (12.09)			
Bond-Lader: *Alert*	1	OC	56.62 (15.96)	52.71 (18.35)	51.90 (18.25)	53.35 (19.48)	NS	NS	NS
		SIF	53.47 (13.22)	45.41 (11.43)	44.19 (17.76)	47.64 (14.23)			
		Placebo	48.68 (19.59)	49.70 (18.71)	55.87 (17.67)	57.72 (15.34)			
	2	OC	54.24 (20.16)	52.26 (22.85)	48.92 (21.80)	57.61 (16.50)	NS	NS	NS
		SIF	50.02 (16.35)	51.19 (17.18)	52.28 (16.39)	49.24 (14.88)			
		Placebo	52.79 (16.92)	52.19 (16.29)	53.28 (17.87)	53.08 (15.73)			
Content	1	OC	68.05 (15.56)	68.08 (17.15)	68.87 (12.39)	66.13 (15.49)	NS	4.82*	NS
		SIF	62.63 (16.56)	54.01 (18.46)	54.20 (13.46)	55.03 (17.68)			
		Placebo	63.01 (17.90)	63.82 (15.17)	66.00 (15.75)	67.82 (16.25)			
	2	OC	63.43 (22.50)	62.89 (19.12)	62.85 (18.76)	67.85 (16.97)	NS	NS	NS

		SIF	59.48 (18.05)	65.68 (16.52)	59.36 (18.52)	60.75 (15.30)			
		Placebo	62.43 (19.72)	60.81 (15.46)	64.37 (18.06)	63.76 (15.16)			
Calm	1	OC	64.21 (10.50)	68.48 (14.99)	68.38 (12.59)	63.48 (15.01)			
		SIF	60.02 (17.12)	57.08 (17.15)	56.02 (10.76)	57.66 (14.82)	NS	3.52*	NS
		Placebo	67.19 (13.30)	64.53 (18.05)	60.25 (14.48)	68.03 (15.82)			
	2	OC	64.34 (18.93)	60.18 (17.78)	65.70 (17.09)	65.98 (12.95)			
		SIF	58.28 (18.01)	60.80 (15.80)	56.31 (11.51)	58.00 (13.36)	NS	NS	2.32*
		Placebo	63.93 (12.67)	61.95 (14.07)	59.91 (13.62)	59.38 (11.46)			
VAS: *Stress*	1	OC	37.55 (19.99)	31.90 (20.84)	31.10 (16.98)	36.59 (23.83)			
		SIF	43.81 (25.87)	39.00 (23.45)	38.90 (20.96)	41.91 (20.73)	NS	NS	NS
		Placebo	37.75 (23.09)	39.56 (21.53)	35.95 (21.48)	36.74 (17.38)			
	2	OC	42.68 (22.88)	40.41 (23.10)	31.55 (21.70)	32.50 (22.98)			
		SIF	47.45 (20.41)	42.45 (22.61)	43.00 (20.24)	39.80 (23.04)	NS	NS	1.96^
		Placebo	37.77 (22.01)	38.05 (18.95)	38.22 (19.20)	40.50 (17.22)			
Mental Fatigue	1	OC	53.30 (23.03)	57.60 (24.91)	56.65 (23.94)	59.55 (24.21)			
		SIF	55.90 (22.02)	63.50 (19.36)	53.95 (23.25)	48.41 (24.19)	NS	NS	NS
		Placebo	48.79 (23.43)	55.17 (19.58)	43.25 (20.80)	48.95 (16.79)			
	2	OC	62.55 (21.65)	57.55 (27.70)	52.40 (29.00)	58.05 (27.21)			
		SIF	53.40 (20.99)	54.10 (24.40)	52.78 (19.71)	50.65 (23.84)	NS	NS	NS
		Placebo	47.86 (20.36)	50.14 (17.21)	47.78 (19.39)	51.79 (17.70)			
POMS: *Total*	1	OC	26.71 (38.31)	24.81 (45.57)	14.65 (27.90)	26.59 (32.87)			
		SIF	48.76 (35.79)	50.00 (41.52)	45.95 (35.48)	43.59 (35.64)	NS	NS	2.25^
		Placebo	35.04 (40.08)	38.78 (48.92)	29.54 (34.48)	18.55 (31.83)			
	2	OC	34.05 (46.40)	34.05 (45.95)	31.00 (51.95)	27.73 (44.60)			
		SIF	46.60 (41.95)	48.00 (42.43)	38.84 (45.61)	38.10 (39.07)	NS	NS	NS
		Placebo	28.23 (37.98)	34.39 (37.54)	27.21 (41.34)	24.58 (42.28)			
Tension-Anxiety	1	OC	9.77 (7.51)	9.33 (8.39)	8.05 (5.83)	9.95 (7.23)			
		SIF	13.29 (7.63)	13.41 (8.02)	12.52 (6.75)	12.05 (8.21)	NS	NS	2.56*
		Placebo	11.13 (7.09)	11.33 (8.79)	10.49 (6.33)	7.80 (7.02)			
	2	OC	10.41 (9.27)	11.27 (9.21)	11.10 (10.37)	9.86 (9.60)			
		SIF	12.70 (8.00)	13.45 (9.09)	11.47 (8.26)	10.65 (7.53)	NS	NS	NS
		Placebo	9.09 (6.03)	11.14 (7.74)	8.86 (7.04)	9.17 (8.12)			

Depression -Dejection	1	OC	10.18 (10.64)	8.95 (12.50)	4.85 (5.85)	8.41 (8.75)	3.22*	3.01^	2.82*
		SIF	15.10 (12.45)	17.35 (13.01)	15.38 (10.69)	13.91 (12.03)			
		Placebo	10.88 (10.60)	12.61 (14.25)	9.35 (10.17)	5.95 (7.94)			
	2	OC	11.59 (14.33)	11.50 (13.61)	11.40 (14.80)	10.00 (12.98)	NS	NS	NS
		SIF	15.90 (13.86)	15.05 (14.06)	13.89 (14.53)	13.60 (12.93)			
		Placebo	10.14 (12.48)	10.72 (10.91)	9.57 (12.94)	8.38 (12.65)			
Anger-Hostility	1	OC	6.27 (6.91)	6.29 (9.61)	3.55 (4.77)	6.09 (6.55)	2.23^	NS	NS
		SIF	12.29 (9.17)	10.76 (8.95)	9.95 (7.59)	10.59 (9.40)			
		Placebo	8.63 (8.88)	8.44 (10.80)	7.20 (8.76)	5.80 (7.32)			
	2	OC	7.77 (9.42)	7.05 (9.23)	7.15 (9.19)	6.09 (7.56)	NS	NS	NS
		SIF	12.15 (9.76)	11.10 (10.17)	11.42 (10.62)	9.55 (9.04)			
		Placebo	8.14 (8.04)	7.71 (8.08)	6.78 (8.63)	6.75 (9.39)			
Vigour-Activity	1	OC	15.33 (7.93)	14.71 (8.16)	15.45 (8.41)	14.68 (7.11)	NS	NS	NS
		SIF	12.67 (6.45)	14.18 (7.19)	13.57 (7.89)	13.00 (5.52)			
		Placebo	14.08 (6.22)	13.67 (7.27)	15.35 (7.29)	15.25 (7.42)			
	2	OC	13.23 (7.66)	14.18 (8.87)	14.10 (9.19)	13.77 (7.93)	NS	NS	2.36*
		SIF	14.00 (5.37)	13.05 (6.07)	15.47 (6.26)	13.90 (7.43)			
		Placebo	15.36 (6.25)	12.52 (4.61)	13.87 (6.50)	15.71 (6.15)			
Fatigue-Inertia	1	OC	9.00 (6.59)	8.38 (6.58)	7.70 (6.01)	9.14 (5.99)	NS	NS	NS
		SIF	11.62 (5.92)	12.24 (7.18)	12.19 (6.51)	10.82 (5.10)			
		Placebo	9.21 (7.06)	10.17 (7.52)	9.40 (5.79)	7.90 (5.63)			
	2	OC	9.82 (6.69)	10.50 (7.10)	8.35 (7.60)	8.50 (6.80)	NS	NS	NS
		SIF	10.20 (6.49)	11.50 (6.79)	9.16 (5.78)	9.80 (5.81)			
		Placebo	7.91 (5.90)	8.67 (6.31)	7.87 (6.04)	8.25 (6.53)			
Confusion-Bewilderment	1	OC	6.82 (5.25)	6.57 (5.08)	5.95 (4.26)	7.68 (5.43)	NS	NS	2.05^
		SIF	9.14 (4.14)	10.41 (4.08)	9.48 (4.33)	9.23 (4.62)			
		Placebo	9.29 (6.02)	9.89 (6.61)	8.45 (5.62)	6.35 (4.90)			
	2	OC	7.68 (5.58)	7.91 (6.56)	7.10 (6.66)	7.05 (6.11)	NS	NS	NS
		SIF	9.65 (4.30)	10.05 (4.36)	8.37 (4.27)	8.40 (3.97)			
		Placebo	8.32 (5.63)	8.67 (5.70)	8.00 (6.06)	7.75 (5.74)			
SPQ: *Total*	1	OC	13.01 (15.15)	12.60 (15.36)	11.43 (13.02)	13.59 (15.14)	NS	NS	NS
		SIF	20.90 (13.70)	18.20 (10.36)	20.24 (14.05)	20.14 (13.39)			

		Placebo	20.66 (17.49)	22.97 (18.80)	19.30 (17.44)	16.30 (14.52)			
	2	OC	11.30 (14.26)	12.95 (15.45)	12.50 (16.39)	12.23 (16.75)			
		SIF	19.32 (12.91)	17.94 (10.91)	16.37 (11.15)	16.99 (12.04)	NS	2.56^	NS
		Placebo	16.23 (13.59)	23.01 (18.49)	20.28 (17.97)	20.07 (18.68)			
Ideas of reference	1	OC	1.59 (1.79)	1.19 (1.54)	1.00 (1.65)	1.68 (2.06)			
		SIF	2.71 (2.76)	2.39 (2.43)	2.38 (2.84)	2.18 (2.65)	NS	NS	NS
		Placebo	2.42 (2.59)	2.67 (3.01)	2.15 (2.78)	1.95 (2.48)			
	2	OC	0.86 (1.39)	1.41 (1.87)	1.05 (1.96)	1.36 (2.15)			
		SIF	2.55 (2.58)	2.20 (2.57)	2.11 (2.85)	2.19 (2.79)	NS	NS	NS
		Placebo	1.73 (2.27)	2.43 (2.71)	2.12 (2.75)	1.95 (2.76)			
Excessive social anxiety	1	OC	2.45 (2.84)	2.29 (2.65)	2.37 (2.55)	2.77 (2.54)			
		SIF	3.24 (2.61)	3.11 (2.54)	3.00 (2.55)	3.00 (2.83)	NS	NS	NS
		Placebo	3.13 (2.72)	3.61 (2.89)	2.85 (2.85)	2.75 (2.83)			
	2	OC	2.27 (2.51)	2.45 (2.69)	2.60 (2.72)	2.45 (2.77)			
		SIF	2.75 (2.57)	2.65 (2.16)	2.37 (2.17)	2.50 (2.46)	NS	NS	NS
		Placebo	2.50 (2.22)	3.33 (3.02)	2.95 (3.01)	2.97 (3.00)			
Odd beliefs and magical thinking	1	OC	0.41 (0.96)	0.33 (0.91)	0.35 (0.93)	0.36 (0.90)			
		SIF	1.67 (1.88)	1.28 (1.23)	1.48 (1.60)	1.50 (1.71)	4.46*	NS	NS
		Placebo	0.93 (1.26)	0.89 (1.88)	1.15 (1.90)	1.20 (1.94)			
	2	OC	0.36 (0.90)	0.23 (0.87)	0.25 (0.91)	0.27 (0.88)			
		SIF	1.50 (1.57)	1.20 (1.54)	1.11 (1.10)	1.25 (1.29)	NS	4.66*	NS
		Placebo	0.95 (1.73)	1.10 (1.70)	1.10 (1.83)	0.93 (1.53)			
Unusual perceptual experiences	1	OC	0.96 (1.66)	1.12 (1.76)	1.00 (1.59)	1.14 (2.03)			
		SIF	1.62 (2.48)	1.74 (2.71)	1.19 (2.18)	1.36 (2.19)	NS	2.80^	NS
		Placebo	2.25 (2.63)	2.56 (2.83)	2.30 (2.62)	1.80 (2.24)			
	2	OC	1.07 (2.00)	0.91 (1.85)	1.00 (1.84)	0.91 (1.74)			
		SIF	1.85 (2.80)	2.04 (2.92)	1.32 (2.47)	1.85 (2.56)	NS	4.20*	NS
		Placebo	1.82 (1.99)	2.86 (2.69)	2.22 (2.68)	2.48 (2.69)			
Odd/ eccentric behaviour	1	OC	1.68 (2.17)	1.38 (2.29)	1.50 (2.28)	1.59 (2.26)			
		SIF	2.05 (2.36)	1.61 (1.97)	2.00 (2.21)	2.05 (2.28)	NS	NS	NS
		Placebo	1.81 (2.68)	1.78 (2.46)	2.00 (2.75)	1.20 (2.14)			
	2	OC	1.20 (1.94)	1.59 (2.30)	1.40 (2.33)	1.23 (2.11)	NS	NS	NS

		SIF	1.50 (1.88)	1.75 (2.10)	1.79 (2.27)	1.60 (2.11)			
		Placebo	1.55 (2.32)	2.05 (2.44)	1.63 (2.35)	1.69 (2.33)			
No close friends	1	OC	1.27 (1.86)	1.52 (2.36)	1.41 (2.07)	1.36 (1.89)	NS	NS	NS
		SIF	2.33 (2.20)	2.06 (2.18)	2.24 (2.34)	2.55 (2.50)			
		Placebo	2.63 (2.86)	2.97 (3.33)	1.95 (2.39)	1.95 (2.04)			
	2	OC	1.52 (2.35)	1.82 (2.42)	1.70 (2.34)	1.68 (2.51)	NS	NS	NS
		SIF	2.25 (2.20)	2.10 (2.10)	1.89 (1.88)	1.95 (2.37)			
		Placebo	1.73 (2.35)	2.63 (3.12)	2.46 (2.83)	2.44 (2.91)			
Odd speech	1	OC	2.14 (2.82)	2.14 (2.82)	1.80 (2.46)	2.09 (2.78)	NS	4.59*	2.48^
		SIF	3.57 (2.82)	3.17 (2.68)	4.05 (2.84)	3.68 (2.71)			
		Placebo	4.08 (2.89)	4.11 (3.14)	3.65 (3.33)	3.15 (2.80)			
	2	OC	1.59 (2.59)	1.95 (2.63)	1.80 (2.78)	1.55 (2.65)	NS	6.63**	NS
		SIF	3.27 (2.55)	2.70 (2.08)	2.74 (2.45)	2.70 (2.52)			
		Placebo	3.55 (2.84)	4.33 (2.97)	3.89 (3.14)	3.89 (3.07)			
Constricted affect	1	OC	1.00 (1.72)	1.10 (1.79)	0.65 (0.99)	1.09 (1.54)	NS	NS	NS
		SIF	1.62 (1.53)	1.44 (1.69)	1.48 (1.69)	1.45 (1.68)			
		Placebo	1.54 (2.06)	1.89 (2.47)	1.60 (2.04)	1.10 (1.29)			
	2	OC	1.09 (1.95)	1.00 (1.72)	1.00 (1.75)	1.00 (1.54)	NS	NS	NS
		SIF	1.45 (1.50)	1.50 (1.40)	1.21 (1.47)	1.45 (1.76)			
		Placebo	1.00 (0.98)	1.86 (2.33)	1.96 (2.40)	1.58 (2.17)			
Suspicious ness	1	OC	1.50 (2.46)	1.52 (2.66)	1.35 (2.35)	1.50 (2.61)	NS	NS	NS
		SIF	2.10 (2.14)	1.48 (1.46)	2.43 (2.54)	2.36 (2.40)			
		Placebo	1.88 (2.71)	2.50 (2.94)	1.65 (2.23)	1.20 (1.74)			
	2	OC	1.32 (2.59)	1.59 (2.67)	1.70 (2.79)	1.32 (2.44)	2.76^	NS	NS
		SIF	2.20 (2.26)	1.80 (1.74)	1.84 (2.12)	1.50 (2.14)			
		Placebo	1.41 (2.20)	2.43 (2.71)	1.96 (2.57)	2.13 (2.79)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Profile of Mood States (POMS): After controlling for baseline scores there was a trend towards a phase x treatment interaction for total mood disturbance scores in the first treatment cycle with OC users having significantly lower mood disturbance scores during the ovulatory phase than both SIF and placebo. For tension-anxiety, in the first treatment cycle there was a significant phase x treatment interaction, with OC use being associated with lower ratings during the ovulatory phase than SIF and placebo. Increased age was also significantly associated with lower tension-anxiety ratings ($F(1,44)=8.87$, $p=0.005$, $d=0.90$).

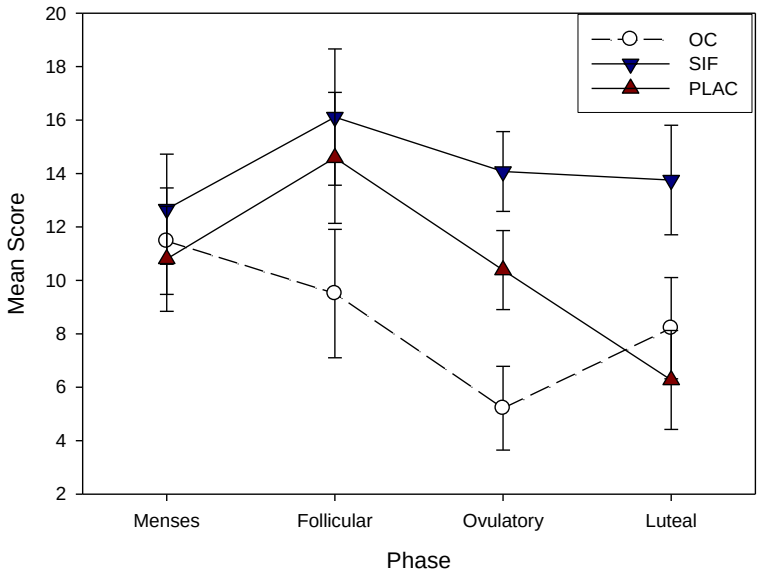

Figure 9.2 Mean ratings on the depression-dejection subscale of the POMS for OC users, women treated with SIF and women treated with placebo during different phases of the first treatment cycle, after controlling for baseline scores.

For depression-dejection there was a significant phase x treatment interaction in the first treatment cycle with OC users having lower scores during the ovulatory phase than SIF and placebo, and with SIF being associated with higher scores during the luteal phase than placebo. There was also a significant main effect of phase with scores in the follicular phase being higher than in the ovulatory and luteal phases, as well as a trend towards a main effect of treatment with SIF being associated with higher ratings of depression-dejection than OC. For anger-hostility there was a trend towards a main

effect of phase in the first treatment cycle with pairwise comparisons revealing higher scores during menses and the follicular phase than the ovulatory and luteal phases. For vigour-activity there was a significant positive association of years of education in the first treatment cycle ($F(1,46)=7.55$, $p=0.009$, $d=0.81$). In the second treatment cycle there was a significant phase x treatment interaction with pairwise comparisons revealing that phase effects were only significant for the placebo group where vigour-activity ratings were significantly higher during the luteal phase than the follicular and ovulatory phases. For confusion-bewilderment there was a trend towards a phase x treatment interaction in the first treatment cycle with OC being associated with lower ratings during the follicular and ovulatory phases than SIF and placebo.

STAI: After controlling for baseline scores, no significant main effects of phase, treatment or their interaction were found for scores on the state or trait subscales in either treatment cycle.

Table 9.2 *Results of post hoc comparisons of estimated marginal means where significant main effects of treatment, phase and their interaction were found on mood outcomes in the two treatment cycles.*

Outcome	Main effect of Phase	Main effect of Treatment	Phase x Treatment Interaction
Beck Depression Inventory (BDI)	NS	TREATMENT CYCLE 1 SIF > OC (t=2.39, p=0.021, d=0.72)	TREATMENT CYCLE 1: Ovulatory: SIF > OC (t=2.62, p=0.011, d=0.84); Plac > OC (t=2.22, p=0.03, d=0.72) Luteal: SIF > OC (t=2.94, p=0.005, d=1.02); SIF > Plac (t=2.90, p=0.005, d=0.98) Plac only: O > L (t=2.32, p=0.025, d=1.20)
Barratt Impulsiveness Scale (BIS-11) Total	NS	NS	TREATMENT CYCLE 2: Menses only: SIF > OC (t=2.51, p=0.015, d=0.80) SIF only: M > O (t=2.90, p=0.005, d=1.45)
BIS-11 Motor Impulsiveness	NS	NS	TREATMENT CYCLE 2: Menses only: SIF > OC (t=2.37, p=0.022, d=0.75) SIF only: M > O (t=2.72, p=0.009, d=1.36)
BIS-11 Attention	NS	NS	TREATMENT CYCLE 2: OC only: O > M (t=2.37, p=0.022, d=1.12); O > F (t=2.82, p=0.007, d=1.33); O > L (t=2.03, p=0.048, d=0.93)
BIS-11 Motor	NS	NS	TREATMENT CYCLE 2: SIF only: M > O (t=3.06, p=0.003, d=1.58); M > L (t=2.85, p=0.006, d=1.38)
BIS-11 Cognitive Complexity	TREATMENT CYCLE 2 M > L (t=2.40, p=0.02, d=0.63) F > L (t=2.80, p=0.008, d=0.74)	TREATMENT CYCLE 2 Plac > OC (t=2.32, p=0.025, d=0.68)	TREATMENT CYCLE 2: Menses: Plac > OC (t=2.43, p=0.018, d=0.75) Follicular: Plac > OC (t=2.24, p=0.03, d=0.71); Plac > SIF (t=2.42, p=0.02, d=0.78) Ovulatory: Plac > SIF (t=2.43, p=0.019, d=0.78)
BIS-11 Perseverance	NS	NS	TREATMENT CYCLE 2: Menses: SIF > OC (t=2.71, p=0.009, d=0.86); Plac > OC (t=2.60, p=0.012, d=0.80) Luteal: SIF > OC (t=2.06, p=0.046, d=0.65) OC only: F > O (t=3.31, p=0.002, d=1.52)

Profile of Mood States (POMS) Total Mood Disturbance	NS	NS	TREATMENT CYCLE 1: Ovulatory only: SIF > OC (t=3.08, p=0.003, d=0.99); Plac > OC (t=2.88, p=0.005, d=0.93)
POMS Tension-Anxiety	NS	NS	TREATMENT CYCLE 1: Ovulatory only: SIF > OC (t=2.91, p=0.005, d=0.93); Plac > OC (t=3.28, p=0.002, d=1.06)
POMS Depression-Dejection	TREATMENT CYCLE 1 F > O (t=3.02, p=0.004, d=0.86) F > L (t=2.66, p=0.01, d=0.76)	TREATMENT CYCLE 1 SIF > OC (t=2.41, p=0.02, d=0.73)	TREATMENT CYCLE 1: Ovulatory: SIF > OC (t=4.01, p<0.001, d=1.29); Plac > OC (t=2.40, p=0.02, d=0.78) Luteal: SIF > Plac (t=2.71, p=0.009, d=0.92)
POMS Anger-Hostility	TREATMENT CYCLE 1 M > O (t=2.04, p=0.046, d=0.55) M > L (t=2.23, p=0.031, d=0.59) F > O (t=2.13, p=0.038, d=0.61) F > L (t=2.01, p=0.048, d=0.57)	NS	NS
POMS Vigour-Activity	NS	NS	TREATMENT CYCLE 2: Plac only: L > F (t=2.27, p=0.027, d=1.04); L > O (t=2.39, p=0.021, d=1.02)
POMS Confusion-Bewilderment	NS	NS	TREATMENT CYCLE 1: Follicular: SIF > OC (t=2.42, p=0.02, d=0.78); Plac > OC (t=2.85, p=0.007, d=0.90) Ovulatory: SIF > OC (t=2.29, p=0.025, d=0.76); Plac > OC (t=2.22, p=0.03, d=0.70)
Schizotypal Personality Questionnaire (SPQ) Total	NS	TREATMENT CYCLE 2 Plac > OC (t=2.20, p=0.035, d=0.65)	NS
SPQ Odd Beliefs and Magical Thinking	TREATMENT CYCLE 1 O > F (t=2.69, p=0.009, d=0.77) L > F (t=2.73, p=0.01, d=0.78)	TREATMENT CYCLE 2 SIF > OC (t=2.96, p=0.005, d=0.90) Plac > OC (t=2.13, p=0.04, d=0.63)	NS
SPQ Unusual Perceptual Experiences	NS	TREATMENT CYCLE 1 Plac > OC (t=2.27, p=0.029, d=0.66) TREATMENT CYCLE 2	NS

		Plac > OC (t=2.90, p=0.005, d=0.86)	
SPQ Odd Speech	NS	TREATMENT CYCLE 1 SIF > OC (t=2.30, p=0.03, d=0.69) Plac > OC (t=2.87, p=0.008, d=0.84) TREATMENT CYCLE 2 Plac > OC (t=3.59, p=0.001, d=1.06) Plac > SIF (t=2.15, p=0.037, d=0.64)	TREATMENT CYCLE 1: Menses: Plac > OC (t=2.47, p=0.017, d=0.75) Follicular: Plac > OC (t=3.01, p=0.004, d=0.99) Ovulatory: Plac > OC (t=2.02, p=0.049, d=0.66); SIF > OC (t=2.89, p=0.006, d=0.93) Luteal: SIF > OC (t=2.11, p=0.041, d=0.73)
SPQ Suspiciousness	TREATMENT CYCLE 2 F > L (t=2.87, p=0.006, d=0.76)	NS	NS
Bond-Lader Contentedness	NS	TREATMENT CYCLE 1 OC > SIF (t=3.06, p=0.003, d=0.92) Plac > SIF (t=2.06, p=0.044, d=0.60)	NS
Bond-Lader Calmness	NS	TREATMENT CYCLE 1 OC > SIF (t=2.50, p=0.016, d=0.75) Plac > SIF (t=2.06, p=0.044, d=0.60)	TREATMENT CYCLE 2: Ovulatory: OC > SIF (t=2.49, p=0.015, d=0.83); OC > Plac (t=2.42, p=0.019, d=0.76) Luteal: OC > SIF (t=2.16, p=0.035, d=0.68); OC > Plac (t=2.55, p=0.013, d=0.77) OC: O > F (t=2.47, p=0.017, d=1.16) Plac: F > L (t=2.13, p=0.038, d=0.98)
Visual Analogue Scales Stress	NS	NS	TREATMENT CYCLE 2: Ovulatory only: SIF > OC (t=2.10, p=0.041, d=0.70) OC only: M > O (t=2.65, p=0.011, d=1.25); F > O (t=2.88, p=0.006, d=1.36)

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; NS, Non-significant; OC, Oral Contraceptive Users; SIF, Soy Isoflavone Group; Plac, Placebo Group

Schizotypal Personality Questionnaire (SPQ): After controlling for baseline scores there was a trend towards a main effect of treatment on total SPQ scores in the second treatment cycle with OC use being associated with fewer schizotypal traits than placebo. In the first treatment cycle there was a significant main effect of phase on odd beliefs and magical thinking with significantly lower scores during the follicular phase than the ovulatory and luteal phases. In the second treatment cycle there was a significant main effect of treatment on odd beliefs, with OC use being associated with significantly lower ratings than SIF and placebo. There was also a significant negative association between years of education and odd beliefs and magical thinking ($F(1,35)=5.52$, $p=0.025$, $d=0.79$). In the first treatment cycle there was a trend towards a main effect of treatment on unusual perceptual experiences with OC users reporting fewer experiences than placebo. In the second treatment cycle this main effect became significant with the difference still lying between OC and placebo. In the first treatment cycle there was a significant positive association between BMI and odd or eccentric behaviour ($F(1,17)=4.57$, $p=0.047$, $d=1.04$). For odd speech, in the first treatment cycle there was a significant main effect of treatment with OC users having lower scores than SIF and placebo, as well as a trend towards a phase x treatment interaction with significantly lower scores with OC compared to placebo in the menses, follicular and ovulatory phases, whereas the difference between OC and SIF lay in the ovulatory and luteal phases. A significant main effect of treatment on odd speech was also found in the second treatment cycle, with placebo being associated with increased odd speech compared to OC and SIF. For suspiciousness, there was a trend towards a main effect of phase in the second treatment cycle with suspiciousness being significantly higher in the follicular than the luteal phase.

Bond-Lader: After controlling for baseline scores there was a significant main effect of treatment on contentedness in the first treatment cycle, with SIF being associated with reduced contentedness compared with OC and placebo. There was also a significant main effect of treatment on calmness with SIF being associated with reduced calmness compared with OC and placebo. In the second treatment cycle a significant phase x treatment interaction was found with OC use being associated with increased calmness during the ovulatory and luteal phases compared with SIF and placebo. In addition, for

the OC group calmness was higher during the ovulatory than the follicular phase whereas for the placebo group calmness was higher during the follicular than the luteal phase. No significant phase effects were found for the SIF group.

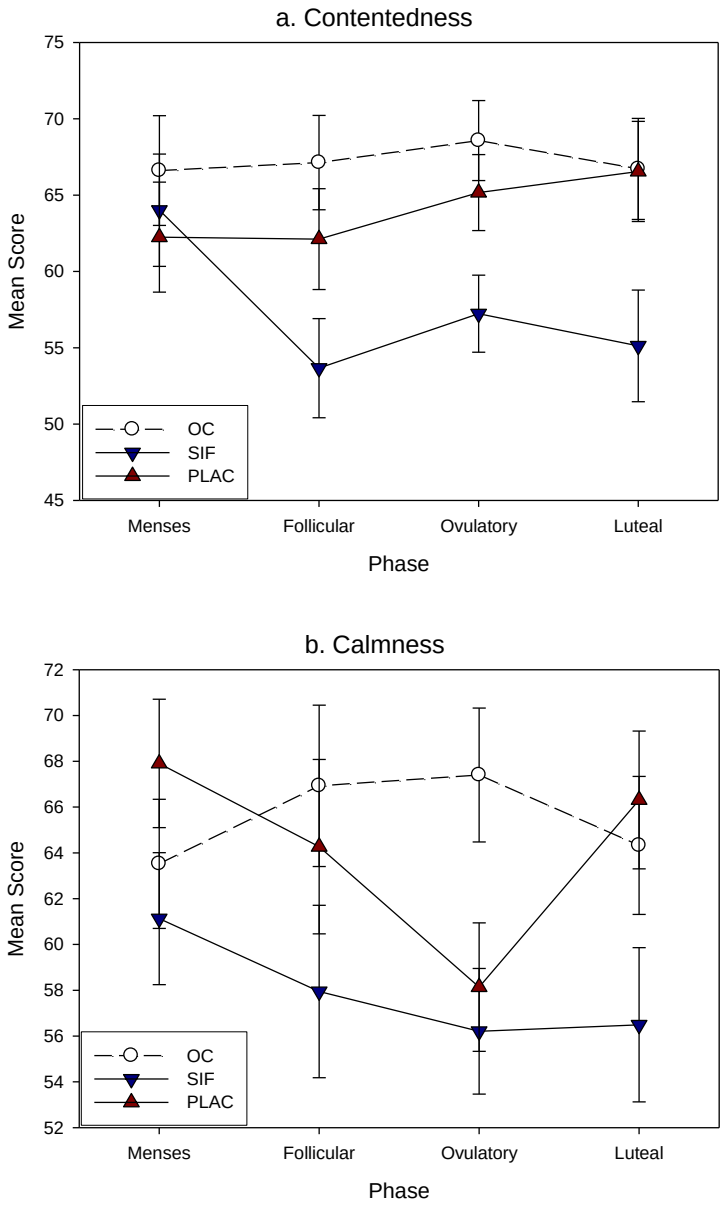

Figure 9.3 Mean ratings of **a.** Contentedness and **b.** Calmness on the Bond-Lader mood scales during the first treatment cycle for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores.

Visual Analogue Scales (VAS): With baseline scores included as a covariate, during the first treatment cycle increased age was significantly associated with lower stress ($F(1,49)=4.50$, $p=0.039$, $d=0.61$). In the second treatment cycle there was a trend towards a phase x treatment interaction, with significantly higher stress with SIF compared to OC during the ovulatory phase. In addition, significant phase effects were only found for the OC group who had lower stress during the ovulatory phase than menses and the follicular phase.

9.5. Premenstrual symptoms and general symptoms

Table 9.3 shows means, standard deviations and statistical values for measures of premenstrual symptoms in both treatment cycles. Where significant main effects or interactions were found, the results of post hoc pairwise comparisons of estimated marginal means are shown in Table 9.4.

Symptom Checklist (SCL): After controlling for baseline scores, there was a trend towards a phase x treatment interaction in the first treatment cycle with pairwise comparisons revealing that OC use was associated with significantly less severe symptoms than SIF during all phases as well as less severe symptoms than placebo during the ovulatory phase, whereas SIF was associated with more severe symptoms than placebo during the luteal phase. There was also a significant main effect of treatment with SIF being associated with more severe symptoms than OC and placebo. Age was also significantly negatively associated with symptom severity ($F(1,46)=6.87$, $p=0.012$, $d=0.77$).

Table 9.3 *Means and standard deviations for measures of premenstrual and general symptoms for during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction*.

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Rest	Luteal	Cycle Phase (P); df=3,54	Treatment Group (T); df=2,51	P x T interaction; df=6,52
SCL	1	OC	42.27 (13.22)	41.67 (14.28)	37.00 (10.16)	43.41 (14.60)	NS	7.43**	2.14^
		SIF	59.71 (17.73)	53.67 (18.22)	57.84 (20.58)	54.82 (18.97)			
		Placebo	51.79 (20.40)	48.34 (19.35)	49.25 (23.11)	42.17 (13.71)			
	2	OC	42.41 (13.18)	44.10 (12.94)	44.15 (18.84)	40.09 (14.84)	NS	NS	NS
		SIF	55.93 (19.04)	54.95 (20.62)	52.47 (18.15)	51.70 (19.10)			
		Placebo	45.91 (17.18)	49.29 (19.37)	46.70 (18.99)	46.49 (20.14)			
DSR: *Total*	1	OC	9.09 (5.22)	7.47 (5.92)	6.57 (6.00)	6.47 (3.36)	NS	NS	2.55*
		SIF	16.35 (10.36)	13.85 (9.95)	11.82 (8.30)	17.05 (11.00)			
		Placebo	11.53 (9.31)	9.14 (7.82)	8.93 (7.99)	9.34 (8.08)			
	2	OC	8.51 (5.56)	5.98 (4.58)	6.11 (4.29)	6.27 (4.56)	2.51^	3.22^	NS
		SIF	17.57 (11.48)	11.93 (9.66)	11.24 (8.99)	14.50 (9.46)			
		Placebo	12.36 (10.54)	7.85 (7.63)	5.63 (4.99)	7.41 (6.49)			
Psychological factor	1	OC	4.03 (3.16)	3.30 (3.07)	3.33 (3.92)	3.01 (2.50)	NS	NS	NS
		SIF	7.32 (6.45)	6.66 (5.78)	6.09 (4.86)	8.53 (5.92)			
		Placebo	4.42 (4.40)	4.59 (4.55)	4.46 (4.68)	4.31 (4.00)			
	2	OC	3.83 (3.41)	2.75 (2.99)	2.94 (3.05)	2.96 (3.49)	NS	2.95^	NS
		SIF	8.35 (6.81)	6.34 (6.04)	5.67 (5.02)	7.18 (5.14)			
		Placebo	5.09 (4.98)	3.78 (3.82)	2.66 (2.39)	3.40 (3.57)			
Physical factor	1	OC	5.06 (3.24)	4.17 (3.42)	3.24 (2.55)	3.46 (2.20)	NS	NS	3.01*
		SIF	9.03 (5.02)	7.19 (4.96)	5.72 (4.01)	8.52 (5.69)			
		Placebo	7.11 (5.28)	4.54 (3.76)	4.47 (3.63)	5.03 (4.52)			

2	OC	4.68 (3.63)	3.23 (2.04)	3.17 (2.16)	3.31 (2.35)			
	SIF	9.22 (5.40)	5.60 (4.14)	5.57 (4.43)	7.32 (5.20)	3.05*	2.57^	NS
	Placebo	7.26 (5.93)	4.07 (4.12)	2.97 (2.83)	4.01 (3.83)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Modified Daily Symptom Report (DSR-20): After controlling for baseline scores, in the first treatment cycle there was a significant phase x treatment interaction for total DSR scores with symptoms being significantly more severe with SIF in comparison to OC during the follicular phase, and in comparison to both OC and placebo during the luteal phase. In the second treatment cycle there was a trend towards a main effect of treatment with SIF being associated with more severe symptoms than OC and placebo although this comparison did not reach statistical significance (p=0.051). There was also a trend towards a main effect of phase with pairwise comparisons revealing more severe symptoms during menses than the follicular and "rest" phases.

In the first treatment cycle higher BMI was significantly associated with higher psychological symptom scores ($F(1,23)=5.26$, $p=0.031$, $d=0.96$). Although no significant interaction was found, pairwise comparisons revealed that SIF was associated with significantly more severe ratings of psychological symptoms during the luteal phase compared with OC and placebo. In the second treatment cycle there was a trend towards a main effect of treatment with SIF being associated with more severe symptoms on the psychological factor than OC use. There was a significant phase x treatment interaction for physical factor scores in the first treatment cycle with more severe symptoms in the SIF group than the OC group during the follicular phase and more severe scores with SIF than placebo during the luteal phase. In the second treatment cycle, there was a significant main effect of phase on physical factor scores with symptoms being more severe during menses than the follicular and "rest" phases. There was also a trend towards a main effect of treatment with significantly more severe symptoms with SIF than OC.

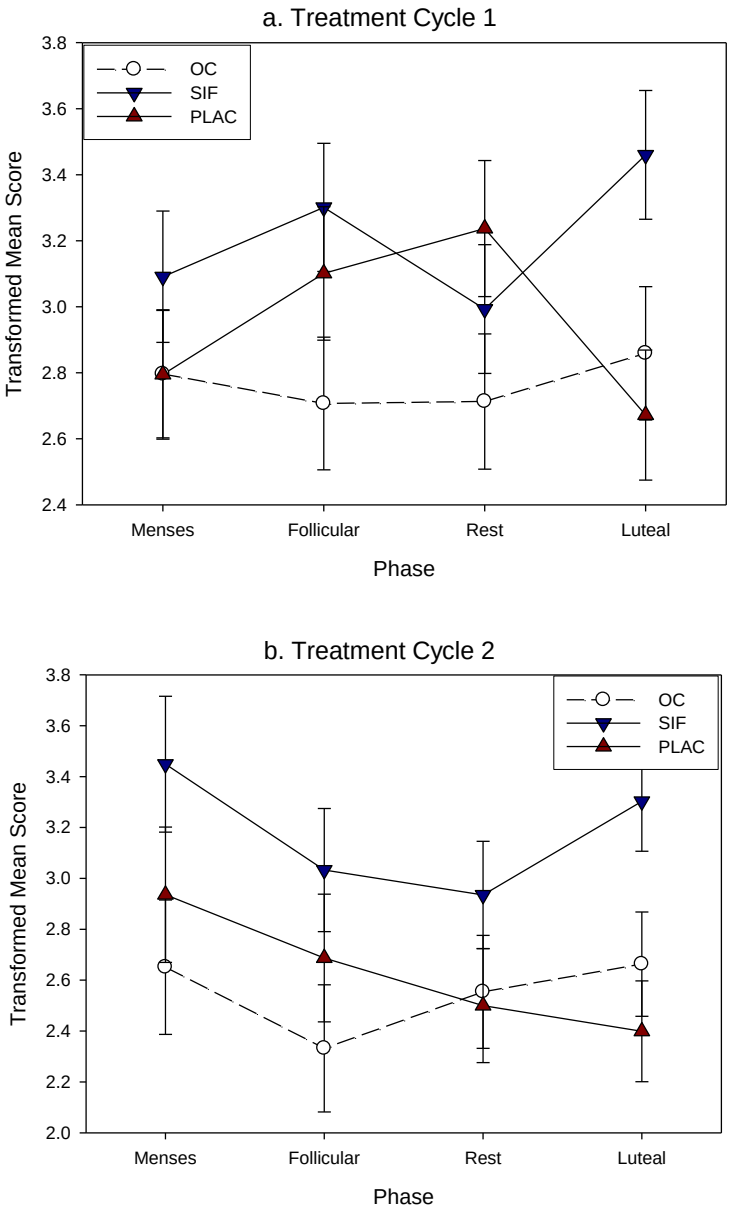

Figure 9.4 Transformed mean ratings of total premenstrual symptoms on the DSR in **a.** the first treatment cycle and **b.** the second treatment cycle for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores.

Table 9.4 *Results of post hoc comparisons of estimated marginal means where significant main effects of treatment, phase and their interaction were found on premenstrual symptoms in the two treatment cycles.*

Outcome	Main effect of Phase	Main effect of Treatment	Phase x Treatment Interaction
Symptom Checklist (SCL)	NS	TREATMENT CYCLE 1 SIF > OC (t=3.86, p<0.001, d=1.16) SIF > Plac (t=2.24, p=0.03, d=0.66)	TREATMENT CYCLE 1: Menses: SIF > OC (t=3.04, p=0.003, d=0.95) Follicular: SIF > OC (t=2.67, p=0.01, d=0.88) Ovulatory: SIF > OC (t=4.52, p<0.001, d=1.45); Plac > OC (t=2.72, p=0.009, d=0.88) Luteal: SIF > OC (t=2.55, p=0.014, d=0.88); SIF > Plac (t=2.52, p=0.015, d=0.85)
Modified Daily Symptom Report (DSR-20) Total	TREATMENT CYCLE 2 M > F (t=2.73, p=0.008, d=0.74) M > R (t=2.08, p=0.042, d=0.57)	TREATMENT CYCLE 2 SIF > OC (t=2.32, p=0.029, d=0.79)	TREATMENT CYCLE 1: Follicular: SIF > OC (t=2.12, p=0.04, d=0.72) Luteal: SIF > OC (t=2.09, p=0.042, d=0.71); SIF > Plac (t=2.86, p=0.007, d=0.98)
DSR-20 Psychological Factor	NS	TREATMENT CYCLE 2 SIF > OC (t=2.25, p=0.032, d=0.76)	TREATMENT CYCLE 1 Luteal: SIF > OC (t=2.63, p=0.012, d=0.79); SIF > Plac (t=2.41, p=0.021, d=0.70)
DSR-20 Physical Factor	TREATMENT CYCLE 2 M > F (t=3.01, p=0.004, d=0.82) M > R (t=2.26, p=0.027, d=0.62)	TREATMENT CYCLE 2 SIF > OC (t=2.09, p=0.045, d=0.71)	TREATMENT CYCLE 1: Follicular: SIF > OC (t=2.09, p=0.042, d=0.71) Luteal: SIF > Plac (t=2.80, p=0.007, d=0.95)

M, Menses; F, Follicular Phase; R, Rest of the Cycle; L, Luteal Phase; NS, Non-significant; OC, Oral Contraceptive Users; SIF, Soy Isoflavone Group; Plac, Placebo Group

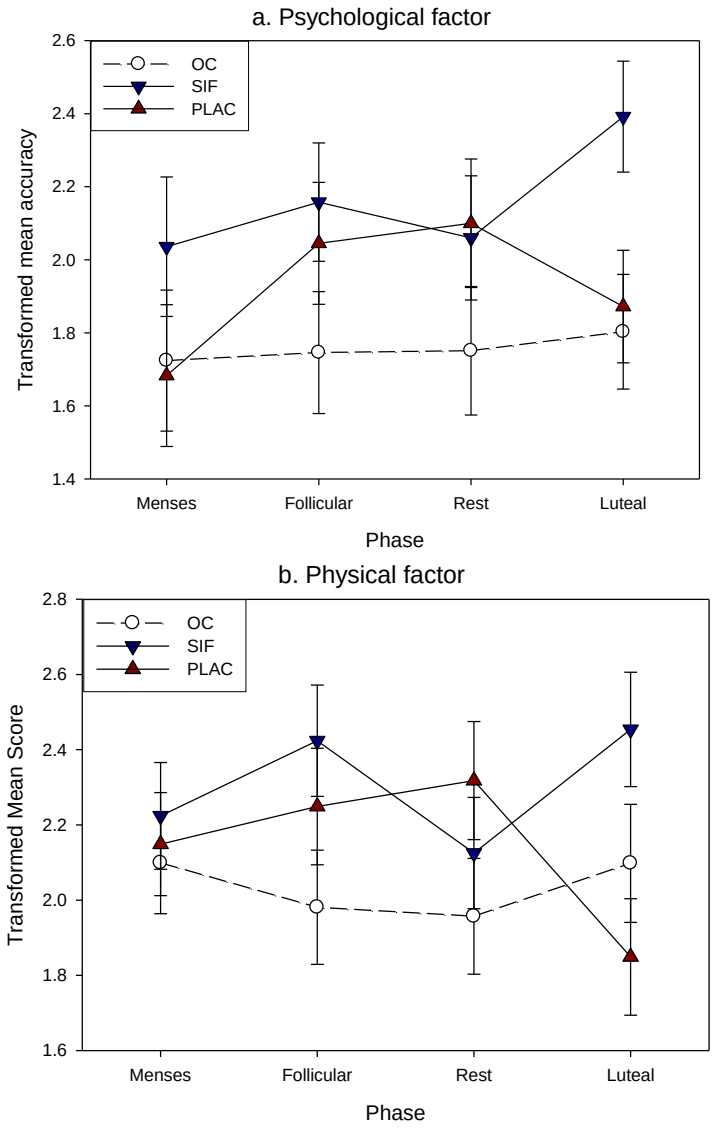

Figure 9.5 Transformed mean scores (±SEM) on **a.** the psychological factor and **b.** the physical factor of the DSR during the first treatment cycle for OC users, women treated with SIF and women treated with placebo across the cycle after controlling for baseline scores.

9.6. Aggression Measures

Mean scores, standard deviations and statistical values for measures of aggression are presented in Table 9.5. Results of post hoc pairwise comparisons are given in Table 9.6.

Buss-Perry Aggression Questionnaire (BPAQ): After controlling for baseline scores there was a trend towards a phase x treatment interaction in the first treatment cycle for total BPAQ scores with the SIF group reporting significantly reduced aggression compared with placebo during menses, and the OC group having significantly lower aggression during the ovulatory phase compared to SIF and placebo. In the second treatment cycle there was a significant main effect of treatment on total BPAQ scores, with OC users reporting significantly lower aggression than SIF and placebo. For the anger subscale there was a significant phase x treatment interaction in the first treatment cycle with placebo being associated with more anger than OC and SIF during menses, whilst in the ovulatory phase OC was associated with reduced anger compared with SIF and placebo, and in the luteal phase OC users were less angry than SIF users. There was also a significant main effect of treatment with OC users being less angry than placebo. In the second treatment cycle there was also a significant main effect of treatment with OC use being associated with less anger than SIF and placebo. For scores on the hostility subscale, in the first treatment cycle there was a trend towards a phase x treatment interaction, with SIF being associated with greater hostility than OC during the ovulatory and luteal phases, as well as being associated with greater hostility than placebo in the luteal phase. For verbal aggression, OC use was associated with significantly lower scores than placebo in both treatment cycles. In the first treatment cycle, there was a significant effect of phase on physical aggression, with the follicular phase being associated with higher physical aggression than the ovulatory and luteal phases. In the second treatment cycle OC use was associated with significantly lower physical aggression than SIF and placebo.

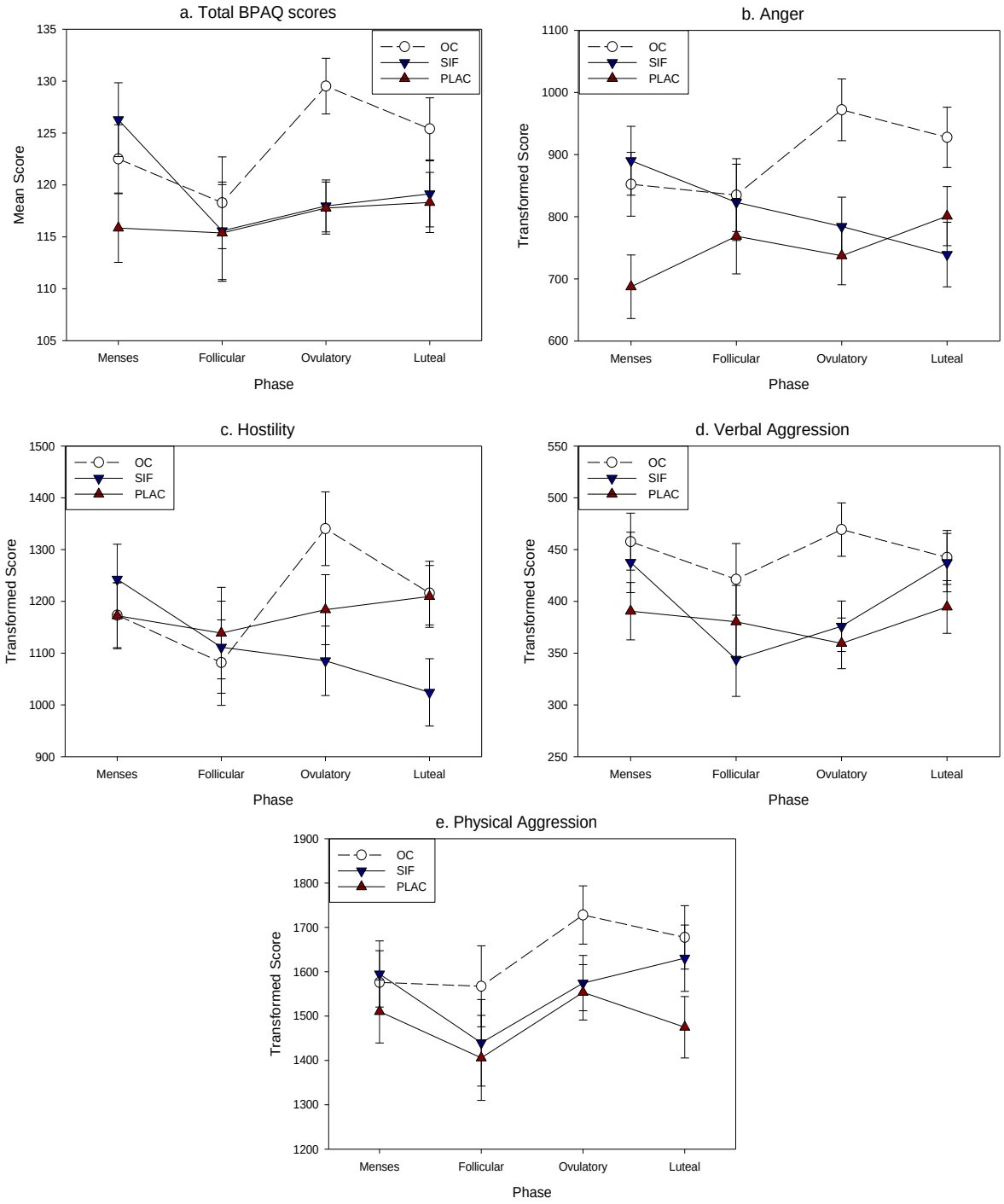

Figure 9.6 Mean aggression ratings on the BPAQ for OC users, women treated with SIF and women treated with placebo during different phases of the first treatment cycle. Graphs depict mean (±SEM) scores for **a.** Total BPAQ scores, **b.** Transformed anger scores, **c.** Transformed hostility scores, **d.** Transformed verbal aggression scores and **e.** Transformed physical aggression scores. A lower score indicates higher aggression.

Table 9.5 *Means and standard deviations for subjective and objective measures of aggression during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction*.

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,54	Treatment Group (T); df=2,67	P x T interaction; df=6,57
BPAQ - Total	1	OC	123.73 (13.15)	121.38 (26.63)	131.53 (10.38)	127.41 (13.14)	NS	NS	2.06^
		SIF	115.14 (22.34)	118.94 (19.22)	118.38 (18.41)	118.77 (20.98)			
		Placebo	113.24 (26.08)	115.08 (27.53)	118.50 (23.71)	120.20 (23.24)			
	2	OC	126.89 (15.86)	126.50 (12.69)	128.20 (12.71)	130.71 (11.29)	NS	4.05*	NS
		SIF	111.25 (25.45)	116.25 (21.99)	114.74 (22.04)	115.55 (22.67)			
		Placebo	116.27 (24.66)	115.14 (26.75)	120.61 (23.62)	118.17 (25.83)			
Hostility	1	OC	33.64 (6.14)	33.52 (8.30)	36.83 (4.01)	34.50 (5.68)	NS	NS	1.98^
		SIF	31.67 (6.53)	33.67 (5.31)	31.71 (7.64)	31.59 (7.42)			
		Placebo	32.63 (8.11)	32.16 (9.26)	33.85 (7.62)	34.65 (6.75)			
	2	OC	34.84 (5.35)	34.55 (5.49)	35.20 (5.02)	36.75 (4.07)	NS	NS	NS
		SIF	30.55 (7.47)	31.65 (7.79)	32.47 (7.13)	32.70 (6.85)			
		Placebo	34.18 (7.27)	33.00 (8.01)	34.35 (7.35)	33.25 (8.10)			
Anger	1	OC	29.05 (3.23)	28.24 (6.46)	31.15 (3.44)	30.18 (3.66)	NS	3.45*	3.40**
		SIF	27.29 (6.11)	28.56 (4.66)	28.00 (5.42)	27.36 (4.88)			
		Placebo	25.36 (6.68)	27.17 (6.09)	26.90 (6.70)	28.10 (6.03)			
	2	OC	29.41 (5.41)	29.45 (4.14)	29.50 (3.83)	30.36 (4.51)	NS	4.15*	NS
		SIF	26.80 (6.32)	27.05 (5.28)	26.21 (6.02)	26.90 (6.06)			
		Placebo	25.91 (7.15)	26.81 (6.01)	27.65 (5.79)	27.46 (6.95)			
Verbal	1	OC	20.77 (3.54)	20.05 (5.20)	21.60 (3.15)	21.41 (3.29)	NS	2.64^	NS
		SIF	18.52 (5.23)	18.67 (5.01)	19.19 (4.49)	20.41 (5.11)			
		Placebo	18.13 (5.24)	18.64 (5.83)	19.40 (4.91)	19.70 (5.41)			
	2	OC	21.32 (3.76)	20.73 (3.67)	21.85 (3.01)	21.86 (3.99)	NS	2.89^	NS
		SIF	18.55 (6.04)	19.35 (5.60)	18.74 (4.85)	18.25 (5.62)			
		Placebo	18.27 (5.57)	18.48 (6.42)	20.13 (4.65)	19.29 (5.35)			

Physical	1	OC	40.27 (3.72)	39.57 (6.85)	41.95 (2.67)	41.32 (3.27)	3.03*	NS	NS
		SIF	37.67 (6.90)	38.06 (6.94)	39.48 (5.12)	39.41 (5.96)			
		Placebo	37.13 (9.06)	37.12 (8.25)	38.35 (7.20)	37.75 (6.93)			
	2	OC	41.32 (3.68)	41.77 (3.62)	41.65 (4.27)	41.73 (2.96)	NS	3.71*	NS
		SIF	35.35 (9.09)	38.20 (6.48)	37.32 (6.97)	37.70 (6.82)			
		Placebo	37.91 (7.14)	36.86 (8.53)	38.48 (7.08)	38.17 (7.12)			
STAXI – S-Anger	1	OC	10.64 (1.59)	10.43 (1.08)	10.10 (0.31)	10.45 (0.91)	3.76*	3.86*	NS
		SIF	12.52 (3.89)	11.56 (2.53)	11.90 (3.49)	12.41 (3.45)			
		Placebo	11.75 (4.83)	12.56 (6.18)	12.05 (4.76)	12.00 (4.14)			
	2	OC	11.36 (3.29)	10.45 (1.10)	10.40 (0.68)	10.55 (1.53)	NS	2.85^	NS
		SIF	13.75 (5.91)	11.65 (3.28)	12.94 (4.29)	12.95 (4.33)			
		Placebo	12.05 (4.81)	12.10 (3.81)	11.57 (3.74)	11.96 (4.04)			
T-Anger	1	OC	15.68 (3.67)	15.76 (5.54)	15.32 (5.96)	16.18 (5.25)	NS	NS	NS
		SIF	18.60 (6.19)	18.76 (6.44)	19.78 (5.69)	19.12 (5.63)			
		Placebo	18.43 (6.14)	17.61 (6.21)	17.80 (6.25)	17.82 (6.92)			
	2	OC	16.30 (5.73)	15.70 (5.20)	14.92 (5.36)	14.88 (4.91)	NS	NS	NS
		SIF	18.90 (6.94)	19.08 (6.21)	20.00 (7.20)	18.70 (6.42)			
		Placebo	18.55 (7.06)	18.67 (6.09)	17.09 (6.10)	17.96 (5.94)			
T-Anger/T	1	OC	5.59 (1.56)	5.57 (2.58)	5.20 (2.59)	5.64 (2.19)	NS	NS	NS
		SIF	7.05 (3.06)	6.72 (3.03)	6.95 (2.62)	6.77 (2.81)			
		Placebo	6.96 (3.29)	6.50 (2.68)	6.55 (2.95)	6.15 (2.94)			
	2	OC	6.00 (2.64)	5.30 (2.20)	5.05 (1.93)	5.18 (1.89)	NS	NS	NS
		SIF	6.65 (3.07)	6.85 (2.98)	7.17 (3.17)	6.70 (3.21)			
		Placebo	6.68 (3.05)	6.95 (2.67)	6.04 (2.25)	6.38 (2.73)			
T-Anger/R	1	OC	7.36 (2.17)	7.52 (2.77)	7.50 (2.84)	7.86 (2.90)	NS	NS	NS
		SIF	8.33 (3.01)	8.89 (3.22)	9.48 (3.22)	8.91 (3.18)			
		Placebo	8.33 (2.53)	8.11 (2.83)	8.10 (2.79)	8.35 (2.94)			
	2	OC	7.59 (2.67)	7.73 (2.80)	7.25 (3.16)	7.32 (2.64)	NS	NS	NS
		SIF	8.90 (3.40)	9.10 (3.01)	9.17 (3.52)	8.70 (3.45)			
		Placebo	8.45 (3.08)	8.10 (2.83)	7.65 (3.11)	8.17 (2.66)			
AX/In	1	OC	16.05 (5.15)	15.67 (4.96)	14.90 (3.71)	15.55 (4.08)	NS	NS	NS
		SIF	18.43 (5.17)	16.44 (5.27)	16.57 (5.91)	17.91 (5.55)			

		Placebo	16.00 (4.70)	16.22 (5.40)	14.80 (3.53)	15.50 (4.16)			
	2	OC	15.59 (3.80)	16.09 (4.28)	15.40 (4.15)	15.18 (3.79)			
		SIF	16.80 (5.15)	17.80 (5.02)	16.61 (5.28)	16.65 (4.45)	NS	NS	NS
		Placebo	15.45 (5.03)	16.45 (5.15)	16.39 (5.36)	16.03 (5.18)			
AX/Out	1	OC	12.59 (3.50)	12.71 (4.45)	12.75 (3.88)	12.91 (3.99)			
		SIF	14.48 (4.25)	14.67 (4.01)	15.14 (4.50)	14.41 (4.65)	NS	NS	NS
		Placebo	16.17 (5.34)	14.72 (3.79)	15.00 (4.33)	14.45 (4.20)			
	2	OC	13.05 (4.20)	13.15 (3.91)	12.65 (3.54)	12.95 (4.16)			
		SIF	14.75 (4.28)	14.50 (3.68)	15.61 (4.65)	14.90 (4.52)	NS	NS	NS
		Placebo	15.20 (4.71)	15.37 (4.05)	14.74 (4.33)	14.92 (4.61)			
AX/Con	1	OC	26.27 (4.80)	25.14 (5.16)	26.50 (5.33)	26.27 (5.07)			
		SIF	22.07 (4.97)	22.11 (4.76)	22.10 (4.97)	21.86 (4.51)	NS	4.42*	NS
		Placebo	22.58 (5.32)	24.56 (5.38)	22.55 (5.53)	22.65 (5.30)			
	2	OC	25.14 (5.41)	26.59 (5.15)	27.55 (4.86)	26.68 (4.71)			
		SIF	21.85 (4.80)	23.15 (4.80)	22.11 (5.79)	21.80 (4.66)	2.36^	5.65**	NS
		Placebo	22.45 (4.73)	21.57 (5.26)	23.30 (5.10)	23.13 (5.56)			
AX/EX	1	OC	18.36 (9.07)	19.24 (10.61)	17.15 (9.31)	18.18 (9.87)			
		SIF	26.83 (9.53)	25.00 (10.50)	25.62 (11.12)	26.45 (10.41)	NS	3.58*	NS
		Placebo	25.58 (12.49)	22.39 (11.65)	23.25 (10.57)	23.30 (11.08)			
	2	OC	19.50 (9.73)	18.65 (9.60)	16.50 (9.56)	17.45 (9.75)			
		SIF	25.70 (10.75)	25.15 (9.95)	26.11 (12.42)	25.75 (10.33)	NS	3.56*	NS
		Placebo	24.20 (11.41)	26.24 (10.25)	23.83 (10.86)	23.82 (12.21)			
DSR - Anger	1	OC	0.35 (0.46)	0.25 (0.36)	0.22 (0.34)	0.22 (0.37)			
		SIF	0.50 (0.61)	0.49 (0.62)	0.43 (0.47)	0.66 (0.65)	NS	NS	NS
		Placebo	0.26 (0.38)	0.34 (0.52)	0.31 (0.44)	0.38 (0.57)			
	2	OC	0.25 (0.52)	0.09 (0.20)	0.20 (0.32)	0.16 (0.31)			
		SIF	0.67 (0.76)	0.43 (0.53)	0.34 (0.49)	0.49 (0.77)	NS	NS	NS
		Placebo	0.40 (0.64)	0.28 (0.47)	0.14 (0.23)	0.20 (0.33)			
Aggression	1	OC	0.32 (0.46)	0.15 (0.27)	0.15 (0.27)	0.10 (0.20)			
		SIF	0.49 (0.60)	0.45 (0.54)	0.44 (0.57)	0.63 (0.60)	NS	NS	2.67*
		Placebo	0.22 (0.33)	0.27 (0.51)	0.25 (0.47)	0.37 (0.62)			
	2	OC	0.31 (0.63)	0.12 (0.23)	0.12 (0.25)	0.08 (0.21)			

		SIF	0.63 (0.79)	0.47 (0.62)	0.47 (0.61)	0.56 (0.84)	NS	3.01^	NS
		Placebo	0.35 (0.64)	0.34 (0.53)	0.18 (0.31)	0.23 (0.46)			
Impulsiveness	1	OC	0.22 (0.27)	0.23 (0.31)	0.22 (0.42)	0.20 (0.38)	NS	NS	NS
		SIF	0.60 (0.68)	0.50 (0.58)	0.52 (0.58)	0.71 (0.78)			
		Placebo	0.31 (0.36)	0.37 (0.42)	0.27 (0.38)	0.42 (0.57)			
	2	OC	0.46 (0.74)	0.23 (0.41)	0.23 (0.44)	0.19 (0.49)	3.15 *	2.73^	NS
		SIF	0.84 (0.93)	0.60 (0.88)	0.52 (0.62)	0.57 (0.57)			
		Placebo	0.47 (0.57)	0.37 (0.53)	0.18 (0.24)	0.27 (0.40)			
Emotional Stroop – Colour	1	OC	634.45 (100.10)	614.03 (100.47)	635.73 (104.88)	605.61 (89.24)	NS	NS	NS
		SIF	631.30 (98.11)	664.40 (103.17)	599.50 (82.03)	618.31 (91.81)			
		Placebo	664.97 (134.83)	607.19 (104.09)	605.04 (114.12)	629.34 (132.64)			
	2	OC	605.73 (94.85)	611.01 (89.03)	588.93 (84.91)	580.28 (75.44)	NS	NS	NS
		SIF	639.00 (89.60)	589.79 (79.46)	597.21 (90.31)	594.52 (83.10)			
		Placebo	621.54 (137.82)	621.18 (113.62)	596.12 (92.70)	624.42 (96.11)			
Direct aggressive	1	OC	593.60 (95.74)	583.31 (73.33)	585.94 (93.02)	591.14 (85.50)	2.89*	NS	NS
		SIF	586.60 (87.01)	603.77 (64.28)	585.18 (62.80)	591.85 (79.97)			
		Placebo	584.04 (108.98)	559.20 (63.39)	576.04 (83.10)	600.26 (93.21)			
	2	OC	580.66 (90.13)	584.94 (77.24)	568.92 (78.46)	575.90 (81.65)	3.98*	NS	NS
		SIF	586.27 (68.24)	591.49 (69.14)	572.33 (56.31)	571.18 (52.96)			
		Placebo	597.76 (100.29)	588.44 (97.31)	587.24 (88.67)	594.78 (82.76)			
Indirect aggressive	1	OC	595.30 (97.68)	579.52 (74.36)	575.33 (84.96)	578.75 (81.63)	NS	NS	NS
		SIF	586.03 (79.82)	608.75 (65.35)	577.94 (69.63)	586.17 (74.58)			
		Placebo	580.15 (99.64)	569.59 (70.21)	574.19 (85.21)	600.14 (101.60)			
	2	OC	585.80 (89.94)	578.52 (73.69)	570.01 (63.56)	577.34 (88.36)	NS	NS	NS
		SIF	586.18 (64.70)	582.11 (66.59)	584.64 (58.71)	572.84 (59.90)			
		Placebo	599.68 (100.08)	589.64 (91.64)	586.97 (80.94)	587.44 (80.57)			
Positive affect	1	OC	592.33 (99.38)	587.43 (74.18)	579.58 (90.12)	593.14 (87.22)	2.70^	NS	NS
		SIF	596.58 (87.08)	609.60 (72.56)	589.02 (67.08)	581.89 (78.19)			
		Placebo	587.34 (103.99)	560.21 (71.09)	575.46 (85.87)	499.74 (111.90)			

	2	OC	586.75 (97.32)	580.35 (69.91)	565.76 (72.62)	572.96 (88.64)	NS	NS	NS
		SIF	589.35 (70.25)	584.45 (62.65)	578.32 (58.02)	570.28 (53.35)			
		Placebo	608.86 (105.20)	593.12 (89.63)	584.53 (81.55)	592.70 (82.75)			
Negative affect	1	OC	584.83 (93.12)	588.04 (68.66)	580.78 (80.96)	592.03 (85.89)	2.93*	NS	NS
		SIF	591.31 (78.59)	601.13 (82.73)	604.39 (78.36)	582.09 (68.06)			
		Placebo	579.22 (103.10)	564.57 (70.40)	578.81 (92.24)	597.73 (104.87)			
	2	OC	585.30 (90.46)	579.92 (72.07)	573.96 (70.63)	572.61 (90.25)	2.76^	NS	NS
		SIF	590.26 (66.66)	586.62 (68.13)	580.99 (60.79)	572.56 (58.16)			
		Placebo	596.37 (103.81)	596.55 (101.88)	583.67 (78.22)	595.34 (81.83)			
Neutral	1	OC	591.83 (90.66)	586.32 (66.93)	579.85 (77.17)	588.35 (83.57)	3.50*	NS	NS
		SIF	591.16 (85.15)	603.19 (63.94)	587.40 (68.31)	587.27 (77.53)			
		Placebo	583.65 (105.35)	564.31 (62.82)	572.18 (92.51)	598.67 (110.78)			
	2	OC	591.97 (89.17)	582.00 (76.47)	570.82 (72.60)	575.58 (83.09)	2.53^	NS	NS
		SIF	594.58 (64.76)	584.77 (58.11)	575.02 (68.90)	579.23 (57.01)			
		Placebo	597.42 (93.67)	588.47 (101.43)	585.39 (85.56)	596.31 (84.52)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

State-Trait Anger Expression Inventory (STAXI): After controlling for baseline scores there was a significant main effect of treatment on state anger (S-Anger) in the first treatment cycle with SIF being associated with increased S-Anger compared with OC. There was also a significant main effect of phase with higher scores in the follicular than the ovulatory phase. In the second treatment cycle there was a trend towards a main effect of treatment with SIF being associated with higher S-Anger than OC.

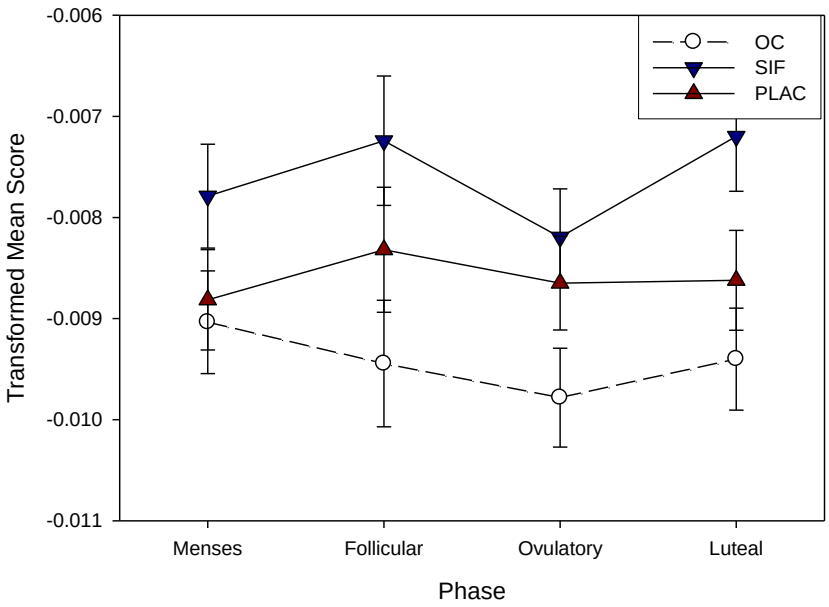

Figure 9.7 Transformed mean ratings of state anger on the STAXI during different phases of the first treatment cycle for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores.

For anger control (AX/Con) there was a significant main effect of treatment in the both treatment cycles with OC use being associated with higher ratings of control than both SIF and placebo. There was also a trend towards a main effect of phase in the second treatment cycle with poorer anger control during menses than the ovulatory phase. In both treatment cycles, there was a significant main effect of treatment on anger expression (AX/EX) with OC use being associated with lower AX/EX than SIF and placebo.

Table 9.6 *Results of post hoc comparisons of estimated marginal means where significant main effects of treatment, phase and their interaction were found on aggression outcomes in the two treatment cycles.*

Outcome	Main effect of Phase	Main effect of Treatment	Phase x Treatment Interaction
Buss-Perry Aggression Questionnaire (BPAQ) Total	NS	TREATMENT CYCLE 2 SIF > OC (t=2.54, p=0.016, d=0.78) Plac > OC (t=2.43, p=0.021, d=0.72)	TREATMENT CYCLE 1: Menses: Plac > SIF (t=2.17, p=0.034, d=0.66) Ovulatory: SIF > OC (t=3.12, p=0.003, d=1.00); Plac > OC (t=3.17, p=0.003, d=1.03)
BPAQ Anger	NS	TREATMENT CYCLE 1 Plac > OC (t=2.62, p=0.013, d=0.77) TREATMENT CYCLE 2 SIF > OC (t=2.44, p=0.018, d=0.74) Plac > OC (t=2.58, p=0.013, d=0.76)	TREATMENT CYCLE 1: Menses: Plac > OC (t=2.27, p=0.027, d=0.69); Plac > SIF (t=2.69, p=0.009, d=0.82) Ovulatory: SIF > OC (t=2.72, p=0.009, d=0.87); Plac > OC (t=3.43, p=0.001, d=1.11) Luteal: SIF > OC (t=2.64, p=0.011, d=0.91)
BPAQ Hostility	NS	NS	TREATMENT CYCLE 1: Ovulatory: SIF > OC (t=2.60, p=0.012, d=0.83) Luteal: SIF > OC (t=2.13, p=0.039, d=0.74); SIF > Plac (t=2.10, p=0.041, d=0.71)
BPAQ Verbal Aggression	NS	TREATMENT CYCLE 1 Plac > OC (t=2.24, p=0.029, d=0.66) TREATMENT CYCLE 2 Plac > OC (t=2.20, p=0.033, d=0.65)	NS
BPAQ Physical Aggression	TREATMENT CYCLE 1 F > O (t=2.94, p=0.005, d=0.84) F > L (t=2.66, p=0.01, d=0.76)	TREATMENT CYCLE 2 SIF > OC (t=2.56, p=0.016, d=0.78) Plac > OC (t=2.10, p=0.044, d=0.62)	NS
State-Trait Anger Expression Inventory (STAXI) State Anger	TREATMENT CYCLE 1 F > O (t=2.31, p=0.025, d=0.66)	TREATMENT CYCLE 1 SIF > OC (t=2.78, p=0.008, d=0.84) TREATMENT CYCLE 2 SIF > OC (t=2.38, p=0.021, d=0.73)	NS
STAXI Anger Control	TREATMENT CYCLE 2 O > M (t=2.54, p=0.014, d=0.69)	TREATMENT CYCLE 1 OC > SIF (t=2.75, p=0.008, d=0.83)	NS

		OC > Plac (t=2.38, p=0.02, d=0.70) TREATMENT CYCLE 2 OC > SIF (t=2.90, p=0.005, d=0.89) OC > Plac (t=2.93, p=0.005, d=0.87)	
STAXI Anger Expression	NS	TREATMENT CYCLE 1 SIF > OC (t=2.54, p=0.014, d=0.77) Plac > OC (t=2.04, p=0.046, d=0.60) TREATMENT CYCLE 2 SIF > OC (t=2.34, p=0.022, d=0.71) Plac > OC (t=2.27, p=0.026, d=0.67)	NS
Modified Daily Symptom Report (DSR-20) Aggression	NS	TREATMENT CYCLE 2 SIF > OC (t=2.45, p=0.019, d=0.83)	TREATMENT CYCLE 1: Luteal: SIF > OC (t=3.61, p=0.001, d=1.22); SIF > Plac (t=2.07, 0.044, d=0.70) SIF only: L > M(t=2.62, p=0.011, d=1.24); L > F (t=2.21, p=0.031, d=1.04); L > R (t=2.81, p=0.007, d=1.33)
DSR-20 Impulsiveness	TREATMENT CYCLE 2 M > F (t=2.59, p=0.012, d=0.71) M > R (t=2.79, p=0.008, d=0.76) M > L (t=2.90, p=0.005, d=0.79)	TREATMENT CYCLE 2 SIF > OC (t=2.32, p=0.026, d=0.79)	NS
Emotional Stroop Direct Aggressive	TREATMENT CYCLE 1 L > M (t=2.89, p=0.006, d=0.76) TREATMENT CYCLE 2 F > M (t=2.75, p=0.008, d=0.74) L > M (t=2.82, p=0.007, d=0.73)	NS	NS
Emotional Stroop Positive Affect	TREATMENT CYCLE 1 O > M (t=2.08, p=0.042, d=0.56) L > M (t=2.61, p=0.012, d=0.69)	NS	NS
Emotional Stroop Negative Affect	TREATMENT CYCLE 1 O > M (t=2.61, p=0.011, d=0.70) L > M (t=2.70, p=0.009, d=0.71) TREATMENT CYCLE 2	NS	NS

	F > M (t=2.03, p=0.047, d=0.54) O > M (t=2.45, p=0.018, d=0.67) L > M (t=2.46, p=0.017, d=0.64)		
Emotional Stroop Neutral	TREATMENT CYCLE 1 O > M (t=2.57, p=0.012, d=0.69) L > M (t=3.06, p=0.003, d=0.80) TREATMENT CYCLE 2 L > M (t=2.66, p=0.011, d=0.69)	NS	NS

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; R, Rest of the Cycle; NS, Non-significant; OC, Oral Contraceptive Users; SIF, Soy Isoflavone Group; Plac, Placebo Group

Modified Daily Symptom Report (DSR-20): For aggression there was a significant phase x treatment interaction in the first treatment cycle with SIF being associated with higher aggression during the luteal phase than both OC and placebo. Furthermore, significant phase effects were found only for the SIF group where aggression was significantly increased during the luteal phase compared to all other phases. In the second treatment cycle there was a trend towards a main effect of treatment with SIF being associated with higher aggression than OC use. For impulsiveness, in the second treatment cycle there was a significant main effect of phase with higher impulsiveness during menses than all other phases. There was also a trend towards a main effect of treatment with SIF being associated with higher impulsiveness than OC.

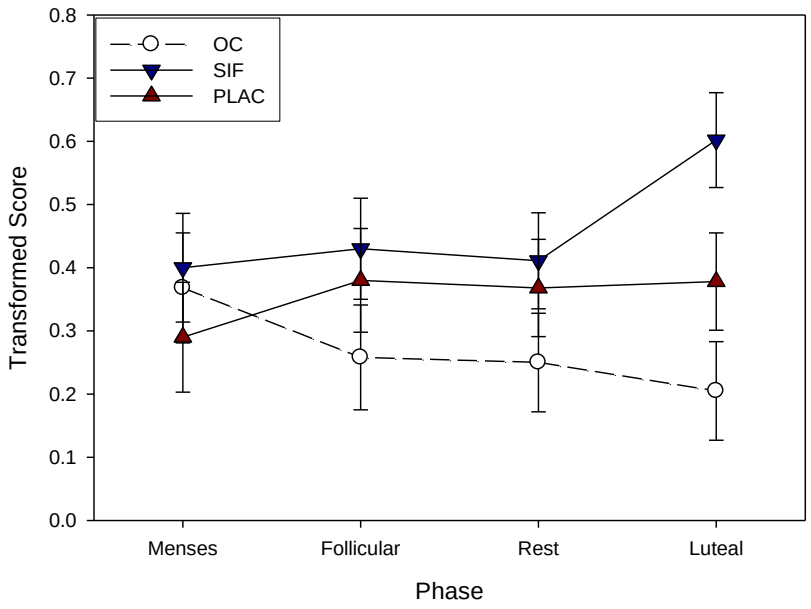

Figure 9.8 Transformed mean ratings on the aggression item of the DSR in the first treatment cycle for OC users, women treated with SIF and women treated with placebo across the cycle after controlling for baseline scores.

Emotional stroop task: After controlling for baseline results there was a significant main effect of phase on response times to direct aggressive stimuli in the first treatment cycle with significantly faster response times in menses than the luteal phase. In the second treatment cycle there was also a significant main effect of phase on response

times to direct aggressive stimuli with menses being associated with faster response times than the follicular and luteal phases. For both positive and affect, there was a trend towards a main effect of phase in the first treatment cycle, with faster response times in menses than the ovulatory and luteal phases. In the second treatment cycle there was a trend towards a main effect of phase on response times to negative affect stimuli, with faster responses during menses than the follicular, ovulatory and luteal phases. There was also a significant main effect of phase on neutral stimuli in the first treatment cycle, with faster response times in menses than the ovulatory and luteal phases. This effect was reduced to a trend in the second treatment cycle where response times were faster in menses than the luteal phase.

9.7. Cognitive Outcomes

Tables 9.7, 9.8, 9.9 and 9.10 show means and standard deviations for tasks of attention and reaction time, working memory, secondary memory and executive function, respectively. The results from post hoc comparisons of estimated marginal means are presented in Table 9.11.

Reaction time: After controlling for baseline scores, in the first treatment cycle there was a trend towards a phase x treatment interaction for choice reaction time with pairwise comparisons revealing that phase effects were only significant for the SIF group where reaction time was faster during menses than the follicular and luteal phases, and faster during the ovulatory phase than the luteal phase. In the second treatment cycle there was a trend towards a main effect of phase on choice reaction time, with slower reaction times during menses than the ovulatory phase.

Rapid Visual Information Processing (RVIP): After controlling for baseline scores there was a significant effect of BMI on false alarms in the first treatment cycle with higher BMI being associated with more false alarms ($F(1,38)=6.47$, $p=0.015$, $d=0.83$).

Card sorting task: After controlling for baseline scores there was a significant phase x treatment interaction for accuracy in the second treatment cycle, with pairwise comparisons revealing that OC use was associated with higher accuracy than placebo during menses, and that for the SIF group, accuracy was significantly poorer during the luteal phase compared with menses and the ovulatory phase, whereas for the placebo group accuracy was poorer during menses than the luteal phase. In the second treatment cycle there was a significant positive association between age and reaction time ($F(1,42)=4.20$, $p=0.047$, $d=0.63$).

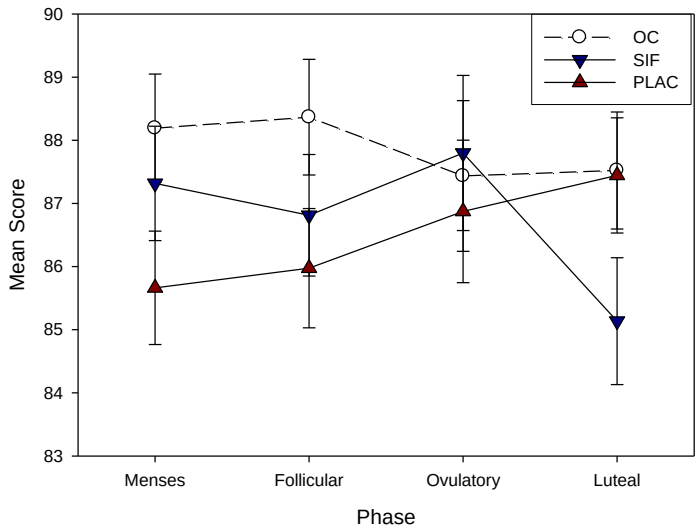

Figure 9.9 Mean accuracy scores on the card sorting task during different phases of the second treatment cycle for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores.

Table 9.7 *Means and standard deviations for tasks of attention and reaction time during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction*.

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values (p-value)		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,50	Treatment Group (T); df=2,61	P x T interaction; df=6,53
Simple reaction time	1	OC	292.03 (62.93)	291.12 (71.24)	332.85 (152.10)	314.45 (78.95)	NS	NS	NS
		SIF	340.38 (108.97)	339.89 (91.40)	305.03 (62.15)	377.83 (199.67)			
		Placebo	312.18 (67.62)	319.74 (90.99)	320.74 (73.25)	331.28 (87.97)			
	2	OC	310.56 (93.60)	295.93 (68.30)	299.93 (80.85)	299.38 (81.44)	NS	NS	NS
		SIF	322.22 (89.45)	326.11 (84.57)	330.19 (119.96)	326.37 (78.98)			
		Placebo	320.25 (67.04)	325.78 (132.06)	333.62 (122.27)	346.37 (150.66)			
Choice reaction time	1	OC	390.25 (80.59)	379.53 (58.03)	379.27 (56.82)	393.45 (80.89)	NS	NS	1.98^
		SIF	391.85 (55.71)	419.53 (62.29)	390.82 (49.34)	428.33 (65.21)			
		Placebo	388.09 (46.94)	405.68 (68.38)	424.77 (152.52)	400.37 (60.93)			
	2	OC	404.12 (132.85)	416.66 (177.64)	402.37 (181.40)	396.37 (113.27)	2.65^	NS	NS
		SIF	475.35 (153.59)	443.99 (119.98)	412.78 (76.18)	410.84 (99.06)			
		Placebo	471.88 (171.86)	414.24 (72.34)	402.79 (80.09)	451.15 (185.40)			
Four choice reaction time	1	OC	459.90 (93.42)	426.34 (53.22)	452.31 (83.42)	451.12 (92.39)	NS	NS	NS
		SIF	458.37 (71.02)	548.47 (194.97)	454.22 (73.17)	476.98 (81.67)			
		Placebo	446.96 (60.94)	487.70 (110.13)	438.57 (45.41)	463.04 (79.13)			
	2	OC	454.71	449.58	478.64 (156.38)	453.54 (127.33)	NS	NS	NS

			(113.70)	(135.51)				
		SIF	469.87 (89.72)	458.12 (68.00)	450.34 (68.70)	441.69 (62.28)		
		Placebo	445.29 (58.41)	462.29 (88.84)	443.37 (54.81)	456.71 (76.05)		
RVIP: *Accuracy*	1	OC	50.92 (18.30)	56.66 (25.66)	51.32 (24.82)	53.12 (22.09)	NS	NS	NS
		SIF	42.78 (22.83)	35.88 (22.79)	40.54 (24.60)	43.93 (20.34)			
		Placebo	37.76 (24.66)	37.17 (25.14)	35.15 (26.06)	32.83 (18.59)			
	2	OC	54.05 (26.92)	55.16 (24.63)	48.55 (23.29)	50.11 (25.10)	NS	NS	NS
		SIF	36.81 (25.86)	38.88 (23.39)	38.45 (27.83)	44.03 (24.26)			
		Placebo	33.90 (25.18)	33.75 (26.41)	36.81 (25.09)	35.92 (27.84)			
False alarms	1	OC	6.90 (10.22)	6.45 (8.15)	7.90 (7.62)	5.68 (6.47)	NS	NS	NS
		SIF	7.89 (7.07)	9.47 (10.40)	6.56 (6.67)	8.95 (11.53)			
		Placebo	6.18 (8.94)	7.00 (11.63)	6.19 (9.14)	5.76 (10.71)			
	2	OC	10.77 (13.14)	7.48 (9.22)	6.90 (7.47)	7.50 (8.50)	NS	NS	NS
		SIF	9.32 (11.75)	11.05 (11.40)	9.07 (12.31)	5.31 (6.61)			
		Placebo	6.22 (10.73)	3.00 (3.16)	5.05 (10.60)	8.90 (13.66)			
Reaction time	1	OC	480.55 (56.55)	484.16 (69.76)	488.96 (57.59)	487.52 (60.23)	NS	NS	NS
		SIF	486.31 (49.56)	479.66 (51.40)	503.19 (45.69)	496.26 (69.50)			
		Placebo	480.10 (84.04)	496.60 (90.80)	480.20 (57.69)	483.34 (75.66)			
	2	OC	476.47 (61.54)	486.10 (55.07)	488.49 (51.05)	493.05 (65.56)	NS	NS	NS
		SIF	477.91 (64.03)	506.83 (82.30)	508.95 (68.93)	481.25 (43.24)			
		Placebo	485.43 (75.48)	457.86 (55.75)	493.60 (65.44)	480.84 (76.38)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Peg and ball task: After controlling for baseline scores there was a trend towards a phase x treatment interaction on accuracy in the second treatment cycle, with pairwise comparisons revealing that OC use was associated with poorer accuracy than placebo during the luteal phase, and that phase effects were only significant for OC users who had better accuracy during the follicular phase than the ovulatory and luteal phases. For thinking time, in the first treatment cycle there were significant positive associations of both age ($F(1,56)=11.22$, $p=0.001$, $d=0.90$) and BMI ($F(1,57)=5.25$, $p=0.026$, $d=0.61$). Similar results were found in the second treatment cycle for age ($F(1,51)=4.91$, $p=0.031$, $d=0.62$) and BMI ($F(1,52)=5.07$, $p=0.029$, $d=0.62$). For completion time, there was a significant positive association of age in the first treatment cycle ($F(1,41)=10.95$, $p=0.002$, $d=1.03$). Although there was no significant phase x treatment interaction, pairwise comparisons revealed that SIF was associated with slower completion times than OC during the luteal phase, and that phase effects were only evident for the SIF group where completion times were slower during the luteal phase than the follicular and ovulatory phases. In the second treatment cycle, only the association of age was significant ($F(1,43)=5.65$, $p=0.022$, $d=0.73$).

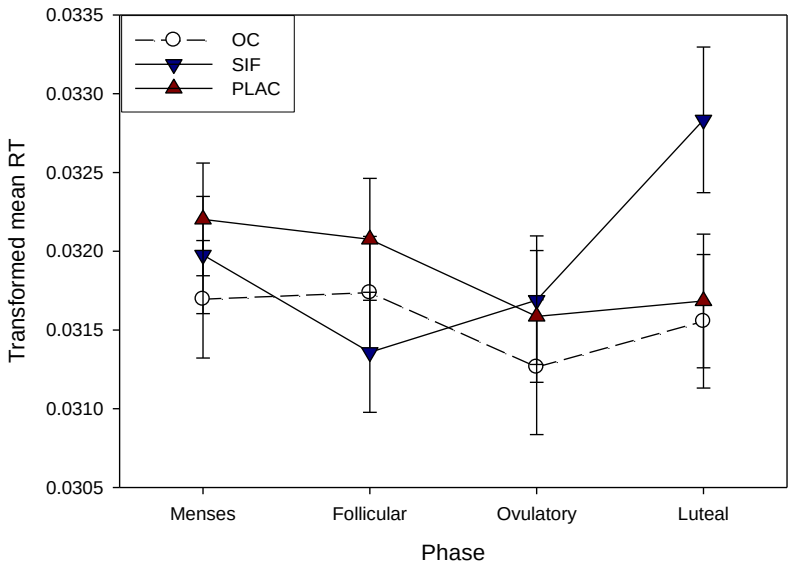

Figure 9.10 Transformed mean completion times on the peg and ball task during different phases of the first treatment cycle for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores.

Serial 3 subtractions: After controlling for baseline scores, there was a trend towards a main effect of treatment with OC use being associated with more total subtractions than SIF and placebo in the first treatment cycle. In the second treatment cycle there was a trend towards a main effect of phase, with more total subtractions during the ovulatory phase than menses. For reaction time, in the first treatment cycle there was a significant negative association of years of education ($F(1,40)=4.36$, $p=0.043$, $d=0.66$).

Serial 7 subtractions: After controlling for baseline scores, in the second treatment cycle there was a significant phase x treatment interaction with pairwise comparisons revealing that SIF was associated with significantly higher accuracy than OC during the luteal phase, and that phase effects were only significant for OC users where accuracy was higher during the follicular phase than the luteal phase.

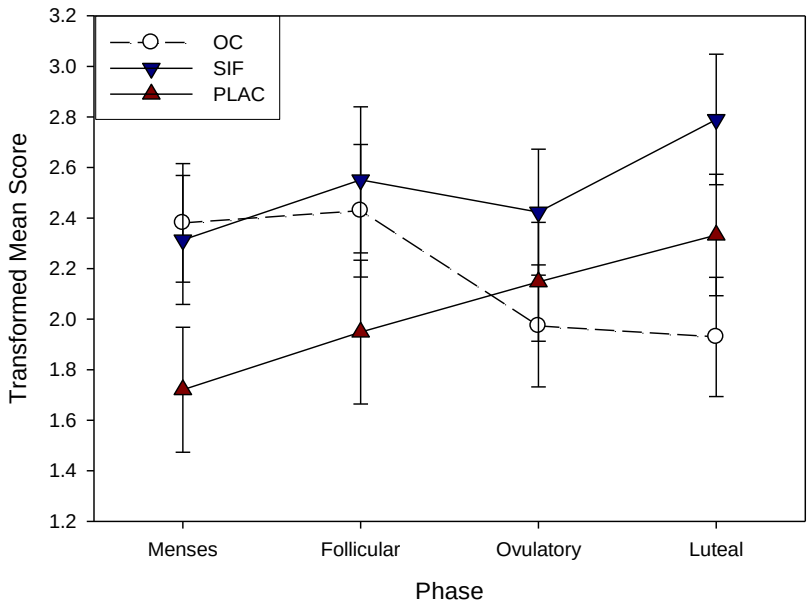

Figure 9.11 Transformed accuracy scores (±SEM) on the serial seven subtractions task during the second treatment cycle for OC users, women treated with SIF and women treated with placebo, after controlling for baseline scores.

Table 9.8 *Means and standard deviations for tasks of executive function during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,56	Treatment Group (T); df=2,54	P x T interaction; df=6,56
Card sort: *Accuracy*	1	OC	86.41 (3.11)	87.52 (3.52)	87.35 (3.60)	88.32 (5.36)	NS	NS	NS
		SIF	87.05 (3.71)	86.69 (4.74)	87.24 (4.29)	85.73 (4.95)			
		Placebo	85.39 (3.30)	85.72 (4.39)	86.21 (3.71)	87.42 (3.99)			
	2	OC	87.86 (4.98)	88.05 (5.70)	88.21 (6.41)	87.45 (4.96)	NS	NS	2.86*
		SIF	87.40 (4.31)	87.00 (4.18)	87.94 (4.68)	86.32 (4.91)			
		Placebo	85.48 (3.80)	86.30 (4.09)	86.00 (5.32)	87.63 (3.67)			
Reaction time	1	OC	1161.00 (259.37)	1110.14 (222.10)	1077.30 (176.15)	1119.67 (214.24)	NS	NS	NS
		SIF	1088.71 (222.05)	1274.17 (284.36)	1086.71 (220.30)	1090.27 (203.59)			
		Placebo	1157.67 (234.28)	1160.22 (239.86)	1144.15 (225.02)	1157.10 (235.64)			
	2	OC	1108.59 (178.55)	1158.50 (238.43)	1128.10 (330.85)	1037.50 (226.85)	NS	NS	NS
		SIF	1120.45 (174.12)	1133.95 (252.73)	1162.22 (344.19)	1160.00 (259.84)			
		Placebo	1176.05 (278.91)	1137.95 (264.40)	1179.74 (274.09)	1104.48 (178.50)			
Peg and ball: *Accuracy*	1	OC	91.19 (9.21)	91.43 (9.10)	90.25 (7.86)	92.73 (6.50)	NS	NS	NS
		SIF	90.95 (6.64)	88.06 (11.00)	90.50 (9.30)	92.27 (6.31)			
		Placebo	90.21 (6.83)	91.11 (10.51)	92.25 (9.80)	90.25 (11.06)			
	2	OC	91.95 (9.06)	93.86 (6.35)	89.25 (8.78)	90.00 (6.17)	NS	NS	2.26^
		SIF	93.00 (8.49)	90.00 (8.58)	91.94 (8.07)	91.00 (7.88)			

		Placebo	88.41 (13.66)	93.10 (6.98)	90.43 (10.10)	92.92 (9.32)			
Extra moves	1	OC	4.00 (4.88)	2.68 (3.70)	4.37 (4.27)	3.27 (4.05)	NS	NS	NS
		SIF	3.67 (3.44)	3.89 (3.85)	4.45 (5.41)	2.82 (3.06)			
		Placebo	3.17 (2.43)	2.88 (3.24)	3.60 (5.30)	3.70 (4.09)			
	2	OC	3.18 (4.10)	2.64 (3.35)	4.15 (4.55)	3.95 (3.02)	NS	NS	NS
		SIF	1.90 (1.94)	3.10 (3.63)	2.33 (2.20)	3.05 (3.19)			
		Placebo	4.27 (5.27)	2.24 (2.28)	3.17 (4.07)	2.75 (4.35)			
Thinking time	1	OC	2082.76 (743.18)	1818.52 (460.46)	1907.15 (531.82)	1822.27 (484.44)	NS	NS	NS
		SIF	1787.45 (260.56)	1979.06 (380.83)	1800.10 (618.22)	1692.05 (350.97)			
		Placebo	1797.58 (399.44)	1821.67 (505.03)	1727.95 (357.72)	1723.30 (412.53)			
	2	OC	1699.38 (411.52)	1600.95 (416.27)	1598.58 (412.62)	1715.95 (658.07)	NS	NS	NS
		SIF	1689.68 (356.49)	1671.00 (345.84)	1558.22 (276.14)	1693.83 (440.38)			
		Placebo	1751.43 (429.34)	1749.35 (512.53)	1626.09 (358.90)	1757.42 (395.75)			
Completion time	1	OC	6041.48 (1370.15)	5789.05 (995.72)	6090.85 (1345.59)	5662.09 (1009.94)	NS	NS	NS
		SIF	5775.05 (879.25)	6033.39 (971.04)	5617.90 (992.63)	5278.82 (799.25)			
		Placebo	5637.58 (852.65)	5845.61 (1559.26)	5689.85 (1078.43)	5505.65 (1085.77)			
	2	OC	5680.82 (1443.72)	5266.95 (1048.74)	5190.00 (853.15)	5418.00 (1522.74)	NS	NS	NS
		SIF	5490.15 (1054.70)	5513.50 (822.92)	5102.78 (750.13)	5177.47 (843.55)			
		Placebo	5684.29 (1227.68)	5391.00 (1011.98)	5281.35 (888.87)	5439.08 (978.85)			
Serial 3s:	1	OC	45.52 (19.88)	47.80 (18.82)	43.70 (15.28)	46.14 (19.57)	NS	2.77^	NS

Total		SIF	38.81 (15.05)	35.83 (14.10)	41.55 (10.79)	42.27 (14.36)			
		Placebo	37.04 (13.90)	39.11 (15.54)	39.50 (14.93)	38.40 (14.32)			
	2	OC	46.45 (15.08)	47.32 (20.27)	47.75 (17.90)	45.91 (17.52)			
		SIF	41.55 (13.72)	46.40 (15.09)	47.72 (15.30)	47.90 (20.77)	2.21^	NS	NS
		Placebo	38.27 (16.13)	40.76 (15.02)	42.09 (17.95)	43.25 (17.86)			
Accuracy	1	OC	92.57 (9.05)	91.54 (10.31)	91.82 (9.57)	90.24 (10.72)			
		SIF	93.47 (5.88)	92.50 (6.83)	94.06 (7.33)	94.77 (3.95)	NS	NS	NS
		Placebo	90.34 (9.16)	90.14 (9.66)	91.24 (9.65)	90.49 (7.99)			
	2	OC	93.43 (5.53)	89.36 (11.70)	93.61 (7.07)	91.02 (9.80)			
		SIF	93.69 (7.55)	95.09 (4.86)	95.92 (4.28)	95.72 (7.19)	NS	NS	NS
		Placebo	92.79 (7.66)	91.53 (7.91)	92.46 (8.57)	93.04 (7.24)			
Reaction time	1	OC	2902.95 (1102.87)	2937.35 (1420.17)	2854.89 (829.24)	2629.95 (810.44)			
		SIF	3013.10 (747.70)	3366.18 (936.16)	3058.80 (919.76)	3044.35 (1103.96)	NS	NS	NS
		Placebo	3517.14 (1475.88)	2994.94 (840.99)	3322.70 (1054.28)	3553.05 (1300.36)			
	2	OC	2687.38 (794.37)	3029.59 (1419.64)	2821.65 (1051.99)	2980.45 (1341.45)			
		SIF	3120.40 (1122.60)	2810.75 (1035.02)	2759.00 (1135.69)	2771.22 (1525.24)	NS	NS	NS
		Placebo	3376.71 (1253.27)	3282.90 (1134.36)	3125.23 (1166.86)	3247.71 (1515.69)			
Serial 7s: *Total*	1	OC	23.71 (11.06)	24.55 (13.52)	25.15 (12.28)	25.95 (12.25)			
		SIF	23.95 (8.82)	23.56 (8.37)	23.50 (9.05)	25.64 (9.50)	NS	NS	NS
		Placebo	20.83 (8.27)	21.44 (7.72)	21.30 (9.41)	21.65 (11.16)			
	2	OC	27.95 (11.83)	28.41 (15.22)	25.05 (11.34)	28.33 (14.97)			
		SIF	24.90 (9.05)	26.15 (8.49)	26.61 (10.14)	28.60 (9.34)	NS	NS	NS
		Placebo	23.45 (10.79)	23.43 (11.38)	23.52 (11.73)	24.63 (12.69)			
Accuracy	1	OC	86.26 (15.15)	87.61 (11.60)	89.28 (13.04)	90.89 (10.12)			
		SIF	87.15 (10.00)	89.92 (8.60)	88.61 (11.65)	88.52 (11.88)	NS	NS	NS
		Placebo	87.74 (12.92)	85.66 (11.02)	84.32 (18.39)	78.64 (21.15)			

	2	OC	91.11 (8.88)	86.93 (15.04)	85.68 (12.93)	85.99 (13.27)	NS	NS	2.34*
		SIF	87.55 (15.43)	91.29 (8.98)	92.54 (5.65)	93.44 (6.99)			
		Placebo	82.25 (19.09)	86.36 (16.43)	84.70 (15.72)	86.68 (16.83)			
Reaction time	1	OC	5925.10 (2615.63)	5810.79 (2891.36)	5305.32 (2371.76)	5368.50 (2240.71)	NS	NS	NS
		SIF	5296.05 (1794.81)	5466.11 (2345.14)	5876.10 (2725.36)	4933.48 (1460.33)			
		Placebo	6058.23 (2529.14)	5951.72 (1927.54)	6269.05 (2915.40)	6321.89 (3242.83)			
	2	OC	4922.45 (2766.42)	4948.91 (2292.41)	5663.25 (2655.89)	5040.81 (2371.16)	NS	NS	NS
		SIF	5060.85 (2094.90)	4934.90 (1584.69)	4924.59 (1866.26)	4475.22 (1534.49)			
		Placebo	6048.91 (2820.66)	5718.75 (2660.95)	6101.83 (2687.42)	5689.30 (2842.86)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Delayed word recognition: After controlling for baseline scores there was a significant positive association between years of education and accuracy scores in the first treatment cycle ($F(1,36)=6.26$, $p=0.017$, $d=0.83$). In the second treatment cycle there was a marginally significant main effect of treatment with OC use being associated with higher accuracy than placebo, as well as a significant negative association between BMI and accuracy ($F(1,46)=5.44$, $p=0.024$, $d=0.69$). For reaction times, in the first treatment cycle there was a significant phase x treatment interaction, with pairwise comparisons revealing that significant phase effects were only found for the OC group where response times were significantly slower during menses than the follicular and ovulatory phases.

Delayed picture recognition: After controlling for baseline scores there was a significant negative association between BMI and accuracy during the second treatment cycle ($F(1,46)=4.10$, $p=0.049$, $d=0.60$).

Sentence Verification: After controlling for baseline scores there was a significant main effect of phase on reaction times in the first treatment cycle with faster reaction times in the follicular phase than the ovulatory and luteal phases. In the second treatment cycle there was a trend towards a main effect of treatment with OC use being associated with faster reaction times than placebo.

Table 9.9 *Means and standard deviations for tasks of secondary memory during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,51	Treatment Group (T); df=2,53	P x T interaction; df=6,51
Immediate word recall	1	OC	8.45 (2.31)	8.28 (2.78)	9.28 (1.89)	9.50 (2.26)	NS	NS	NS
		SIF	8.19 (2.32)	8.75 (2.73)	8.69 (2.50)	8.84 (2.74)			
		Placebo	8.90 (2.21)	8.64 (2.35)	8.70 (2.99)	8.75 (2.73)			
	2	OC	9.14 (1.49)	9.11 (2.36)	9.40 (1.96)	9.07 (2.58)	NS	NS	NS
		SIF	8.40 (3.15)	8.80 (2.77)	8.14 (2.21)	9.13 (2.32)			
		Placebo	8.84 (2.77)	8.98 (2.94)	8.72 (2.66)	8.50 (2.57)			
Delayed word recall	1	OC	6.43 (2.16)	5.85 (2.48)	6.73 (2.06)	7.09 (2.29)	NS	NS	NS
		SIF	5.67 (1.87)	6.50 (2.89)	6.00 (2.34)	6.18 (2.38)			
		Placebo	6.23 (2.78)	6.36 (2.77)	5.88 (3.50)	6.15 (3.38)			
	2	OC	6.69 (1.62)	7.00 (2.14)	6.88 (2.08)	6.74 (2.27)	NS	NS	NS
		SIF	5.80 (2.78)	6.53 (2.40)	6.53 (2.42)	6.55 (2.73)			
		Placebo	6.18 (3.41)	6.10 (3.13)	5.59 (3.00)	5.75 (2.82)			
Word recognition accuracy	1	OC	82.58 (8.60)	80.17 (10.09)	83.50 (8.82)	85.30 (7.54)	NS	NS	NS
		SIF	81.11 (12.75)	80.19 (11.29)	83.81 (8.18)	82.12 (12.06)			
		Placebo	84.31 (9.03)	84.44 (6.76)	84.33 (9.37)	84.67 (8.47)			
	2	OC	84.85 (6.15)	85.61 (8.81)	85.83 (6.39)	85.15 (8.46)	NS	3.19^	NS
		SIF	82.17 (12.49)	83.83 (10.61)	82.41 (11.25)	85.00 (11.21)			
		Placebo	82.73 (11.80)	82.22 (9.96)	82.61 (7.52)	82.92 (8.36)			
Word recognition RT	1	OC	816.33 (184.10)	764.63 (164.47)	749.55 (163.75)	776.59 (177.27)	NS	NS	2.74*
		SIF	822.85 (254.79)	963.59 (327.07)	865.57 (235.42)	849.92 (278.64)			
		Placebo	871.84 (235.85)	868.89 (270.28)	778.74 (143.30)	838.59 (220.92)			

	2	OC	749.67 (153.27)	803.71 (235.62)	747.89 (180.57)	776.50 (224.45)	NS	NS	NS
		SIF	873.00 (219.12)	883.05 (281.56)	846.25 (260.73)	850.14 (252.78)			
		Placebo	841.77 (181.21)	845.50 (183.74)	844.00 (182.87)	844.50 (170.15)			
Picture recognition accuracy	1	OC	87.50 (10.53)	87.28 (11.82)	91.04 (6.80)	85.42 (11.98)	NS	NS	NS
		SIF	87.50 (12.50)	86.34 (11.05)	87.70 (9.54)	85.42 (13.22)			
		Placebo	87.15 (11.91)	87.27 (10.83)	90.62 (10.80)	91.25 (9.64)			
	2	OC	88.64 (8.05)	86.17 (11.38)	88.96 (9.68)	88.64 (10.14)	NS	NS	NS
		SIF	86.46 (9.64)	87.08 (10.55)	85.42 (12.48)	89.04 (7.63)			
		Placebo	85.04 (12.11)	89.48 (13.67)	89.13 (8.68)	87.33 (10.95)			
Picture recognition RT	1	OC	735.86 (142.33)	711.58 (95.52)	724.00 (110.93)	742.00 (146.49)	NS	NS	NS
		SIF	722.19 (130.68)	845.67 (196.20)	727.31 (127.43)	700.04 (114.15)			
		Placebo	729.30 (138.79)	752.00 (155.74)	713.35 (95.19)	765.67 (167.72)			
	2	OC	771.23 (147.24)	737.43 (171.99)	683.63 (125.15)	689.48 (111.59)	NS	NS	NS
		SIF	729.68 (122.62)	793.40 (182.69)	739.18 (164.69)	725.97 (163.86)			
		Placebo	747.41 (100.82)	702.86 (111.16)	718.91 (103.86)	746.92 (137.09)			
Sentence verification accuracy	1	OC	95.23 (7.63)	96.32 (5.49)	97.00 (4.41)	95.23 (5.45)	NS	NS	NS
		SIF	94.74 (6.12)	91.67 (8.22)	91.19 (10.60)	91.90 (8.14)			
		Placebo	93.50 (8.75)	96.67 (4.20)	93.50 (9.75)	90.79 (10.44)			
	2	OC	96.14 (5.10)	93.86 (8.85)	93.50 (6.71)	93.18 (8.80)	NS	NS	NS
		SIF	92.00 (8.65)	92.75 (9.93)	91.18 (9.93)	92.54 (8.21)			
		Placebo	92.27 (10.43)	91.19 (9.21)	92.17 (7.81)	92.08 (8.84)			
Sentence verification	1	OC	1231.18 (317.01)	1128.47 (184.13)	1113.00 (140.23)	1211.41 (317.82)	3.77*	NS	NS

RT		SIF	1422.00 (485.39)	1368.25 (428.35)	1385.75 (454.59)	1405.25 (425.63)			
		Placebo	1524.20 (647.46)	1357.24 (528.09)	1423.89 (501.33)	1500.16 (660.92)			
	2	OC	1149.86 (273.82)	1163.09 (198.97)	1083.00 (237.39)	1103.00 (204.05)	NS	2.44^	NS
		SIF	1469.65 (533.22)	1454.79 (522.96)	1403.38 (363.48)	1341.47 (478.66)			
		Placebo	1418.32 (485.94)	1355.81 (489.28)	1359.96 (438.06)	1378.25 (472.08)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Table 9.10 *Means and standard deviations for tasks of working memory during the first and second treatment cycles, with statistical values for the effects of cycle phase, treatment and their interaction.*

Outcome	Treatment Cycle	Treatment	Mean score (SD)				F values		
			Menses	Follicular	Ovulatory	Luteal	Cycle Phase (P); df=3,57	Treatment Group (T); df=2,61	P x T interaction; df=6,57
Alphabetic working memory: *Accuracy*	1	OC	94.39 (3.83)	94.33 (3.40)	94.17 (3.16)	92.01 (6.20)	NS	NS	NS
		SIF	94.29 (2.88)	92.78 (4.78)	94.18 (3.60)	93.28 (4.32)			
		Placebo	92.69 (5.28)	94.08 (3.64)	91.78 (4.66)	93.09 (4.66)			
	2	OC	93.76 (4.05)	93.08 (5.21)	94.63 (4.16)	91.72 (5.73)	NS	NS	NS
		SIF	93.72 (4.75)	92.83 (5.58)	93.52 (3.66)	92.83 (6.07)			
		Placebo	93.43 (5.47)	92.01 (5.81)	91.16 (4.97)	93.43 (3.60)			
Reaction time	1	OC	401.45 (100.55)	393.60 (110.07)	376.55 (89.14)	414.77 (106.70)	3.48*	NS	NS
		SIF	414.79 (64.86)	409.54 (50.54)	399.27 (75.47)	404.76 (70.31)			
		Placebo	437.08 (87.05)	412.37 (118.24)	424.68 (111.52)	425.39 (117.17)			
	2	OC	404.59 (123.47)	409.21 (115.13)	366.60 (93.11)	384.35 (100.52)	NS	NS	NS
		SIF	399.40 (62.31)	419.85 (92.71)	382.03 (72.76)	417.35 (116.32)			
		Placebo	421.58 (123.09)	399.44 (89.36)	415.71 (109.40)	411.65 (127.71)			
Numeric working memory: *Accuracy*	1	OC	93.84 (3.64)	94.61 (4.42)	94.22 (5.21)	94.13 (4.78)	NS	NS	NS
		SIF	93.86 (4.66)	93.09 (6.00)	94.34 (3.75)	94.39 (3.27)			
		Placebo	93.49 (5.48)	92.35 (6.05)	93.56 (2.81)	92.37 (5.28)			
	2	OC	94.72 (3.46)	93.60 (5.40)	94.08 (5.54)	94.09 (4.41)	NS	NS	NS
		SIF	95.06 (4.55)	93.95 (4.00)	93.89 (3.35)	93.33 (5.51)			
		Placebo	93.18 (5.24)	94.13 (5.11)	93.08 (4.90)	92.61 (5.38)			
Reaction time	1	OC	411.21 (102.21)	397.02 (104.14)	392.40 (88.85)	409.13 (79.77)	NS	NS	NS
		SIF	425.05 (84.96)	418.17 (47.77)	401.98 (74.32)	408.00 (71.41)			
		Placebo	415.67 (85.51)	448.74 (149.81)	428.43 (126.19)	449.76 (135.92)			
	2	OC	379.54 (82.57)	403.62 (92.65)	397.32 (110.22)	360.77 (62.09)	NS	NS	NS
		SIF	405.52 (62.19)	433.63 (116.08)	399.23 (61.49)	386.67 (72.34)			

		Placebo	417.62 (105.40)	421.08 (134.13)	424.32 (120.74)	415.42 (116.74)			
Nback: *Accuracy*	1	OC	88.18 (9.01)	90.83 (10.42)	88.83 (11.46)	89.24 (9.81)	2.25^	3.73*	2.10^
		SIF	82.54 (17.98)	76.48 (20.66)	81.91 (17.91)	81.51 (16.61)			
		Placebo	81.35 (17.45)	75.37 (20.17)	75.50 (20.33)	78.50 (18.96)			
	2	OC	88.33 (11.49)	87.88 (11.89)	89.67 (11.69)	89.39 (10.97)	NS	2.59^	NS
		SIF	80.33 (21.00)	84.17 (15.85)	80.57 (18.66)	80.67 (24.75)			
		Placebo	80.15 (18.87)	79.05 (19.18)	77.39 (19.87)	79.86 (14.53)			
Reaction time	1	OC	787.32 (166.75)	813.82 (253.63)	793.49 (342.26)	725.42 (293.49)	NS	2.63^	NS
		SIF	975.87 (379.57)	1057.62 (381.43)	872.86 (282.76)	848.52 (261.23)			
		Placebo	789.96 (242.90)	915.82 (359.23)	871.31 (229.80)	762.61 (167.21)			
	2	OC	682.31 (302.27)	744.21 (193.69)	718.73 (263.45)	631.49 (155.08)	NS	NS	2.16^
		SIF	911.48 (307.39)	868.50 (373.32)	809.01 (244.48)	789.48 (328.20)			
		Placebo	821.19 (318.69)	815.30 (294.95)	808.08 (313.09)	877.90 (357.34)			
Corsi blocks: *Span score*	1	OC	6.22 (1.23)	5.97 (1.33)	6.18 (1.17)	5.82 (1.40)	NS	NS	NS
		SIF	5.65 (1.18)	6.15 (1.24)	5.95 (1.14)	5.67 (1.32)			
		Placebo	5.82 (1.43)	6.09 (1.14)	5.88 (1.19)	6.10 (1.10)			
	2	OC	5.96 (1.25)	5.91 (1.58)	6.03 (1.65)	5.85 (1.55)	NS	NS	NS
		SIF	5.70 (1.35)	5.49 (1.33)	6.19 (1.39)	6.07 (1.34)			
		Placebo	6.43 (1.11)	6.14 (0.95)	6.13 (0.94)	5.88 (1.12)			
Reaction time	1	OC	4974.00 (1552.03)	4684.20 (1381.72)	4721.30 (1101.63)	4791.29 (1446.89)	NS	NS	NS
		SIF	5028.67 (1887.85)	5491.94 (2133.86)	4913.35 (1748.67)	4773.32 (1532.70)			
		Placebo	5172.04 (1934.16)	4824.44 (1296.86)	4567.85 (866.37)	5115.63 (1624.83)			
	2	OC	4821.27 (1873.44)	4529.32 (1463.70)	4620.63 (1737.86)	4547.68 (1442.47)	NS	NS	NS
		SIF	4950.30 (2297.26)	4835.95 (1869.43)	4660.18 (1091.52)	4985.93 (1717.77)			
		Placebo	5411.05 (1616.92)	5305.45 (1613.47)	5300.65 (1267.33)	4799.00 (1366.76)			

Corsi blocks reversed: *Span score*	1	OC	4.52 (2.64)	4.52 (2.95)	4.32 (2.70)	4.06 (2.56)	NS	NS	NS
		SIF	2.97 (2.57)	3.41 (2.69)	3.72 (2.78)	3.53 (2.53)			
		Placebo	3.57 (2.59)	4.37 (2.64)	3.83 (2.81)	3.57 (2.55)			
	2	OC	4.45 (2.37)	4.46 (2.61)	4.50 (3.03)	4.33 (2.69)	NS	NS	NS
		SIF	3.37 (2.78)	3.15 (2.89)	3.57 (2.75)	3.25 (3.05)			
		Placebo	3.82 (2.52)	3.32 (2.89)	3.36 (2.63)	3.56 (2.67)			
Reaction time	1	OC	4210.52 (1444.10)	3937.60 (1349.92)	4141.95 (1618.54)	3819.62 (1146.76)	NS	NS	NS
		SIF	3575.86 (1319.31)	4037.06 (1454.85)	3684.65 (1217.57)	3375.30 (920.39)			
		Placebo	3773.07 (1289.71)	4506.89 (1532.47)	4180.20 (1652.75)	4121.59 (1693.40)			
	2	OC	4062.32 (1179.26)	4100.50 (1642.58)	4293.80 (1484.34)	3724.82 (1125.25)	NS	NS	NS
		SIF	3873.65 (1552.26)	3842.72 (1545.75)	3706.79 (1501.51)	3584.25 (1974.82)			
		Placebo	3827.32 (1206.44)	3956.43 (1054.75)	3442.45 (855.59)	3990.46 (1853.86)			

*, p<0.05; **, p<0.01; ^, p<0.1 (trend); NS, Non-Significant

Table 9.11 *Results of post hoc comparisons of estimated marginal means where significant main effects of treatment, phase and their interaction were found on cognitive outcomes in the two treatment cycles.*

Outcome	Main effect of Phase	Main effect of Treatment	Phase x Treatment Interaction
Choice Reaction Time	TREATMENT CYCLE 2 M > O (t=2.57, p=0.013, d=0.70)	NS	TREATMENT CYCLE 1: SIF only: F > M (t=2.39, p=0.021, d=1.23); L > M (t=2.38, p=0.021, d=1.09); L > O (t=2.54, p=0.014, d=1.17)
Card Sorting Task Accuracy	NS	NS	TREATMENT CYCLE 2: Menses only: OC > Plac (t=2.05, p=0.045, d=0.63) SIF only: M > L (t=2.57, p=0.013, d=1.25); O > L (t=2.41, p=0.02, d=1.25) Plac only: L > M (t=2.27, p=0.027, d=1.02)
Peg and Ball Task Accuracy	NS	NS	TREATMENT CYCLE 2: Luteal phase only: Plac > OC (t=2.01, p=0.049, d=0.61) OC only: F > O (t=2.52, p=0.015, d=1.19); F > L (t=2.55, p=0.014, d=1.17)
Peg and Ball Task Completion Time	NS	NS	TREATMENT CYCLE 1: Luteal phase only: SIF > OC (t=2.04, p=0.047, d=0.70) SIF only: L > F (t=2.61, p=0.012, d=1.31); L > O (t=2.32, p=0.024, d=1.06)
Serial 3 subtractions Total	TREATMENT CYCLE 2 O > M (t=2.47, p=0.017, d=0.67)	TREATMENT CYCLE 1 OC > SIF (t=2.06, p=0.045, d=0.62) OC > Plac (t=2.07, p=0.043, d=0.61)	NS
Serial 7 subtractions Accuracy	NS	NS	TREATMENT CYCLE 2: Luteal only: SIF > OC (t=2.46, p=0.017, d=0.78) OC only: F > L (t=2.19, p=0.036, d=0.79)
Delayed Word Recognition Accuracy	NS	TREATMENT CYCLE 2 OC > Plac (t=2.49, p=0.016, d=0.74)	NS
Delayed Word Recognition	NS	NS	TREATMENT CYCLE 1: OC only: M > F (t=2.12, p=0.039, d=0.61); M > O (t=3.00, p=0.004, d=0.86)

Reaction Time			
Sentence Verification Reaction Time	TREATMENT CYCLE 1 O > F (t=2.98, p=0.007, d=0.85) L > F (t=2.79, p=0.008, d=0.80)	TREATMENT CYCLE 2 Plac > OC (t=2.11, p=0.041, d=0.62)	NS
Alphabetic Working Memory Reaction Time	TREATMENT CYCLE 1 L > M (t=2.07, p=0.042, d=0.54) O > F (t=2.02, p=0.049, d=0.58) L > F (t=3.20, p=0.003, d=0.91)	NS	NS
Nback Accuracy	TREATMENT CYCLE 1 M > L (t=2.43, p=0.02, d=0.74)	TREATMENT CYCLE 1 OC > SIF (t=2.04, p=0.049, d=0.62) OC > Plac (t=2.57, p=0.015, d=0.75) OC > Plac (t=2.27, p=0.028, d=0.67)	TREATMENT CYCLE 1: Follicular phase: OC > SIF (t=3.08, p=0.004, d=1.02); OC > Plac (t=2.63, p=0.012, d=0.87) Ovulatory phase: OC > Plac (t=2.36, p=0.023, d=0.76) SIF only: M > F (t=3.10, p=0.003, d=0.90); M > L (t=2.28, p=0.028, d=0.70) TREATMENT CYCLE 2: Menses: SIF > Plac (t=2.36, p=0.022, d=0.73) SIF only: M > F (t=3.04, p=0.004, d=0.94); M > O (t=2.62, p=0.011, d=0.70); M > L (t=2.08, p=0.041, d=0.54)
Nback Reaction Time	NS	TREATMENT CYCLE 1 SIF > OC (t=2.14, p=0.034, d=0.65)	TREATMENT CYCLE 2: Menses: SIF > OC (t=3.37, p=0.002, d=1.07); Plac > OC (t=2.41, p=0.02, d=0.74) Luteal: Plac > OC (t=2.04, p=0.046, d=0.62) OC only: F > M (t=3.09, p=0.004, d=1.06); O > M (t=2.08, p=0.044, d=0.65)

M, Menses; F, Follicular Phase; O, Ovulatory Phase; L, Luteal Phase; NS, Non-significant; OC, Oral Contraceptive Users; SIF, Soy Isoflavone Group; Plac, Placebo Group

Alphabetic working memory: After controlling for baseline scores there was a significant main effect of phase on reaction times in the first treatment cycle, with pairwise comparisons revealing faster reaction times in menses compared with the luteal phase, and faster responses in the follicular phase than the ovulatory and luteal phases.

Nback: After controlling for baseline scores, in the first treatment cycle there was a significant main effect of treatment with OC users having higher accuracy than both SIF and placebo. There was also a trend towards a main effect of phase with higher accuracy in menses than the luteal phase, as well as a trend towards a phase x treatment interaction, with pairwise comparisons revealing that the difference between OC and SIF was only significant during the follicular phase and that the difference between OC and placebo was significant during the follicular and ovulatory phases. Furthermore, significant phase effects were only found for the SIF group, where accuracy was higher during menses than the follicular and luteal phases.

In the second treatment cycle, only a trend towards a main effect of treatment was found with OC use being associated with significantly higher accuracy than placebo. However, pairwise comparisons also revealed that SIF was associated with significantly higher accuracy during menses compared with placebo, and that significant phase effects were found only for the SIF group, where accuracy was higher during menses than all other phases.

In terms of reaction time on the Nback task there was a trend towards a main effect of treatment in the first treatment cycle, with SIF being associated with significantly slower reaction times than OC. In the second treatment cycle there was a trend towards a phase x treatment interaction with pairwise comparisons revealing that OC use was associated with significantly faster response times during menses compared with SIF and placebo, and that OC use was associated with faster response times in the luteal phase compared with placebo. Furthermore, significant phase effects were only found for the OC group where reaction times were faster during menses than the follicular and ovulatory phases.

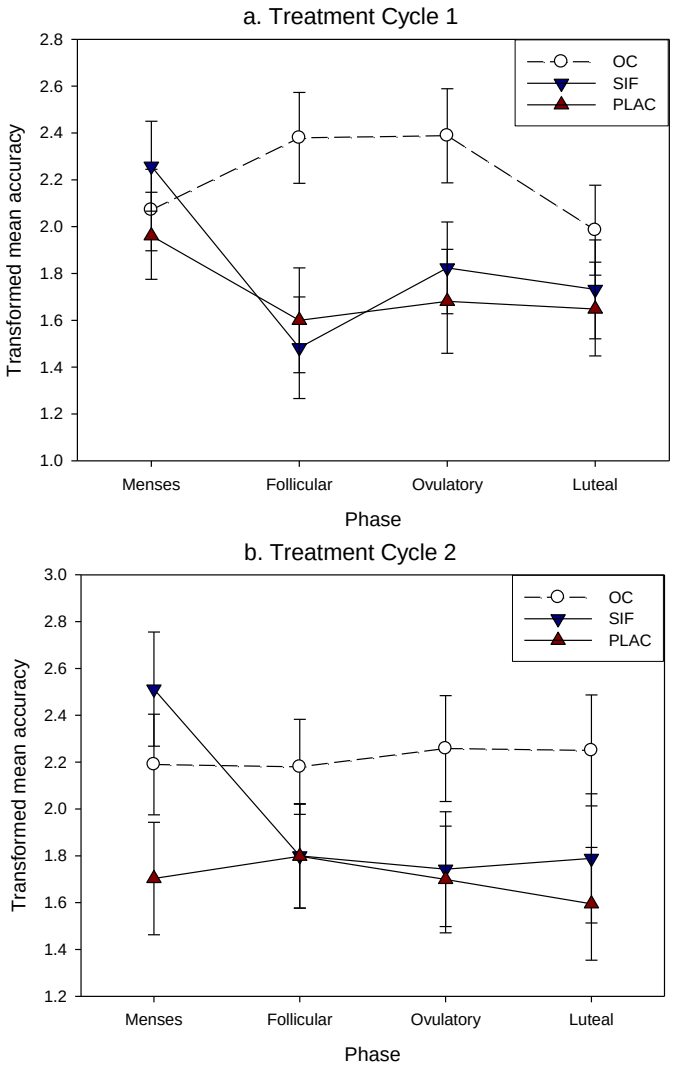

Figure 9.12 Transformed mean accuracy scores on the N-back task for OC users, women treated with SIF and women treated with placebo after controlling for baseline scores. Graphs depict **a.** Mean (±SEM) accuracy during the first treatment cycle and **b.** Mean (±SEM) accuracy during the second treatment cycle.

Corsi blocks (forward and reversed): In the first treatment cycle, after controlling for baseline scores age contributed significantly to the model for reaction time for corsi blocks forward with increased age being associated with slower reaction times ($F(1,41)=6.30$, $p=0.016$, $d=0.78$). In the first treatment cycle there was also a significant positive association between years of education and reaction time on the reversed corsi

blocks task (F(1,49)=6.41, p=0.015, d=0.72). There were no effects of treatment or phase on these tasks.

9.8. EEG measures: Behavioural results

9.8.1. Standard go/nogo task

25% nogo condition

After controlling for baseline scores there was a trend towards a main effect of phase on the number of errors of commission to up arrows with more errors during the luteal phase than menses (F(1,22)=3.54, p=0.073). There was also a trend towards a treatment x phase interaction for response times to go stimuli (F(2,25)=2.58, p=0.096) with pairwise comparisons revealing that placebo was associated with slower response times than OC during menses only (t=2.23, p=0.034, d=0.95) and that there was a trend towards an effect of phase for the placebo group only where responses were slower during menses than the luteal phase (t=1.84, p=0.079).

25% go condition

After controlling for baseline scores there were no significant effects of treatment or phase on any of the outcomes.

9.8.2. Go/nogo task with face emotion stimuli

Neutral faces as the 25% nogo stimulus

After controlling for baseline values there was a significant main effect of treatment on response times to happy stimuli (F(2,20)=4.05, p=0.034) with pairwise comparisons revealing that SIF was associated with significantly slower reactions compared with OC (t=2.84, p=0.009, d=1.21). No other effects of treatment or phase were found.

Neutral faces as the 25% go stimulus

There was a significant contribution of age to the models for correct response inhibitions to happy faces ($F(1,30)=4.63$, $p=0.04$, $d=0.73$) as well as angry faces ($F(1,29)=6.03$, $p=0.02$, $d=0.83$). No effects of treatment or phase on any of the outcomes were found.

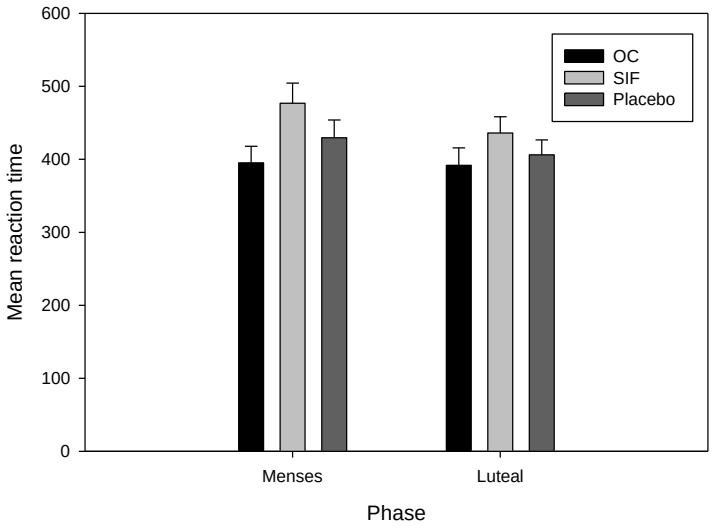

Figure 9.13 Mean reaction times to happy go stimuli when neutral faces were the 25% nogo stimulus. Graph depicts mean reaction times during menses and the luteal phase of the post-treatment cycle for OC users, women treated with SIF and women treated with placebo, after controlling for baseline scores.

Angry faces as the 25% nogo stimulus

After controlling for baseline scores, there were no significant effects of treatment or phase on accuracy or reaction times in responding to happy and neutral faces, nor were there any significant effects on the number of errors of commission to angry faces.

Angry faces as the 25% go stimulus

There was a significant contribution of age to the models for correct inhibitions to happy faces ($F(1,34)=4.68$, $p=0.038$, $d=0.73$) and neutral faces ($F(1,32)=4.89$, $p=0.034$, $d=0.75$) with increased age being associated with more correct response inhibitions. No effects of treatment or phase on any of the outcomes were found.

Table 9.12 *Mean (SD) number of responses/inhibits and reaction times to each stimulus type for OC users, women treated with SIF and women treated with placebo during each phase of the post-treatment cycle.*

Task	OC		SIF		Placebo	
	Menses	Luteal	Menses	Luteal	Menses	Luteal
Up arrow 25% nogo Correct go response	185.50 (65.42)	194.09 (37.06)	161.42 (62.77)	181.36 (51.27)	131.36 (84.28)	164.83 (61.09)
Go reaction time	284.50 (44.34)	288.00 (40.35)	338.50 (85.32)	346.27 (93.49)	323.45 (50.49)	314.33 (51.09)
Errors of commission	8.83 (8.54)	10.36 (9.66)	7.50 (8.19)	7.27 (7.40)	7.82 (7.40)	7.92 (5.63)
Up arrow 25% go Correct go response	61.58 (23.39)	65.45 (12.23)	56.50 (18.06)	61.09 (17.48)	57.82 (21.69)	60.33 (20.62)
Go reaction time	367.67 (53.00)	349.18 (36.24)	416.92 (84.88)	414.64 (78.69)	388.18 (58.20)	383.50 (57.84)
Neutral 25% nogo Angry response	72.70 (43.10)	82.55 (33.41)	77.00 (40.28)	92.00 (27.46)	72.45 (45.65)	73.75 (42.91)
Angry reaction time	426.30 (70.47)	392.91 (76.38)	499.18 (150.94)	447.60 (129.53)	424.45 (83.24)	447.50 (105.83)
Happy response	74.20 (44.88)	83.09 (34.40)	76.45 (39.58)	91.10 (27.22)	73.00 (45.32)	74.25 (40.86)
Happy reaction time	395.10 (66.54)	374.55 (60.29)	470.36 (126.90)	455.30 (131.56)	411.82 (73.57)	409.50 (70.47)
Errors of commission	4.50 (4.09)	8.36 (8.27)	6.55 (8.80)	6.60 (9.94)	9.09 (8.23)	6.00 (5.03)
Neutral 25% go Angry inhibit	110.11 (4.17)	109.91 (5.22)	111.00 (4.90)	111.10 (4.20)	114.18 (4.12)	111.33 (5.35)
Happy inhibit	110.11 (5.62)	111.55 (5.15)	113.18 (4.79)	112.90 (5.11)	114.82 (3.97)	113.00 (5.34)
Neutral response	59.89 (20.72)	63.45 (13.96)	52.27 (19.33)	58.33 (11.10)	51.00 (28.86)	52.00 (22.61)
Neutral reaction time	493.11 (82.24)	465.64 (52.04)	531.09 (97.44)	544.44 (80.83)	519.73 (98.31)	499.08 (71.98)
Angry 25% nogo Happy response	73.67 (38.95)	82.64 (37.03)	88.33 (26.28)	84.11 (30.37)	79.50 (44.79)	76.58 (35.26)
Happy reaction time	411.75 (62.45)	408.45 (93.56)	462.08 (107.05)	450.22 (112.44)	446.50 (143.06)	435.58 (69.31)
Neutral response	73.33 (39.01)	81.64 (38.67)	86.50 (29.32)	77.00 (40.20)	76.50 (44.14)	73.42 (35.60)
Neutral reaction time	406.83 (62.23)	396.00 (93.17)	453.25 (97.72)	469.20 (108.35)	442.90 (116.33)	451.83 (84.41)
Errors of commission	9.33 (8.97)	11.09 (8.75)	7.50 (6.14)	7.78 (11.74)	10.20 (6.56)	7.08 (4.58)
Angry 25% go Happy inhibit	111.64 (5.48)	111.00 (5.60)	111.33 (5.19)	110.18 (6.01)	110.90 (5.00)	112.58 (5.73)
Neutral inhibit	111.55 (6.02)	113.18 (6.48)	112.92 (4.40)	111.18 (7.86)	114.40 (4.88)	113.33 (5.14)
Angry response	58.55 (23.62)	61.09 (20.63)	55.42 (24.67)	64.60 (15.54)	53.90 (23.12)	52.42 (22.37)
Angry reaction time	468.73 (54.73)	464.09 (58.13)	519.83 (87.65)	517.80 (99.34)	495.00 (87.06)	501.42 (93.32)

OC, Oral Contraceptive; SIF, Soy isoflavone

9.9. EEG measures: Electrophysiological results

9.9.1. Standard go/nogo task

After controlling for baseline scores there were no significant effects of treatment or phase on the nogo minus go difference in amplitude or latency of either component.

9.9.2. Go/nogo task with face emotion stimuli

Neutral faces as the 25% stimulus

After controlling for baseline values there was a trend towards a main effect of phase on N2d mean amplitude with larger N2d mean amplitude during menses than the luteal phase ($F(1,28)=3.16$, $p=0.086$). There was also a trend towards a treatment x phase interaction for N2d latency, with pairwise comparisons revealing that SIF was associated with shorter N2d latency during the luteal phase compared with placebo ($t=2.39$, $p=0.025$, $d=0.63$) and OC although the latter was marginal ($t=2.04$, $p=0.052$).

There was a trend towards a treatment x phase interaction for P3d peak amplitude ($F(2,22)=2.71$, $p=0.088$) with pairwise comparisons revealing that phase effects were significant only for OC users, where P3d peak amplitude was larger during the luteal phase than during menses ($t=2.98$, $p=0.007$, $d=0.74$). A significant main effect of phase was also found, with larger P3d peak amplitude in the luteal phase than during menses ($F(1,22)=4.73$, $p=0.041$, $d=0.93$). There was also a trend towards a treatment x phase interaction for P3d mean amplitude, with phase effects only evident in OC users where P3d mean amplitude was larger during the luteal phase than during menses ($t=2.32$, $p=0.028$, $d=0.60$). For P3d latency, there was a trend towards a main effect of treatment ($F(2,26)=3.15$, $p=0.059$) with pairwise comparisons revealing significantly longer P3d latency with SIF compared to OC ($t=2.39$, $p=0.024$, $d=0.76$) as well as placebo although this did not reach significance ($t=1.87$, $p=0.073$). There was also a trend towards a main effect of phase with longer P3d latency during menses than the luteal phase ($F(1,18)=4.35$, $p=0.051$).

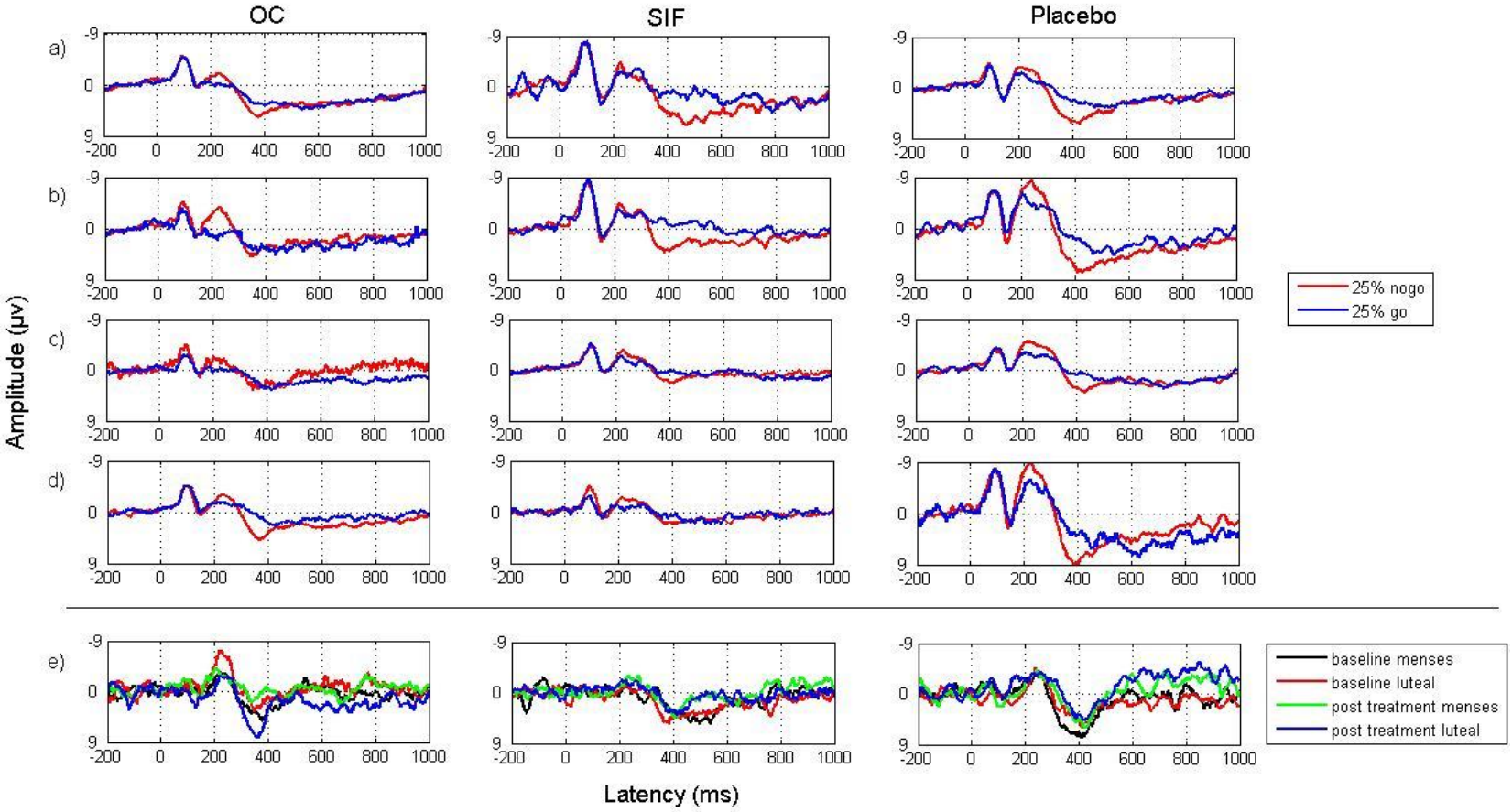

Figure 9.14 ERP waveforms to 25% nogo and 25% go neutral face stimuli for OC users (left), women treated with SIF (middle) and women treated with placebo (right). Graphs depict waveforms following nogo (red) and go (blue) stimuli during **a.** menses of the baseline cycle, **b.** the luteal phase of the baseline cycle, **c.** menses of the post treatment cycle, and **d.** the luteal phase of the post treatment cycle. In addition, **e.** depicts nogo minus go difference waves for each cycle phase.

Table 9.13 *Mean (SD) ERP values for N2 and P3 nogo-go difference wave peak amplitudes, mean amplitudes and peak latencies for OC users, women treated with SIF and women treated with placebo during each phase of the post-treatment cycle.*

Task	OC		SIF		Placebo	
	Menses	Luteal	Menses	Luteal	Menses	Luteal
Standard go/nogo						
N2d peak amplitude	-5.39 (2.85)	-5.18 (3.37)	-4.61 (2.60)	-4.25 (1.72)	-4.88 (1.46)	-4.80 (2.18)
N2d mean amplitude	0.79 (1.25)	0.45 (2.38)	0.67 (2.20)	0.24 (1.49)	1.09 (3.34)	0.55 (2.20)
N2d peak latency	214.09 (27.18)	216.45 (16.75)	232.25 (25.46)	226.27 (21.67)	242.00 (33.42)	218.83 (37.96)
P3d peak amplitude	7.98 (3.31)	7.96 (3.21)	6.77 (3.08)	5.70 (3.31)	7.02 (2.47)	8.56 (5.29)
P3d mean amplitude	2.02 (1.46)	1.56 (2.18)	1.63 (2.69)	1.61 (2.25)	1.45 (1.73)	1.45 (2.65)
P3d peak latency	356.09 (65.48)	366.73 (88.89)	392.25 (98.41)	417.36 (108.64)	360.45 (34.81)	385.75 (61.71)
Neutral go/nogo						
N2d peak amplitude	-3.62 (1.76)	-3.99 (2.44)	-3.44 (1.97)	-3.39 (2.90)	-4.39 (2.35)	-4.97 (3.76)
N2d mean amplitude	-0.64 (1.86)	0.61 (1.37)	-1.89 (1.42)	-0.23 (1.28)	-1.09 (2.00)	-0.37 (2.64)
N2d peak latency	227.25 (55.42)	250.27 (58.75)	240.27 (56.31)	190.90 (62.22)	246.09 (33.59)	255.58 (51.20)
P3d peak amplitude	3.79 (2.56)	7.68 (4.67)	5.05 (2.59)	4.60 (2.41)	5.39 (1.75)	6.00 (4.19)
P3d mean amplitude	-0.05 (2.16)	1.49 (1.94)	1.07 (1.77)	0.25 (1.33)	0.54 (1.49)	0.43 (2.29)
P3d peak latency	463.88 (151.09)	402.18 (95.18)	497.36 (110.35)	507.00 (103.95)	468.36 (98.77)	423.25 (66.31)
Angry go/nogo						
N2d peak amplitude	-2.96 (2.23)	-2.48 (0.70)	-2.93 (1.53)	-4.07 (2.72)	-4.72 (1.53)	-4.25 (2.14)
N2d mean amplitude	-0.26 (1.49)	0.21 (1.45)	0.15 (1.78)	-0.99 (1.62)	-0.78 (1.30)	-0.28 (2.02)
N2d peak latency	246.18 (60.60)	242.82 (60.76)	241.92 (45.04)	244.18 (55.27)	272.70 (51.71)	262.33 (51.96)
P3d peak amplitude	8.45 (5.13)	5.98 (2.33)	5.71 (2.95)	6.57 (2.29)	7.38 (3.78)	6.78 (3.56)
P3d mean amplitude	2.07 (2.33)	1.81 (1.57)	1.69 (1.94)	1.47 (1.09)	1.65 (1.50)	1.03 (1.78)
P3d peak latency	425.27 (96.35)	458.00 (89.58)	449.33 (86.85)	502.09 (83.09)	466.50 (75.35)	452.75 (99.96)

OC, Oral Contraceptive; SIF, Soy Isoflavones

Angry faces as the 25% stimulus

After controlling for baseline values, although there were no significant main effects or interactions for any of the outcomes pairwise comparisons of estimated marginal means revealed that for P3d latency phase effects were significant only for the SIF group where P3d latency was longer during the luteal phase than during menses (t=2.11, p=0.047, d=0.75).

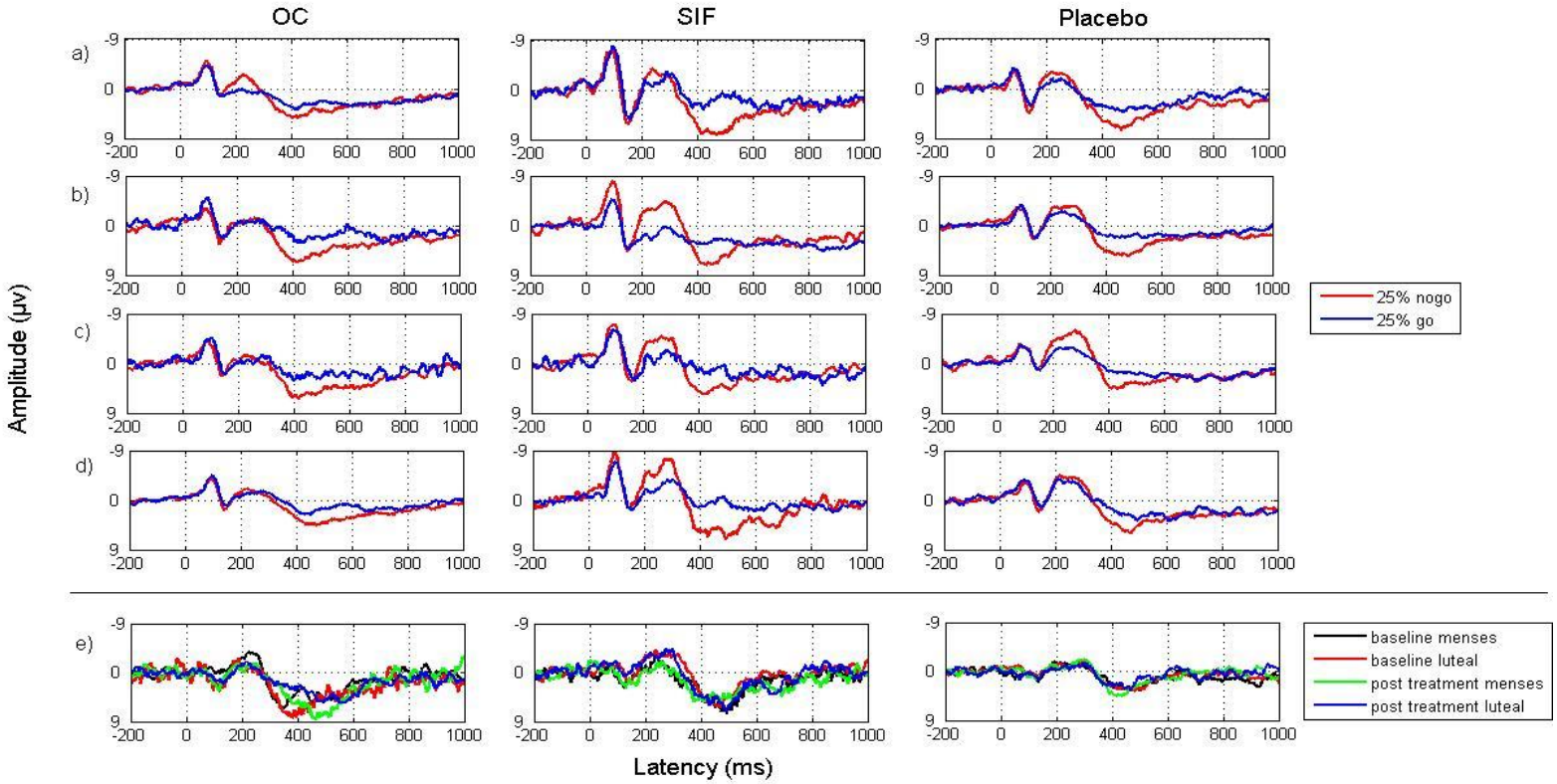

Figure 9.15 ERP waveforms to 25% nogo and 25% go angry face stimuli for OC users (left), women treated with SIF (middle) and women treated with placebo (right). Graphs depict waveforms following nogo (red) and go (blue) stimuli during **a.** menses of the baseline cycle, **b.** the luteal phase of the baseline cycle, **c.** menses of the post treatment cycle, and **d.** the luteal phase of the post treatment cycle. In addition, **e.** depicts nogo minus go difference waves for each cycle phase.

Chapter 10

Discussion of Post Treatment Effects of Soy Isoflavones across the Menstrual Cycle

10.1 Overview and main findings

In the current study, contrary to the hypotheses that mood, premenstrual symptoms and aggression would improve following SIF treatment, women receiving SIF actually rated these symptoms as more severe than women receiving placebo. For most outcomes these effects were only apparent during the first treatment cycle, with few significant differences between SIF and placebo during the second cycle. The negative effects of SIF were also most pronounced during the luteal phase, with some measures showing improvements with SIF compared to placebo during menses. These effects were largely consistent across most measures. There were few effects on cognitive performance, however the results show that for some cognitive outcomes women treated with SIF show different cyclical variation than those treated with placebo. Specifically, SIF may be associated with improved performance of some cognitive tasks during menses but poorer performance during the luteal phase. The effects of SIF supplementation on ERP components appeared to be confined to the luteal phase and were also dependent on stimulus type. No effects of SIF were observed on the N2 and P3 to abstract stimuli, but larger differences between nogo and go P3 latencies to both neutral and angry faces were observed with SIF supplementation, as well as smaller differences between nogo and go N2 latencies to neutral stimuli. It should be noted that the effect sizes for the majority of significant findings in this study were medium to large (0.5 – 0.8) as defined by Cohen (1988). These findings will be discussed in more detail within the context of the broader literature below, and possible explanations for these findings will be considered.

10.2 Effects of soy isoflavone supplementation on mood and premenstrual symptoms

Some surprising effects were found regarding the effects of SIF on various parameters of mood. Whilst anxiety was not affected, SIF was associated with significantly higher ratings of depression than OC overall and higher depression ratings than placebo during the luteal phase only. SIF was also associated with reduced contentedness and calmness compared with both OC and placebo. These effects were restricted to the first treatment cycle, with no significant treatment effects on these outcomes in the second treatment cycle. However, SIF appeared to stabilise calmness during the second cycle since no phase effects were evident in this group whereas OC users and women receiving

placebo showed cyclical variation. Also in the second treatment cycle SIF supplementation was associated with an increase in total impulsivity scores on the BIS during menses. This appeared to be driven by increases in motor impulsiveness rather than any other form of impulsivity.

These findings of poorer mood with SIF are in contrast to those of previous studies which found reduced depression and other improvements in mood with SIF treatment (Casini et al., 2006; Ishiwata, Melby, Mizuno, & Watanabe, 2009). However, those studies investigated the effects in postmenopausal women rather than younger women with normal menstrual cycles. The few studies conducted in younger women have generally found either slight improvements in mood outcomes that did not reach statistical significance (Ishiwata, Uesugi, & Uehara, 2003) or no significant differences compared with placebo (Bryant et al., 2005). The negative findings in the current study may be due to the higher dose of isoflavones administered, with 130mg/day isoflavone aglycones administered in this study compared with 40 mg/day administered by Ishiwata et al. (2003) and 68mg/day administered by Bryant et al. (2005). The participants in the current study were also asymptomatic women whereas in the previous studies only PMS sufferers were included, which may suggest a differential effect of SIF on mood depending on existing experiences of PMS symptoms.

It should be noted that the negative effects of SIF on mood were mostly restricted to the luteal phase. In fact, there was little difference between groups during menses on all the outcomes. This suggests that SIF may worsen mood when endogenous female sex hormone levels are higher, whereas when endogenous steroid hormones are low these negative effects are not apparent. This is in line with evidence that isoflavones have anti-estrogenic effects when levels of endogenous estrogens are high, whereas when endogenous estrogen levels are low isoflavones have estrogenic effects (Kuiper et al., 1998). An alternative explanation is that the poorer mood outcomes may be due to the withdrawal of SIF from peak plasma levels as the half-life of isoflavones is fairly short. Further insight could have been gained from the measurement of isoflavones at the time of testing, although this was not possible in the current study.

For depression ratings it is clear that the difference between SIF and placebo was actually due to improvements in depression in the luteal phase for the placebo group. This may be a placebo effect, with expectations of improvements in premenstrual mood

influencing ratings of depression, or may reflect improved mood with increased steroid hormones in the luteal phase. Either way, the lack of this effect in women treated with SIF suggests a negative effect of SIF supplementation during the luteal phase. In addition, ratings of contentedness and calmness were substantially lower with SIF compared to placebo in all phases except menses where there was little difference between groups. This effect was due to poorer outcomes in these phases for SIF users rather than improved outcomes for the placebo group as was the case for depression ratings, indicating a genuine negative effect of the high dose of SIF used in this study on mood.

SIF was also associated with more severe symptoms as measured using the symptom checklist during the luteal phase compared with placebo, and compared with both OC and placebo overall. Premenstrual symptoms measured using the DSR were also more severe during the luteal phase following SIF supplementation compared with placebo and OC. Although only ratings on the physical factor were found to differ significantly between groups in the first treatment cycle, a similar pattern emerged for ratings on the psychological factor with pairwise comparisons revealing higher ratings with SIF in the luteal phase. As with the effects on other mood measures, these differences partially lay in the improvement of symptoms in this phase in the placebo group, potentially indicating a larger placebo effect than treatment effect in this study. However, it is clear from examination of Figure 5.16 that although symptoms were reduced in the placebo group, they were also increased in the SIF group which demonstrates a worsening of both physical and psychological symptoms during the luteal phase with SIF use.

This is the first study to demonstrate negative effects of SIF use on both psychological and physical premenstrual symptoms, with previous studies showing either beneficial effects of SIF administration or no difference between SIF and placebo treatment (e.g. Bryant et al. 2005; Ferrante, Fusco, Calabresi, & Cupini, 2004; Ishiwata et al., 2003; Kim, Kwon, Kim, & Reame, 2006). As mentioned above, these discrepancies may be due to the higher dose of SIF administered in the current study, or a differential effect of SIF in asymptomatic women as opposed to PMS sufferers.

10.3 Effects of soy isoflavone supplementation on aggression

Findings regarding the effects of SIF on aggression levels in the current study were rather unclear. Ratings on the aggression item of the DSR suggested an increase in aggression during the luteal phase with SIF supplementation compared with both placebo and OC, and little difference between groups during other phases. On the other hand, total BPAQ scores suggested reduced aggression with SIF use compared to placebo during menses, with little difference between SIF and placebo during other phases. However, different subscales of the BPAQ appeared to be differentially affected by SIF use suggesting that some forms of aggression may indeed be increased during the luteal phase with SIF use. Whilst ratings on all subscales showed reduced aggression during menses, anger and hostility gradually increased towards the end of the cycle with SIF supplementation, with ratings on both scales being most severe during the luteal phase. A significant difference between SIF and placebo was in fact found for the hostility subscale during the luteal phase, although for anger the increase in severity was only significant in comparison to OC users and not placebo. It is therefore possible that the increased ratings on the aggression item on the DSR reflect an increase in hostility during the luteal phase. However, the anger item of the DSR showed no significant effects of SIF treatment in any cycle phase, contrasting with the findings of the BPAQ. A further difference between these measures lies in the lack of significantly poorer outcomes associated with SIF use during the second treatment cycle for any of the subscales on the BPAQ, whereas for the aggression item of the DSR, SIF was associated with significantly more severe ratings than OC during the second treatment cycle.

In contrast, neither verbal nor physical aggression ratings were negatively influenced by SIF compared to placebo in any phase. The lack of effect on verbal aggression is somewhat surprising given the evidence that estrogen may be implicated in this form of aggression (Brambilla et al., 2010), however since no significant cyclical variation in verbal aggression was evident at baseline in the current sample this may explain the lack of significant findings. Interestingly, SIF users were less aggressive on all subscales of the BPAQ during menses of the first cycle, although this finding was not significant for all subscales, suggesting an overall improvement in aggression with short term SIF use during periods of low endogenous hormones. This may be due to activation of ERβ, since isoflavones preferentially bind to this ER (Kuiper et al., 1997) and ERβ activation is thought to inhibit aggressive behaviour (Nomura et al., 2002; Ogawa, Lubahn,

Korach, & Pfaff, 1997). The increases in aggression observed during periods of high circulating steroid hormones may be due to the antagonistic effects of SIF on estrogen activity that are known to occur when levels of endogenous estrogen are high (Kuiper et al., 1998). SIF lowers levels of endogenous hormones by increasing sex hormone binding globulin (SHBG) production (Adlercreutz et al., 1998), and may block full estrogenic activity when endogenous estrogen levels are high by occupying ERs, thus resulting in less estrogenic activity.

The finding that SIF supplementation is associated with increased aggression is supported by the higher ratings of state anger on the STAXI. However, no cyclical variation was evident for this outcome, with ratings for the SIF group being higher than both other groups during all cycle phases. This is in contrast to both the anger item of the DSR, which showed no effects of SIF treatment, and the anger subscale of the BPAQ, where SIF treatment was associated with reduced anger during menses and increased anger during the luteal phase. No other subscales of the STAXI showed an effect of SIF use, suggesting that not all components of anger are affected by SIF supplementation.

Although slightly different findings were observed for the different measures of aggression, taken together these findings suggest an increase in aggression with SIF supplementation, particularly during the luteal phase when levels of endogenous steroid hormones are high. Conversely, aggression may be reduced during menses when endogenous hormones are at their nadir although this effect was not seen for state anger or for the aggression item of the DSR. These findings add to the existing body of literature currently published on the effects of SIF on aggression, some of which also found increases in aggression following administration of SIF (Patisaul & Bateman, 2008; Simon, Kaplan, Hu, Register, & Adams, 2004; Wasserman et al., 2012).

10.4 Effects of soy isoflavone supplementation on cognition

Similar to the observed effects on mood and aggression, some cognitive outcomes were negatively impacted by SIF use during the luteal phase. Accuracy on the card sorting task, which assesses the executive function of rule learning and reversal, was impaired

during the luteal phase compared with menses and the ovulatory phase for women treated with SIF, whereas for those treated with placebo accuracy was highest during the luteal phase. This effect was not observed until the second treatment cycle when the negative effects on mood were no longer evident, suggesting that this effect was not mediated by deteriorations in mood. However, in the first treatment cycle reaction time was slower in the luteal phase on the choice reaction time task and completion times for the peg and ball task were also slower for SIF users only.

Gleason et al. (2009) also reported detriments on tasks of executive function after six months SIF supplementation, however only older men and women were included in that study. Those findings suggest that SIF may impair executive function when endogenous female sex hormones are low, whereas in the current study performance on the card sorting task and peg and ball task measuring executive function were impaired when endogenous hormones were high. This may be due to effects on progesterone, since accuracy was highest during the ovulatory phase when estrogen levels are high but progesterone levels are low. The findings of the current study partially refute those of File et al. (2001) who found improvements in rule learning and reversal as well as a planning task in females, however in that study cycle phase was not controlled for and the impairments in the current study were observed during the luteal phase only.

Only one cognitive outcome showed any improvement during menses, which was accuracy on the N-back task. Again, this effect was not evident until the second treatment cycle suggesting that the improvement in performance was not due to the improvements in mood during this phase that were only evident during the first treatment cycle. Islam, Sparks, Roodenrys, and Astheimer (2008) also found improvements in working memory following a dietary soy intervention during menses, however the positive effects in that study were evident following short term supplementation whereas in the current study the effects did not emerge until the second treatment cycle. As in that study, no differences in performance were observed on this task between phases at baseline in the current study, nor for women treated with placebo, suggesting that the effects of SIF on working memory as measured using the N-back task may be mediated by non-hormonal mechanisms.

A possible mechanism underlying the beneficial effects of SIF on working memory concerns the neuroprotective effects of isoflavones, particularly in the hippocampus.

Dendritic spine density has been shown to increase in the CA_1 region following a high phytoestrogen diet (Luine et al., 2006), and SIF are also known to protect against neuronal loss (Azcoitia, Moreno, Carrero, Palacios, & Garcia-Segura, 2006). These neuroprotective effects are thought to stem from the ability of SIF to increase brain derived neurotropic factor (BDNF) which promotes cell survival (Hartikka & Hefti, 1988) and synaptic plasticity (McAllister, Katz, & Lo, 1999). Phytoestrogen supplementation has been found to increase BDNF gene expression and BDNF concentration in the hippocampus of female rats (Pan et al., 2010). The hippocampus is known to be crucial for working memory processes (Olton, Becker, & Handelmann, 1979), therefore the promotion of cell survival in this region may explain the improvements in working memory observed with SIF supplementation in the current trial. However, why this effect was observed only during menses and not in other phases of the cycle is as yet unclear.

Performance on the serial seven subtractions task was actually found to improve with SIF use during the luteal phase of the second treatment cycle. This is surprising given that mood and other cognitive outcomes mentioned above were negatively affected by SIF use during this phase. Although the difference between SIF and OC users during the luteal phase was due to a decline in accuracy in OC users during this phase, the SIF group were consistently more accurate than both other groups across all phases. The lack of effect on serial three subtractions suggests that this improvement with SIF use may only be evident in tasks with higher cognitive demand. The N-back task is also cognitively demanding, and although performance was improved during different cycle phases, taken together these findings suggest an improvement in performance only on cognitively demanding tasks. This may be due to the effects of SIF on the cholinergic system, since recruitment of this system increases with higher cognitive demand reflecting more effortful processing (Sarter, Hasselmo, Bruno, & Givens, 2005). The cholinergic system is also important for attention and memory encoding (Gais & Born, 2004; Rogers & Kesner, 2003), processes that are employed in performance of the serial sevens task. SIF increases cholinergic activity by increasing choline acetyltransferase (ChAT) activity and reducing acetylcholinesterase (AChE) activity, as well as increasing the density of cholinergic neurons in the medial septum and CA_1 region of the hippocampus (Lee et al., 2004). Increases in cholinergic activity therefore provide a

plausible explanation for the enhanced performance of cognitively demanding tasks with SIF supplementation.

Contrary to the hypothesis that spatial working memory would be improved with SIF use, no effect on this outcome was found. This is in contrast to the findings of Thorp et al. (2009) who found improved spatial memory with a similar dose and duration of SIF supplementation to that used in the current study. However, an important difference between these studies is that Thorp et al. investigated the effects in men only, who were not included in the current study. A further explanation for the discrepant findings is that at baseline no effects of cycle phase were seen for this outcome in NC women, suggesting that hormonal status did not affect performance of this task in the women in the current study. This is perhaps due to ceiling effects, and future studies should increase task difficulty to further explore the relationship between SIF use and spatial working memory.

10.5 Effects of SIF on inhibition and associated brain electrical activity

10.5.1 Behavioural findings

Contrary to the expectations that SIF supplementation would result in improved response inhibition performance, there were few effects of chronic SIF supplementation on behavioural indices of inhibition in the current study. The number of errors of commission did not vary between groups on either the standard or the face emotion tasks, however response times to happy faces were slower with SIF compared with OC users when neutral faces were the nogo stimulus. This may suggest an increased difficulty in discriminating positive and neutral faces with SIF use, although no differences between SIF and placebo were found. It could tentatively be hypothesised that this may reflect increased hostility with SIF compared with OC use as hostile individuals tend to rate happy faces as less friendly (Knyazev et al., 2008), and indeed SIF supplementation was associated with increased self-rated hostility, although only during the luteal phase (see Chapter 5). However, the lack of effect of SIF on any other behavioural outcome in this experiment suggests that this effect may be due to chance.

The lack of an effect of SIF supplementation on errors of commission is somewhat surprising given the findings that women treated with SIF rated their levels of motor impulsivity as higher during menses compared with OC users during the second treatment cycle. This would suggest that more errors of commission would be made by women treated with SIF during menses in this experiment, which was not the case. However, SIF supplementation was also associated with reduced aggression during menses, which would suggest fewer errors of commission during this phase since aggression is known to be closely related to impulsivity (Barratt et al., 1999; Vigil-Colet & Codorniu-Raga, 2004). The lack of effect of SIF supplementation compared with placebo on accuracy in this task suggests that although personal perceptions of impulsivity may be influenced by SIF, actual impulsivity is not. Furthermore, since impulsivity is more closely related to reactive rather than instrumental aggression, this suggests that any effects on self-reported aggression may reflect changes in less reactive forms of aggression, such as hostility, rather than more impulsive forms such as physical aggression. Indeed, physical aggression ratings were not affected by SIF supplementation in the current study.

10.5.2 Effects on ERP components

Despite the lack of performance differences on either the standard or emotional go/nogo tasks in the current study, some electrophysiological effects of SIF were found. SIF supplementation was associated with a smaller difference in N2 latency between nogo and go conditions during the luteal phase when neutral faces were the infrequent stimulus type, but a larger difference in P3 latency between nogo and go conditions regardless of cycle phase. Whilst no group differences were found when angry faces were the infrequent stimulus type, only women treated with SIF showed any phase effects with a larger difference between nogo and go P3 latency during the luteal phase compared with during menses. Importantly, no differences were found in the standard go/nogo task, supporting the hypothesis that salience of the stimulus is important when investigating the effects of changes in hormone levels on tasks of response inhibition (Johnston & Wang, 1991).

N2 latency is thought to reflect speed of processing of a stimulus (Folstein & Van Petten, 2008), suggesting that SIF may be associated with faster early processing of

neutral face stimuli. Previous studies have not investigated the effects of SIF on the N2 and P3, however it has been reported that N2 latency to nogo stimuli is shorter during menses, when endogenous hormone levels are low, compared with the follicular and luteal phases (Walpurger et al., 2004). It could therefore be postulated that the shorter N2 to nogo stimuli during the luteal phase with SIF supplementation is due to the effects of SIF on lowering circulating hormone levels during periods when endogenous hormones are high (Kuiper et al., 1998). Unfortunately, due to the lack of reliable hormone assays this theory could not be tested in the current study and will require further investigation in future trials.

Existing literature regarding the effects of female sex hormones on P3 latency are rather unclear. Whilst some studies have found longer latency during the ovulatory phase than during menses and the luteal phase (Tasman et al., 1999), others have found shorter latency during the ovulatory phase (O'Reilly et al., 2004). Several studies have also found no variations in P3 latency across the menstrual cycle, suggesting that for abstract stimuli at least P3 latency is not affected by fluctuating steroid hormones (Baker & Colrain, 2010; Ehlers et al., 1996; Walpurger et al., 2004).

The increase in P3 latency to nogo stimuli with SIF supplementation in the current study suggests a slowing of neural processing speed to facial stimuli, since P3 latency is thought to be a reliable measure of processing speed (Polich, 1996). Whilst this effect was evident regardless of cycle phase when neutral faces were the target stimuli, P3 latency was increased only during the luteal phase in women treated with SIF when angry faces were the target stimuli. This could be interpreted in two ways. Since emotional processing is disrupted in aggressive individuals (Hall, 2006) this slowing of processing speed may reflect an increased difficulty in discriminating different emotions during the luteal phase in women treated with SIF, possibly due to the increases in aggression with SIF supplementation that were found in this study. On the other hand, the P3 has also been suggested to reflect the direction of attentional resources to a stimulus (Polich, 2003), and since aggressive individuals show an attentional bias towards angry faces (van Honk et al., 2001) this suggests that the longer P3 latency to angry faces during the luteal phase may reflect reduced attention towards these stimuli and therefore reduced aggression. Whilst the exact implications of these effects cannot be discerned from the data in the current study, these findings do support those of

Johnston and Wang (1991) who suggested that the salience of emotional stimuli may vary with fluctuations in hormone levels, and that ERP components would also be affected depending on the salience of the stimulus.

There is some evidence to suggest that SIF supplementation may reduce dopaminergic activity in the vas deferens (Velis et al., 2008). Since dopamine is thought to be involved in eliciting both the N2 and P3 in tasks of response inhibition (Beste et al., 2010; Polich & Criado, 2006) this may go part way to explaining the effects of SIF on these components. It has been suggested that the effects of SIF on dopaminergic activity may vary depending on brain region, and in particular dopamine transporter (DAT) expression in the prefrontal cortex was found to increase with administration of genistein (Neese et al., 2010). In addition, isoflavones are reported to protect against insult to the dopaminergic system by attenuating induced reductions in dopamine uptake and loss of dopaminergic neurons in mesencephalic cultures (Chen, Sun, Wang, Xu, & Jin, 2011). Whilst the mesencephalon has not been implicated in eliciting either the N2 or the P3, the prefrontal cortex has been suggested to be involved in eliciting the N2 (Huster et al., 2010; Lavric, 2004). This may explain the faster N2 latency with SIF supplementation, since dopaminergic activity may be increased in the N2 source generator. Further research using techniques with higher spatial resolution could aid in extrapolating these effects and investigating activity in brain regions associated with both components following SIF supplementation.

10.6 Limitations and future directions

The strength of the current study lies in the use of testing during different cycle phases across multiple cycles and the inclusion of OC users as a comparison group. However, several limitations should be considered. Firstly, the lack of reliable salivary measures of circulating estradiol means that estimated cycle phase based on the counting backwards method could not be confirmed. Although care was taken to preserve all samples, degradation of some samples may have occurred due to the length of time between sample collection and analysis. This resulted in a large number of samples where estradiol concentration was below the threshold for detection. There was also large variation in estradiol concentrations in participants from the OC group, with some

participants having more than a 10-fold increase in concentration between phases. Since estradiol levels in this group should remain consistently relatively low, this suggests a methodological error and therefore the saliva samples were deemed to be unreliable. This is likely due to the method of collection, since cotton-based sample collection has been shown to interfere with immunoassay results and to introduce error of sufficient magnitude to attenuate the association between serum and saliva levels (Shirtcliff, Granger, Schwartz, & Curran, 2001). Additionally, the lack of reliable estradiol assays means that theories of the mechanisms of action underlying the observed effects can only be speculative.

To reduce the likelihood of errors in the estimation of cycle phase, where there was doubt over which phase a participant had been tested in these data were excluded from the analysis. This resulted in a large amount of missing data. Despite all participants who were eligible for the trial claiming to have regular cycles, for many of the NC women their cycle lengths were not as expected during the trial. In order to overcome this in future studies, researchers could utilise ovulation test kits as a reliable method of estimating ovulation. This was not possible in the current study due to budget restraints, however the use of linear mixed models for analysis enabled the use of all reliable data. It should also be noted that only around 60% of women aged 20-24 ovulate during every cycle (Metcalf & Mackenzie, 1980). Since ovulation was not verified in this study it cannot be assumed that all the women had ovulated when they were tested during the ovulatory phase, or that circulating hormone levels followed the expected pattern.

As the go/nogo task is a measure of response inhibition and provides information regarding levels of impulsivity, any links between the electrophysiological measures used in this study and aggression can only be made with caution. In particular, although impulsivity has been consistently shown to be related to reactive aggression (Barratt et al., 1999; Netter et al., 1998; Vigil-Colet & Codorniu-Raga, 2004) it may not be so closely related to other forms of aggression such as hostility. This is of particular concern since the effects of SIF were found on hostility and anger and not overt forms of aggression such as physical and verbal aggression. In addition, the effects of SIF on self-reported aggression were found primarily during the first treatment cycle, with few effects during the second treatment cycle. Since EEG measures compared only baseline

with the second treatment cycle, and did not include measures during the first treatment cycle, this may have resulted in fewer significant findings.

It could be argued that the use of only two face emotions as target stimuli in the EEG tasks is a limitation. Previous studies have found differences in the processing of fearful and happy faces between individuals with high and low levels of aggression, including non-impulsive forms of aggression such as hostility (Hall, 2006; Knyazev et al., 2008). Although happy faces were included as stimuli in the current study, due to time constraints within testing sessions it was not feasible to run the go/nogo task with happy faces as the target stimuli in this study. Since behavioural effects of SIF supplementation were found only for responses to happy faces in this experiment, future studies should investigate the effects of SIF on cortical activation to happy faces. Previous studies have also shown that the effects of hormonal status on ERPs depend on subjective ratings of pleasantness and intensity of the stimulus (Johnston & Wang, 1991). Participants in the current study were not asked to rate the different stimulus categories, therefore further research could explore whether the effects of SIF on the N2 and P3 do depend on these subjective ratings.

The current study could have been improved by measuring circulating isoflavone levels, and in particular equol. It has been suggested that the effectiveness of isoflavone supplementation in hormone-dependent studies depends on whether or not the recipient is an equol producer (Setchell, Brown, & Lydeking-Olsen, 2002). Blood pressure was found to decrease following soy protein supplementation in equol producers, whereas in non-producers blood pressure actually increased (Kreijkamp-Kaspers et al., 2005). Since only around 30-50% of the population produce equol (Rowland, Wiseman, Sanders, Adlercreutz, & Bowey, 2000; Setchell et al., 1984), it would be beneficial to know the percentage of the sample used in this study who were producers. However, in a study of the effects of SIF on PMS symptoms, effectiveness of treatment did not differ between equol producers and non-producers (Bryant et al., 2005). Future studies should aim to measure isoflavones as well as levels of circulating steroid hormones in order to better understand the mechanisms of action of these compounds.

10.7 Summary and Conclusions

The results of this exploratory study indicate that supplementation with 200mg/day total isoflavones is associated with short term worsening of mood and premenstrual symptoms during the luteal phase in healthy, asymptomatic women. In particular, SIF is associated with increased depression, reduced contentedness and reduced calmness, as well as an increase in severity of physical symptoms during the luteal phase in comparison to placebo. These effects were only evident during the first treatment cycle, with no difference between groups during the second treatment cycle suggesting only a short term worsening of symptoms with SIF use which may indicate a development of tolerance with prolonged use.

Aggression also appeared to be increased with SIF supplementation in the current study, although the effects during different phases were rather unclear. Whereas anger may be increased compared to placebo regardless of phase, other components of aggression such as hostility appear to increase during the luteal phase only, and overall aggression may actually be reduced during menses.

Few cognitive tasks showed any effect of SIF supplementation in the current study, however the findings do suggest a possible detriment to tasks of executive function during the luteal phase, whereas working memory may be improved with SIF supplementation, particularly during menses. In contrast to the effects on mood the majority of effects on cognitive function did not emerge until the second cycle, suggesting that these effects may not be evident with short term isoflavone use.

Taken together the findings of the current study suggest that in normally cycling, asymptomatic women, a high dose of SIF may be associated with poorer mood and increased aggression during the luteal phase, whereas beneficial effects may be seen during menses. This may be due to the effects of SIF on endogenous hormones, with antagonistic effects being apparent when endogenous hormone levels are high, or could reflect a withdrawal effect as peak plasma levels of SIF fall. In addition, executive function may be impaired during the luteal phase, whereas the current findings suggest an improvement in working memory with long term SIF treatment. In contrast, OC use appears to have beneficial effects in long term users on various measures of premenstrual symptoms (see Chapters 7 and 8). These effects are consistent across cycles and phases, suggesting they may be a preferable treatment over SIF for women experiencing premenstrual symptoms.

Chapter 11

General Discussion

This thesis reported the findings of two studies investigating the effects of chronic soy isoflavone (SIF) supplementation on aggression, mood and cognitive function, as well as changes in brain activity that may underlie any effects on these outcomes, in healthy young women using a randomised, double-blind, placebo-controlled methodology. The effects of oral contraceptive (OC) use and menstrual cycle phase on these outcomes were also examined. The discussion will begin with a summary of the key findings of the experimental chapters, including how these findings relate to the broader literature. This will be followed by a discussion of the potential mechanisms of action of SIF as they relate to these data. The limitations of the study will be outlined and implications of the findings will be discussed, as well as future directions for research in this area.

11.1 Summary of key findings and their implications

There were several aims of the current thesis. The primary purpose was to examine the effects of chronic SIF supplementation on aggression in healthy young women. Although several authors have reported neuro-regulatory effects of SIF (e.g. Linford & Dorsa, 2002; Luine et al., 2006), the effects of SIF on brain activity in humans had not been previously studied. Therefore, to assess the potential mechanisms underlying any effects of SIF on aggression, ERP measures during tasks of response inhibition, which is closely linked to aggression, were also taken in order to examine whether changes in brain electrical activity may underlie changes in aggression. Further aims were to examine the effects of SIF on other facets of mood and premenstrual symptoms, as well as cognitive function. In addition, the effects of OC use on these outcomes were examined as a positive control. A final aim was to determine whether these effects varied across the menstrual cycle.

Contrary to the hypothesis that administration of SIF would result in lower levels of aggression compared with placebo, the results demonstrated an increase in self-rated aggression with SIF supplementation, particularly during the luteal phase. This effect was evident only for certain forms of aggression, such as anger and hostility, whilst physical and verbal aggression were not affected by SIF. Whilst an overall increase in aggression was observed with SIF supplementation, there was some evidence to suggest that SIF may reduce aggression specifically during menses. Again, this effect was not

consistent across measures, suggesting differential effects of SIF on various forms of aggression. Previous studies have reported both increases (Simon et al., 2004; Patisaul & Bateman, 2008) and reductions in aggression (Wizniewski et al., 2005; Zeng et al, 2010) associated with SIF supplementation in animals, however this is the first trial to investigate such effects in humans. These findings suggest that the effects of SIF on aggression may depend on endogenous levels of steroid hormones, which may explain the divergent effects found in previous animal studies. The changes in self-reported aggression with SIF supplementation were not reflected in treatment effects on performance of the go/nogo task, suggesting that relational rather than impulsive forms of aggression may be affected by SIF.

Other mood outcomes and premenstrual symptoms were also found to worsen during the luteal phase in the SIF group as compared to placebo. In particular, depression and physical symptoms were increased, although anxiety was not affected. These findings contrast with those of several other studies which have found improvements in premenstrual symptoms with SIF supplementation (Bryant et al., 2005; Ishiwata et al., 2003; Kim et al., 2006). Physical symptoms particularly have been reported to benefit from SIF supplementation (Bryant et al., 2005; Ishiwata et al., 2003). These discrepant findings may be explained by the inclusion of only asymptomatic women in the current study, as well as the higher dose of isoflavones administered. Future studies should aim to discover the optimal dose for the reduction of both physical and psychological symptoms. It should also be noted that women in the current study experienced more severe symptoms during menses rather than the luteal phase. As symptoms were improved during menses with SIF compared to placebo, women who experience more severe symptoms during the luteal phase may show improvements with SIF during that phase.

Few effects of SIF were observed on cognitive function in the current study. The findings suggest possible detrimental effects on tasks of executive function and reaction time during the luteal phase associated with SIF supplementation. This is in contrast to several prior reports of improved executive function following administration of SIF (e.g. Duffy et al., 2003; File et al., 2001). However, consistent with previous research showing improved working memory performance associated with SIF consumption during menses (Islam et al., 2008), performance of the N-back task in the current study

was enhanced by SIF during menses. In addition, accuracy on the serial sevens subtraction task was shown to benefit from SIF supplementation in the current study. This supports the notion that only tasks with a high level of cognitive demand may show improvements following administration of SIF (Duffy et al., 2003; Pilsakova et al., 2009).

Regarding the effects of SIF on brain activity, contrary to expectations there was no effect of SIF on N2 or P3 amplitude. Although SIF and placebo did not differ in their effects on the amplitude of inhibition-related components, there were differences in terms of latency of these components. Whilst SIF was associated with shorter N2 latency to nogo neutral face stimuli during the luteal phase, P3 latency was longer with SIF use to both nogo neutral face stimuli regardless of phase, and nogo angry face stimuli during the luteal phase compared with menses. Shorter N2 latency is thought to reflect faster early processing of the stimulus (Folstein & Van Petten, 2008), therefore the shorter N2 to neutral faces during the luteal phase may reflect an increased salience of these stimuli to women administered SIF during the luteal phase. On the other hand, the longer P3 latency to face stimuli suggests a differential slowing of neural processing speed to these stimuli (Polich, 1996). This may underlie the increases in aggression seen with SIF use, since studies have shown disruptions to emotional processing in aggressive individuals (Hall, 2006). Alternatively, the longer P3 latency may be responsible for the slower reaction times seen with SIF use on several cognitive measures. However, this is unlikely given the lack of behavioural differences between groups on the go/nogo task, and the fact that longer P3 latency was only observed with face stimuli rather than abstract stimuli. These findings are therefore consistent with the hypothesis that the longer latency to these stimuli reflects the attentional resources devoted to processing the different stimuli.

Regarding the effects of OC use, as predicted OC users reported being less aggressive than NC women across several self-report measures of aggression. This is consistent with our previous findings of reduced aggression in OC users (Perry et al., under review), although in the current study no interactions with cycle phase were observed, and there were no effects of contraceptive status on the objective measure of aggression in the current study. This may be due to the finding that NC women did not report experiencing more severe aggression during the luteal phase in the current study,

whereas in the previous study ratings of aggression were significantly higher during the luteal phase than any other phase for NC women.

Although there was no difference in amplitude of either ERP component between OC users and NC women in the standard go/nogo task, differences were observed when faces were presented, such that OC users demonstrated enhanced N2 to nogo neutral faces. As aggressive individuals have been shown to have attenuated N2 in the go/nogo task (Chen et al., 2005) this effect may be part of the same process underlying the increased aggression in NC women. Indeed, physical aggression was found to differ between OC users and NC women, and has previously been shown to be closely related to impulsivity (e.g. Barratt et al., 1999). However, it could also be postulated that the enhanced N2 in OC users reflects an increased ease of inhibition and ability to inhibit responses in this group, as reaction times were faster for OC users than NC women. On the other hand, compared with OC users NC women had an enhanced P3 difference between go and nogo neutral faces, which could be suggested to indicate an increased salience of these stimuli to NC women. In addition, the N2 to nogo angry faces was enhanced in NC women compared with OC users, although this effect was restricted to the luteal phase. This may suggest improved early inhibitory control for aggression-related stimuli during periods of high circulating steroid hormones. These findings also highlight the importance of including stimuli with different emotional valence when investigating the effects of female sex hormones.

Consistent with several prior studies (Berenson et al., 2008; Mordecai et al., 2008; Toffol et al., 2012) OC users were found to have less severe ratings of mood and other premenstrual symptoms compared with NC women. It was expected that the difference between OC and NC groups would vary during different cycle phases due to greater fluctuations in symptoms in the NC group and more stable symptoms over time in the OC group. However, few interactions between contraceptive status and menstrual cycle phase were observed. This is likely due to the finding that in the current study, symptoms were most severe during menses for both OC users and NC women, rather than being most severe during both the luteal phase and menses for NC women as is often reported in the literature (Romans et al., 2012).

Also consistent with predictions was the superior performance of OC users across several cognitive domains. Reaction times on several tasks were faster with OC use, and

performance of tasks involving a high level of cognitive effort was also superior in OC users compared with NC women. This is similar to the finding of improved performance with SIF for only tasks with a heavy cognitive load, and suggests that the effects of female sex hormones on cognitive performance may only be evident when task difficulty is high. In addition, whereas NC women showed cyclical variation in reaction times on several tasks, this cyclicity was not evident in OC users. This finding is consistent with previous research demonstrating that OC use has a stabilising effect on cognitive function (Rosenberg & Park, 2002). Reaction times on the go/nogo task were also faster for OC users compared with NC women, a finding which was accompanied by shorter latencies in nogo minus go wave differences, indicating faster processing speed (Polich, 1996). Taken together these findings suggest that long term OC use is associated with enhanced neural processing speed.

The findings of this thesis suggest that unlike synthetic hormones, administration of phytoestrogens does not stabilise premenstrual symptoms and in fact may result in poorer outcomes during periods where endogenous hormone levels are high. However, it should be taken into consideration that the negative effects of SIF were largely confined to the first treatment cycle, suggesting that these negative effects may improve with longer term treatment. This makes any comparison with OC use difficult, since only long term OC users were included in the current study. In addition, since symptoms were improved during menses, the phase of peak symptom severity, this does suggest that SIF may be an effective therapy during this phase for asymptomatic women who do not tolerate OCs well. Further research is needed to confirm whether these beneficial effects would be apparent long term. Overall, it appears that OC use may be more beneficial than SIF in women who can tolerate it as this has consistent positive effects across phases and cycles.

The lack of effects of SIF on amplitude of the inhibition-related ERP components suggest that the negative effects of SIF on aggression may not be due to changes in brain activity in regions associated with impulsivity. However, group differences in latency of these components may explain some of the effects observed. A discussion of other proposed mechanisms which may contribute to the observed behavioural effects will follow.

11.2 Putative mechanisms of action of soy isoflavones and oral contraceptives

The following sections will discuss potential mechanisms of action of the SIF supplement administered in the current study which may have mediated the observed effects on aggression, mood and cognitive function as well as other premenstrual symptoms. It is important to note that it is beyond the scope of this thesis to isolate which, if any, of the isoflavones contained in the supplement contributed most to these effects. Without data regarding the absorption of these compounds, or their physiological effects on endogenous hormone levels, any putative mechanisms can only be speculated on. Nevertheless it is worth considering these findings in the context of previous research focusing on the known effects of SIF on endogenous hormones, neurotransmitters and brain activity, as well as their effects on cardiovascular function.

11.2.1 Alterations in circulating endogenous steroid hormone levels

The finding that the effects of SIF on aggression varied with menstrual cycle phase suggests that these effects may be mediated by endogenous steroid hormone levels. Specifically, administration of SIF appears to reduce aggression during menses when endogenous hormone levels are at their nadir. Conversely, during the luteal phase when levels of both estrogen and progesterone are high, SIF administration increased severity of aggression. These findings are consistent with evidence suggesting that SIF may exhibit estrogenic activity when levels of endogenous estrogen are low, whereas when endogenous estrogen levels are high, administration of SIF can have anti-estrogenic effects (Kuiper et al., 1998) as well as reducing levels of other endogenous hormones through increased production of sex hormone binding globulin (SHBG; Adlercreutz et al., 1998). Activation of ERβ by SIF during periods of low circulating estradiol provides a particularly convincing explanation for the improvements in aggression observed during menses, as activation of ERβ is thought to have inhibitory effects on aggression (Nomura et al., 2002) and SIF preferentially bind to this receptor type (Kuiper et al., 1997). In asymptomatic women, aggression may be reduced when levels of both estrogen and progesterone are high (Ritter, 2003), therefore the potential reduction in

activity of these steroids with SIF supplementation through increased SHBG and occupation of ERs by SIF during the luteal phase may explain the increases in aggression. This is likely mediated through effects on neurotransmitters as will be discussed in the following section.

The effects of SIF on endogenous steroid hormone levels may also explain the effects on mood and other premenstrual symptoms observed in the current study. Similar to the effects on aggression, several parameters of mood as well as physical symptoms showed negative effects of SIF supplementation compared with placebo, particularly during the luteal phase. The negative effects of SIF on mood were not evident during menses, when endogenous hormones are low. Estrogen has been implicated in promoting positive mood, with lowered estrogen levels being associated with increased depression (Warnock et al., 2000; Young et al., 2000), therefore the anti-estrogenic effects of SIF when endogenous estrogen levels are high (Kuiper, 1998) may underlie the poorer mood outcomes observed during the luteal phase. However, several studies have shown no differences in endogenous hormone levels between women with PMS/PMDD and asymptomatic women (e.g. Gingnell et al., 2012), therefore without evidence of changes in endogenous hormone levels these suggestions can only be speculative.

The findings of the current study also suggest a potential role for the effects of SIF on progesterone levels in mediating some cognitive outcomes. In particular, the negative effects of SIF on tasks of executive function during the luteal phase are more likely to reflect the effects on progesterone than estrogen, since performance was actually highest during the ovulatory phase when endogenous estrogen levels are high but progesterone is low. Since executive functions did show cyclical variation at baseline, this suggests that current hormonal status is an important factor in the performance of these tasks. However, it is unlikely that the positive effects of SIF on cognitively demanding working memory tasks were mediated by the effects on endogenous hormones, since these tasks did not show cyclical variation at baseline. The neuroprotective effects of SIF and effects on neurotransmitters more likely underlie these effects as will be discussed in the following sections.

11.2.2 Possible effects on neurotransmitter systems

Whether the actions of SIF on aggression, mood and cognitive function in the current study were due to their direct effects or through effects on endogenous hormone levels, it is likely that at least some of these effects were mediated by changes in neurotransmitter activity. Regarding the effects of SIF on aggression, it is unlikely that changes in aggression levels were mediated by alterations to the dopaminergic system since the N2 and P3 amplitudes in the standard go/nogo task were not affected by SIF supplementation. The dopaminergic system is implicated in eliciting both of these components in tasks of response inhibition (Polich et al., 2006; Beste et al., 2010). Therefore if SIF supplementation resulted in altered dopaminergic activity, these components might be expected to show effects of SIF. The finding that latency of these components was affected when faces were the target stimulus does, however, suggest that dopaminergic activity may be altered in brain regions not related to response inhibition, as will be discussed below.

Effects on the serotonergic system, on the other hand, may provide an explanation for the changes in aggression observed with SIF supplementation. Phytoestrogens have been shown to increase serotonergic function in ovariectomised (OVX) monkeys when administered at a similar equivalent human dose to that used in the current study (Shively et al., 2003). This suggests that when endogenous hormones are low, administration of SIF may increase serotonergic activity. As increased serotonin is associated with reduced levels of aggression (Cleare & Bond, 1995), this may explain the lowered levels of aggression observed during menses following SIF supplementation. On the other hand, the antagonistic effects of SIF on estrogenic activity during the luteal phase may result in reduced serotonergic activity as endogenous estrogen has been shown to be positively associated with serotonin synthesis (Hiroi et al., 2006). This would explain the increases in aggression during the luteal phase with SIF supplementation compared to placebo. The effects of SIF on serotonergic function may also explain the findings of poorer mood during the luteal phase, since serotonin is also implicated in the promotion of positive mood, and reduced serotonergic function is associated with increased mood disturbances (Baldwin & Rudge, 1995; Neumeister, 2002).

Regarding the effects of SIF on cognitive function, a likely mechanism underlying the improvements observed in performance of cognitively demanding tasks of working memory concerns the effects of SIF on cholinergic activity. Previous research has demonstrated an increase in the density of cholinergic neurons in the hippocampus following administration of SIF (Lee et al., 2004), a region known to be crucial for working memory performance (Olton et al., 1979; Tesche & Karhu, 2000). In addition, SIF increases activity of choline acetyltransferase (ChAT) and reduces activity of acetylcholinesterase (AChE). These combined effects result in an overall increase in cholinergic activity. Since the cholinergic system is important for the performance of tasks with a high level of cognitive demand (Sarter et al., 2005), and only tasks requiring a high level of cognitive effort were improved in this study, these findings suggest cholinergic involvement in the improvements in working memory with SIF supplementation observed in the current study.

11.2.3 Suggested direct effects in the brain and neuroprotective effects

In addition to the above mentioned potential mechanisms of action of SIF, it is possible that some of the observed effects arose from direct actions of SIF in the brain. The lack of effect of SIF on amplitude or latency of either the N2 or P3 in the standard go/nogo task suggests that SIF did not directly affect brain regions associated with response inhibition, such as the midcingulate cortex (MCC). However, since the latency of these components did show effects during processing of face stimuli, this suggests possible effects in brain regions associated with social and emotional processing. In particular, the prefrontal cortex is a region that warrants further investigation in future trials, as this region is thought to be involved in eliciting the N2 (Huster et al., 2010). SIF have been reported to increase dopamine transporter (DAT) activity in the prefrontal cortex, suggesting that this brain region may be a potential site for the actions of SIF.

The neuroprotective effects of SIF have been demonstrated in several previous studies (Donzelli et al., 2010; Luine et al., 2006). The hippocampus has been the brain region most studied to date in this context, with research demonstrating an increase in dendritic spine density in the CA_1 region induced by phytoestrogens (Luine et al., 2006). Genistein in particular may be responsible for these neuroprotective effects, as genistein

was found to protect cells against insult in both the CA_1 region (Donzelli et al., 2010) and the dentate gyrus (Azcoitia et al., 2006). However, researchers have suggested that although genistein alone does have neuroprotective effects, the synergistic effects of a combination of isoflavones are greater than those of genistein alone (Zhao et al., 2009). These neuroprotective effects may be mediated by direct effects on ERs in the brain, since the blocking of ERs in the prefrontal cortex (PFC) reduced the protective effects of genistein in this region (Linford & Dorsa, 2002). An alternative hypothesis is that SIF exert their effects through increasing concentrations of brain derived neurotropic factor (BDNF). Phytoestrogens have been shown to increase BDNF in both the frontal cortex (Lephart et al., 2002; Pan et al., 1999) and the hippocampus (Pan et al., 2010). BDNF is known to promote cell survival (Hartikka & Hefti, 1988) and also increases synaptic plasticity (McAllister et al., 1999).

Taken together these findings suggest that a potential mechanism underlying the positive effects of SIF on working memory performance in the current study may be via the same mechanisms which underlie their neuroprotective effects, particularly in the hippocampus. Whilst the effects on mood were observed in the first treatment cycle, the effects on working memory did not emerge until the second treatment cycle, suggesting a more long term effect of SIF on these outcomes which may share similar underlying mechanisms as their neuroprotective effects. The neuroprotective role of SIF therefore provides a plausible explanation for these findings. In addition, as the tasks that showed improvement rely on hippocampal function, this explanation makes sense as the neuroprotective effects of SIF are known to be exerted in this region. The effects of SIF on BDNF may also be important for the improvements seen in working memory tasks, as BDNF is crucial for long term potentiation (LTP) induction, which is important for memory functions (Kovalchuk et al., 2002). Future studies should aim to elucidate whether the cognitive effects of SIF are due to individual compounds or to a synergistic effect of these compounds in order to better understand these potential mechanisms.

11.2.4 Potential involvement of cardiovascular mechanisms in the effects of SIF

The enhancement in performance of cognitively demanding working memory tasks following SIF supplementation may be explained by the effects of SIF on

cardiovascular function. Isoflavone supplementation has been shown to reduce arterial pressure in older adults (Carlson et al., 2008) as well as in healthy normotensive participants (Welty et al., 2007). In addition, isoflavones have been shown to reduce arterial stiffness and improve endothelial function (Nestel et al., 2007), supporting the notion that consumption of isoflavone-containing foods may improve cardiovascular function. The antioxidant properties of SIF (Behl et al., 1995) may underlie these improvements in cardiovascular function through increasing the availability of nitric oxide, although further research will be needed in order to ascertain whether other actions of SIF are involved. Impaired cardiovascular function is associated with detriments to cognition, with high arterial pressure being associated with vascular dementia and Alzheimer's disease (Breteler, 2000). Protection of cardiovascular function may therefore also protect against cognitive impairment.

The finding that only tasks of working memory with a high level of cognitive demand were improved with SIF supplementation in the current study, whereas no other cognitive domains were improved, suggests that improvements in cardiovascular function may underlie these positive effects. Future studies of the cognitive effects of SIF should aim to include measures of cardiovascular parameters such as blood pressure, arterial stiffness and cholesterol in order to further investigate the possibility that the cardiovascular effects of SIF underlie improvements in working memory.

11.3 Limitations

Several limitations of this trial were identified in the baseline and post-treatment discussion chapters. Perhaps the biggest shortfall of the current thesis is the lack of hormonal assays to assess the effects of SIF and OC use on levels of circulating estradiol, as well as the lack of biochemical analysis of circulating isoflavones, in particular equol. This means that any discussion of the potential mechanisms of action of SIF in the current trial can be necessarily speculative. Several other issues relating to the sample and methodology used in the current study should also be addressed.

It is important to consider the possibility that due to the number of hypotheses tested in this thesis, a Type 1 error may have been made. In studies where multiple comparisons are made, there is a greater chance of incorrectly rejecting the null hypothesis (Shaffer,

1995). Due to the exploratory nature of this study, corrections for multiple comparisons were not made, therefore the findings should be interpreted with caution. This is particularly important where effects reached only trend level or borderline significance.

With regard to the sample used in the current trial, the inclusion of only non-help-seeking women may limit the generalizability of the findings to women who suffer from PMS/PMDD. This is particularly relevant since the findings of the current trial suggest a worsening of symptoms during the luteal phase, which was not characterised by more severe symptoms at baseline, whereas a slight improvement in symptoms was observed during menses, which was characterised by the most severe symptoms at baseline. Therefore in women who experience severe premenstrual symptoms during the luteal phase the effects of SIF supplementation may provide more benefits than those found here. In addition, these findings cannot be generalised to males, and a replication of the current study could ascertain the effects of SIF on aggression and brain activity in males.

Finally, the soy germ extract itself should also be considered. The product used in the current study was extracted from the soy germ using ethanol, and there is some evidence to suggest that this method of extraction can remove phytoestrogens from soy (Coward, Smith, Kirk, & Barnes, 1998). Although the extract in the current study was standardised to 40% isoflavones, the possibility that ethanol extraction may have affected the isoflavones should be taken into consideration. In addition, due to the inclusion of three isoflavones in the supplement it is not known whether the effects found in the current study are due to only one of these or a combination. Future studies could examine the effects of the individual isoflavones separately in addition to the combined effects.

Despite these limitations, the findings of the current study highlight the importance of accounting for menstrual cycle phase when investigating the effects of SIF in young women. The finding that the effects of SIF differed depending on endogenous hormonal status may contribute to explaining some of the discrepant findings in the literature, since the majority of studies did not account for menstrual cycle phase. In addition, the current findings add to the existing body of literature on the effects of menstrual cycle phase in women without premenstrual complaints, and support the findings of Meaden et al. (2005) who reported more severe symptoms during menses rather than the

worsening of symptoms during the luteal phase that is most commonly described (Reed et al., 2008; Sakai et al., 2013; Seippel & Backstrom, 1998).

11.4 Conclusions and future directions

This study aimed to investigate the effects of a high dose of soy isoflavones on aggression, mood and cognitive function in a sample of healthy young women without premenstrual complaints. Specifically, these effects were examined during different phases of the menstrual cycle in order to elucidate whether the effects vary according to endogenous hormone levels. In addition, the effects of oral contraceptives on these outcomes were assessed through comparisons between current contraceptive users and normally cycling women.

The current trial was the first study into the effects of SIF on human aggression, and animal studies have yielded inconsistent results. Similarly, findings from studies of the effects on mood and cognitive function in humans have been somewhat conflicting to date although generally the outcomes appear to be positive. Contrary to expectations, the findings of the current study demonstrated a worsening of several aggression, mood and cognitive measures during the luteal phase of the menstrual cycle with SIF supplementation. These findings suggest negative effects of SIF during periods of high endogenous steroid hormones. On the other hand, some outcomes were improved during menses, suggesting that SIF may benefit aspects of mood and cognition when endogenous steroid hormones are low. In addition, the findings suggest possible improvements in tasks with a high level of cognitive demand, although less demanding tasks may not be affected by SIF supplementation. To maximise the likelihood of capturing cognitive enhancements in healthy young women, future studies could focus on the inclusion of more cognitively demanding tasks.

OC use, on the other hand, was found to be associated with improved mood outcomes, aggression and several cognitive measures, particularly those involving high cognitive demand or reaction times. These findings were consistent across cycles and across all cycle phases. Although some individuals do not tolerate OC use as well as others (see Chapter 3), these findings suggest that for those women who do not experience negative side effects of OC, this may be a preferable treatment alternative over SIF.

Prior research has shown that whilst hormonal status may not have an effect on aggression levels *per se*, hormone levels may affect aggressive responding during negative situations (Van Goozen et al., 1996). It would therefore be interesting for future researchers to investigate the effects of both SIF and OC use on objectively measured aggressive responding under provocation.

One of the more interesting findings of the current trial was that the negative effects of SIF were apparent during only the first treatment cycle, whereas during the second cycle few negative effects were observed. It is likely that supplement users in real life situations would continue using the supplement for longer than the two menstrual cycles assessed in the current study. Therefore future researchers could conduct trials of longer duration with larger samples. These trials should also focus on elucidating the possible mechanisms of SIF underlying the mood deteriorations seen during the luteal phase, as well as those underlying the enhancement of cognitive function in mentally demanding tasks. In addition, as the dose used in the current study was higher than that used in previous studies, the effects of different doses should be studied in order to determine the optimal dose for improving aggression and mood in young women. Finally, as the current findings are generalizable only to asymptomatic women, future trials should aim to examine whether similar cyclical effects are observed in women with premenstrual complaints.

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Appendices

Appendix A: Ethics declaration

To: Ms Naomi Perry for Prof Andrew Scholey, FLSS

Dear Naomi,

SUHREC Project 2009/224 Chronic effects of isoflavones on cognition and aggression
Prof Andrew Scholey, FLSS; Ms Naomi Perry et al
Approved Duration Extended to 30/05/2013
[Modified March, June, September, October 2011, February 2012, March 2012, June/July 2012, Extension granted December 2012]

I refer to your request, simply to extend the ethics clearance for the project to complete approved research procedures, as per your email of 5 December 2012 with attached progress report.

There being no change to the approved protocol as approved to date, I am authorised here to issue a simple extension of ethics clearance in line with ethics clearance conditions previously communicated and reprinted below.

The standard conditions for on-going ethics clearance are as follows.

- All human research activity undertaken under Swinburne auspices must conform to Swinburne and external regulatory standards, including the*National Statement on Ethical Conduct in Human Research* and with respect to secure data use, retention and disposal.

- The named Swinburne Chief Investigator/Supervisor remains responsible for any personnel appointed to or associated with the project being made aware of ethics clearance conditions, including research and consent procedures or instruments approved. Any change in chief investigator/supervisor requires timely notification and SUHREC endorsement.

- The above project has been approved as submitted for ethical review by or on behalf of SUHREC. Amendments to approved procedures or instruments ordinarily require prior ethical appraisal/ clearance. SUHREC must be notified immediately or as soon as possible thereafter of (a) any serious or unexpected adverse effects on participants and any redress measures; (b) proposed changes in protocols; and (c) unforeseen events which might affect continued ethical acceptability of the project.

- At a minimum, an annual report on the progress of the project is required as well as at the conclusion (or abandonment) of the project.

- A duly authorised external or internal audit of the project may be undertaken at any time.

Please contact me if you have any queries about on-going ethics clearance, citing the SUHREC project number. Please retain copies of this email clearance as part of project record-keeping.

Best wishes for the extended project.

Yours sincerely

Keith Wilkins
Secretary SUHREC

Ethics declaration: The author would like to state that all ethics conditions pertaining to the ethics clearance were properly met, and all annual reports have been submitted.

SWINBURNE UNIVERSITY OF TECHNOLOGY

Informed Consent Form

Centre for Human Psychopharmacology, Swinburne University of Technology, Hawthorn

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Full Project Title: Chronic effects of Isoflavones on Cognition and Aggression in a female population across the menstrual cycle

Principal Researcher: *Professor Andrew Scholey*[1]

Associate Researchers: *Dr Pat Johnson*[2]*, Ms Marni Kras*[1]

Student Researcher: *Ms Naomi Perry*[1]

Research Assistant: *Ms Michayla Bush*

[1] *Centre for Human Psychopharmacology, Swinburne University of Technology, Australia*

[2] *Department of Psychology, University of York, UK*

Consent

I,…… (Name of participant) agree to participate in a research project entitled: Chronic effects of isoflavones on cognition and aggression.

Conducted by: Professor Andrew Scholey, Doctor Patrick Johnston, Ms Marni Kras, Ms Michayla Bush and Miss Naomi Perry

My agreement is based on the understanding that:

- I agree to participate in this activity, realizing that my identity will remain confidential (other than terms specified above with regards to Clinical Trials Connect), and that I may withdraw at any time.
- I freely agree to participate in this project according to the conditions on the Participant Information.
- I will be given a copy of the Participant Information and Consent Form to keep.
- My consent to participate in this project is given freely.
- The researcher has agreed not to reveal my identity and personal details if information about the project is published or presented in any public form.
- I understand the time involved in each of the practise and testing sessions.
- I am a non smoker
- I am English speaking
- I am aged between 18 and 35 years
- I do not have a history of anxiety, depression, epilepsy or psychiatric disorders

- I am not taking any medications, for example anti-coagulants, anti-depressants, anti-cholinergics or acetycholinesterase inhibitors
- I am not taking any herbal extracts, vitamin supplements or illicit drugs
- I do not have any health conditions that would effect food metabolism including the following: food allergies, kidney disease, liver disease and/or gastrointestinal diseases (e.g. Irritable bowel syndrome, celiac disease, peptic ulcers)
- No history of low or high blood pressure or heart disease
- I am not pregnant or breast feeding.
- I am either using a monophasic oral contraceptive, or no hormonal contraception
- I am a non-regular soy consumer

I have read this document and I understand the purposes, procedures and risks of this research project as described within it.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project, as described.

I understand that I will be given a signed copy of this document to keep.

Participant's name (printed) ……

Signature Date:

Declaration by researcher*: I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Researcher's name (printed) ……

Signature Date:

Appendix B: Isoflavones Food Frequency Questionnaire

Subject ID | | | | | | |

Instructions

Please fill in the circle that best represents your usual pattern of consumption of that food **over the last 12 months**. Please fill in one circle for every food listed, even if you never eat that particular food.

When reading through the list of foods, please think back over the last 12 months and your usual weekday and weekend eating patterns, which might be different. Also think carefully about foods and beverages consumed way from home and when on holiday as well as those foods prepared and consumed at home. Think about all eating occasions.

Example of holiday food
If you eat grilled or steamed fresh fish 2 -3 times a week when you are on holiday during summer and fried fish once on most weekends during the remainder of the year, then fill in the circle 'less than once per month' for the fish - steamed/baked/grilled and the '1-3 times per month' for the fish fried/battered/crumbed.

Examples of seasonal eating
If you eat nectarines 4 times per week for 2-3 months of the year when they are in season and tinned apricots once per week for the rest of the year fill in the circle 'once per week' for peach/nectarine/plum/apricot/cherries.

Examples of mixed food
Some mixed foods have been listed as a single item to make it easier for you to answer. These include casseroles and meat cooked in simmer sauces, mixed vegetable and stir fry dishes, mixed salads in a sandwich or as a side salad, pizza and hamburger. Do not count foods which are part of a mixed dish when filling in the questionnaire. If you usually eat a pizza once a week, fill in the circle 'once per week' for pizza and do not count the ham, pineapple, tomato or any other ingredients in the pizza.

Examples of sandwiches and filled rolls
For sandwiches and filled rolls, record the bread/roll and filling separately. If you usually eat a wholemeal ham and salad sandwich made without any spread 3 times a week and no other wholemeal bread, ham or mixed salad during the week, fill in the '2-4 times per week' circle for each of wholemeals bread, ham, and green/mixed salad.

Please fill in one circle for each food item listed

Average no of times consumed in the last 12 months

	Never	Less than once per month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4+ times per day
Dairy Foods Flavoured milk/soy drink (e.g. milkshake, iced coffee, hot chocolate)									
Milk/soy milk as a drink									
Milk/soy milk on breakfast cereals									
Milk/soy milk in hot beverages (e.g. in tea)									
Cream or sour cream									
Ice-cream									
Yoghurt including plain, frozen, flavoured etc.									
Cottage or ricotta cheese									
Cheddar and all other cheese									
Bread and Cereal foods White bread, toast or rolls									
Wholemeal or mixed grain bread, toast or rolls									
English muffin, crumpet, focaccia or flat bread									
Dry or savoury biscuits, crispbread, crackers									
Muesli									
Cooked porridge									
Breakfast cereal									
Rice including white or brown									
Pasta including filled pasta, noodles									
Meat, Fish, Eggs Mince dishes (e.g. bolognaise sauce, meatloaf, rissoles)									
Mixed dishes with beef/veal (e.g. stir fry in simmer sauce or as casserole)									
Beef/veal – roast, chop, steak									
Mixed dishes with lamb (e.g. stir fry in simmer sauce or as casserole)									
Lamb – roast, chop, steak									
Mixed dishes with pork (e.g. stir fry in simmer sauce or as casserole)									

Meat, Fish, Eggs continued on next page

	Never	Less than once per month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4+ times per day
Meat, Fish, Eggs - continued Pork – roast, chop, steak									
Sausages, frankfurters									
Bacon									
Ham									
Luncheon meats (cold cuts) – salami, devon, chicken loaf									
Liver including pate									
Mixed dishes with chicken, turkey, duck (e.g. stirfry, cooked in simmer sauce or as a casserole)									
Chicken, turkey, duck – roast, steamed, BBQ, fried									
Canned fish (e.g. tuna, salmon, sardines)									
Fish – steamed, baked, grilled									
Fish – fried, battered, crumbed									
Oily fish (e.g. sardines, salmon, trout, anchovies, mackerel)									
Non-oily fish (white fish)									
Other seafood (e.g. prawns, oysters, calamari)									
Eggs or egg dishes									
Vegetables (Fresh, frozen or tinned) Green/mixed salad as main dish (e.g. lettuce, tomato, cucumber, onion)									
Green/mixed salad as side salad or in a sandwich									
Stirfry and mixed cooked vegetables including vegetable soups									
Vegetables – excluding the above dishes Potato cooked without fat (boiled, mashed, dry baked)									
Potato cooked with fat (chips, wedges, sautéed, roast)									
Carrots									
Sweet potatoes and other root vegetables									
Peas									
Green beans									
Silverbeet or spinach									
Salad greens including lettuce,									

rocket, spinach leaves									

Vegetables continued on next page

	Never	Less than once per month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4+ times per day
Vegetables - continued Celery, asparagus or bean sprouts									
Broccoli									
Cauliflower									
Brussel sprouts and cabbage (coleslaw, chinese, red)									
Pumpkin									
Zucchini, eggplant or squash									
Capsicum									
Tomatoes (including tinned)									
Tomato products (e.g. dried, paste, sauce)									
Avocado									
Onion or leeks									
Sweetcorn or corn on the cob									
Mushrooms									
Soybean or tofu									
Baked beans									
Other beans/peas (e.g. kidney, borlotti, chickpeas, lentils, dhal, split peas etc.)									
Fruits (Fresh, frozen or tinned) Mixed fruit and fruit salad									
Excluding mixed fruit how often do you eat: Apple or pear									
Orange, mandarin or grapefruit									
Peach, nectarine, plum, apricot or cherries									
Banana									
Mango or paw-paw									
Pineapple									
Berries (e.g. strawberries, blueberries)									
Other fruit (e.g. grapes, melon, kiwi fruit)									
Dried fruit (e.g. sultanas, apricots, prunes									
Baked goods and snacks Meat pie, sausage roll or other savoury pastries									
Pizza									
Hamburger with bun									
Cakes, sweet muffins, scones or pikelets									

Sweet pies or sweet pastries									
Other puddings and desserts									

Baked goods and snacks continued on next page

	Never	Less than once per month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4+ times per day
Baked goods and snacks - continued Plain sweet biscuits									
Fancy biscuits including jam/cream filled, chocolate, fruit and nut									
Milk/white chocolate including chocolate bars (e.g. Mars, Twix)									
Dark chocolate including chocolate bars									
Other confectionery									
Nuts									
Potato chips, corn chips, twisties and other similar snacks									
Sugar, spreads and dressings Sugar, syrup or honey									
Jam or marmalade									
Peanut butter or other nut spreads									
Butter, dairy blends or other margarine									
Vegemite, marmite or promite									
Oil and vinegar dressing									
Mayonnaise or other creamy dressing									
Non-milk beverages Fruit juice									
Cordial									
Soft drinks including flavoured mineral water									
Electrolyte or sports drinks (e.g. Gatorade)									
Energy drinks (e.g. Red Bull)									
Water including unflavoured mineral water, soda water, tap water									
Coffee (caffeinated)									
Coffee (decaffeinated)									
Tea (Caffeinated)									
Herbal teas									
Beer – low alcohol									
Beer – regular									
Red wine									
White wine or									

champagne/sparkling wine									
Sherry or port									
Spirits or liquers									
Other alcohol drinks									

Section 2 – Supplementary Questions

Please fill in the circle that best describes how often you usually consume each of the following:

How often do you usually consume each type of milk?	Most of the time	Some of the time	Rarely
Full cream			
Low/reduced fat or skim			
Evaporated or sweetened condensed			
Soy milk			
Flavoured milk			
Acidophilus milk (e.g. Yakult)			
Other (Specify)_____			

How often do you usually consume each type of bread?	Most of the time	Some of the time	Rarely
White fibre enriched			
Other white			
Wholemeal			
Rye			
Mixed grain with soy/linseed			
Other mixed/multi grain			
Other (Specify)_____			

How often do you usually consume each type of breakfast cereal?	Most of the time	Some of the time	Rarely
Wheat flakes (e.g. Weetbix)			
Corn/rice based (e.g. rice bubbles, special K, cornflakes)			
Bran based (e.g. All bran)			
Cereals with added sugar (nutri grain, coco pops)			
Mueslis			
Other cereals with fruit/nuts (e.g. sultana bran)			
Breakfast bars			
Other (Specify)_____			

How often do you usually consume each soft drink?	Most of the time	Some of the time	Rarely
Flavoured mineral water			
Diet cola drinks			

Regular cola drinks			
Other diet soft drinks			
Other (Specify)_____			

How often do you take the following vitamins/supplements?	Most of the time	Some of the time	Rarely
Multivitamins			
Multivitamins with extra iron or minerals			
Vitamin A, C or E			
Calcium and Vitamin D			
Fish oils (e.g. cod liver oil)			
Other oils (e.g. evening primrose)			
Other (Specify)_____			

How many serves of fruit do you eat per day? A serve of fruit is equal to one medium piece, two small pieces, or one cup of diced fruit

I eat _____ serves per day

☐ I don't eat fruit every day

☐ Don't know/unsure

How many serves of vegetables do you usually eat per day? A serve of vegetables is equal to half a cup of cooked vegetables or 1 cup salad

I eat _____ serves per day

☐ I don't eat fruit every day

☐ Don't know/unsure

Appendix C: Symptom Checklist

Below is a list of problems people sometimes have. Please read each one carefully and fill in the circle that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU IN THE PAST 7 DAYS INCLUDING TODAY. Blacken the circle for only one number for each problem and do not skip any items. If you change your mind or make a mistake simply cross it out and fill in the correct response. If you have any questions please ask them now.

	Not at all	Somewhat	Unsure	Moderately	Very much so
I have a change in energy					
My skin feels irritated more than usual					
I feel cold					
I feel hot					
I feel dizzy					
I am sweating more than usual					
I have blurred vision					
I feel nauseous					
My heart is racing					
I have a dry mouth					
I have stomach pains					
I feel pain in my eyes					
I feel pain in my ears					
I feel a change in my bowel patterns					
I find that I bruise easily					
I feel that I have a shortness of breath					
I feel hungrier more than normal					
I feel thirstier more than normal					
I feel constipated more than usual					
I feel that I need to urinate more than usual					
I feel fatigued more than usual					
I feel stressed more than usual					
I feel anxious more than usual					
I am moody more than usual					
I feel a change in my ability to remember events/ places/ people					
I feel more attentive than usual					
I feel a change in my sleeping patterns					
I feel tremors/tingling more than usual					