Development of a Continuous Respiration Olfactometer for Odorant Delivery Synchronous With Natural Respiration During Recordings of Brain Electrical Activity

Caroline M. Owen, John Patterson, and David G. Simpson

Abstract—The continuous respiration olfactometer (CRO) was designed as a respiration-synchronous method for delivering odorants during recordings of brain electrical activity, providing control and monitoring of the timing of the delivery as well as the quantities of odorant involved. The CRO incorporates a purpose-built electronic system designed with very specific temporal and quantitative characteristics, and is composed of four main parts: the respiratory monitoring apparatus, the odorant/air delivery system, the serial interface device and the respiratory monitoring software. Tests were undertaken to determine the performance of the system with reference to the accuracy and precision of timing and control of odorant delivery. Tests were also undertaken to determine the effects of variations in natural respiration between subjects on the capability of the respiratory monitoring system, using a group of 50 subjects, to test the success of a variable gain control to optimize the range of the digitized respiratory output. The delivery system was able to provide information concerning quantities of air or odorant delivered, and the stimulus timing information required for integration with neurophysiological recording techniques.

Index Terms—Brain topography, delivery apparatus, olfactometry, olfactory, respiratory monitoring.

I. INTRODUCTION

In Olfactory testing, the relevance of the results to the research objectives can be strongly influenced by the nature of the stimulus delivery system, and the degree to which it can be precisely monitored and controlled. The mode of stimulus delivery to the nose should therefore be selected to functionally meet the desired objective of the research.

Recording brain electrical activity is of interest in that it may reveal underlying olfactory processes or, at least, their temporal and spatial dynamics. The odorant delivery system used while determining these dynamic brain processes must provide precise temporal presentation of the odorant and avoid concomitant excitation of other sensory systems [1]. Guidelines for the collection and reporting of chemosensory event-related potentials (ERPs) have suggested that the use of artificial methods for administering odorous stimuli (such as odorant pulses or “blast” olfactometry) should be discouraged in view of the possible interaction with tactile trigeminal components of sensation (pain or irritation) [2]. One possible factor contributing to variations in the monitoring of human olfactory responses is the variations in sampling behavior of the subject during odorant presentation [3]. Human olfactory research has not typically involved the measurement of sniffing or breathing parameters, however, evidence has suggested that variations in these parameters may influence measures of odorant perception [3], [4].

Research investigating breathing and odorant responses has found that nostril flowrate effects the detection of odorous stimuli [5], [6] and in particular, the breathing techniques used during odorant administration can influence the recording of olfactory responses [4], [7]. The potential benefit of synchronizing odorant stimulus delivery with natural respiration has been recognized [8]–[12] and confirmed by research demonstrating the effect of odorants on respiratory behavior [13]–[16]. Differences have been reported in chemosensory ERPs amplitudes and latencies associated with passive breathing and active breathing techniques (such as sniffing or velopharyngeal closure) [4], [5]. The adoption of specific breathing techniques can also introduce the confounding affect of divided attention, with the subject attending to breathing instructions and techniques rather than to odorant presentation [4].

This paper reports the development of an odorant delivery system that was devised for use with different brain electrical recording techniques, such as continuous electroencephalogram (EEG) and ERP studies. The continuous respiration olfactometer (CRO) delivers odorants synchronous with natural respiration, thereby avoiding an unnecessary distraction of attention. This system utilizes respiratory information to deliver the odorant stimuli into peak inspiratory air flow thereby providing greater opportunity for odorant perception.
While also providing information to monitor any odorant induced respiratory changes, in order to monitor changes in brain electrical activity associated with odorant responses induced during natural respiration for comparison with electrical activity associated with breathing odorant-free air.

### II. Materials and Method

All research was conducted within the parameters and procedures established by the Swinburne University of Technology’s policies on Code of Conduct, Human Experimental Ethics Clearances and Intellectual Property.

#### A. Continuous Respiration Olfactometer

The CRO provides control and monitoring of the timing of odorant delivery, as well as the quantities of odorant involved, and incorporates a purpose-built electronic system designed with very specific temporal and quantitative characteristics. The CRO is composed of four main parts: the respiratory monitoring apparatus, the odorant/air delivery syringe system, the serial interface device and the respiratory monitoring software. A schematic representation of the system can be seen in Fig. 1.

1) **Respiratory Monitoring Apparatus:**

The respiratory monitoring system was designed with safety, convenience, and reliability in mind, using standard respiratory physiology device components. This respiratory apparatus is composed of a disposable medical facemask (model ISG1 150 000; Support and Fittings, Melbourne, Vic. Australia), attached to a two-way nonrebreathing valve (Series 1410A; Hans Rudolph; Kansas City, MO), which is attached to a nonheated Linear Pneumotachometer (Series 4700; Hans Rudolph; Kansas City, MO). The pneumotachometer (PNT) has a flow range of 0–160 L/min (suitable for adults at rest and children), and can be connected via polyester elastometer tubing (Series 9030; Hans Rudolph; Kansas City, MO) to a 5 L Douglas bag containing a supply of medical air (BOC; Melbourne, Vic. Australia). A supply of medical air can be included in the design to ensure that subjects would not breath room air contaminated with any odorants, but in a well-ventilated room may be unnecessary. The PNT is connected to the inhalation port of the nonrebreathing valve and the exhalation port of the nonrebreathing valve can be connected to a second Douglas bag to collect the expired air (also optional). The various connections are made using medical-grade connectors (Series 7023; Hans Rudolph; Kansas City, MO); all parts can be fully sanitized between uses. The disposable facemask is connected to the mouth port of the nonrebreathing valve. The differential pressure transducer on the serial device is connected to the signal pressure taps of the PNT. The flow-related differential pressure supplied by the PNT is converted by the pressure transducer into an analog electrical value as a voltage proportional to flowrate.

2) **Odorant Delivery Syringe System:**

Two 50-mL gas-tight glass syringes with Teflon-sealed plungers (model 009 660; SGE Scientific; Melbourne, Vic. Australia) are used to deliver small volumes of air or odorant into the facemask (typically as a 1-mL aliquot). The air or odorant are passed through separate small bore PFA fluoropolymer tubes [(perfluoroalkoxy; -T2-030, inner diameter (ID) 1 mm; North Melbourne Valve and Fittings, Melbourne, Vic. Australia). This translucent PFA tubing was selected because it is chemically inert, nontoxic, virtually nonporous, and has excellent crack- and stress-resistance. These PFA tubes are inserted into the facemask and positioned close to the external nares (nostrils). Movement of the syringe plungers is affected by stepper motors (model FD-55GFR; TEAC Corporation; Tokyo, Japan), connected to the syringe plungers with recirculating ball screws (model MTF0601; Linear Bearings; Knoxfield, Vic. Australia), enabling accurate control of the quantity and timing of air or odorant delivery.

3) **Serial Interface Device:**

The serial interface device incorporates a differential pressure transducer (model PK 8772 4; Microswitch, Honeywell; Melbourne, Vic. Australia) connected to the PNT, analog-to-digital converters (ADCs), a serial communication device (RS232), motor outputs and a microprocessor (68HC05C8; Motorola; Oak Hill, TX). Because an ADC is necessary, a stand-alone serial communication microprocessor-based design was used for the serial device (rather than a PC-installed ADC). This was to avoid potential problems of differing PC environments which would make the host software more complex. This externalization of interfacing has ensured that the system can be implemented with different types of PC environments (PC or Mac; however host software would have to be written for other than the Mac environment). The microprocessor runs at clock speed, sampling at the serial communication baud rate (19 200 baud). The microprocessor controls the ADC and the syringe stepper motor boards, handles the serial communications device, and is connected to the monitoring computer (originally a Powerbook 520c; Apple; Cupertino, CA). The 8-bit ADC has a 0–10 V range and a resolution of 40 μV. The motor output consists of eight transistors to buffer the output circuitry and provide the high current drive to each stepper motor board. A stepper motor controller board (model FD-55GFR; TEAC Corporation; Tokyo, Japan) controls each of the stepper motors driving the syringes.

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1Available internationally through Cole-Parmer, Vernon Hills, IL.
The microprocessor is programmed to process the incoming respiratory information (from the pressure transducer and the ADC), to send the information to the monitoring computer (via the serial communications device), and to send the appropriate driving current from the transistors to the stepper motor boards. The device incorporates a variable gain control (a gain of five times with a 20-turn potentiometer of 20 kΩ value and an integrated bar graph ten-element LED display) to ensure that the analog output of the respiratory circuit has the widest dynamic range for each situation prior to the signal being fed into the ADC (to maximize the utilization of the 256-step range of the ADC). This was a simple solution for reducing variations between individuals found in the level of airflow during natural respiration; all respiratory signals can be adjusted to within an acceptable range for analysis by the monitoring computer to optimize the range of the digitized respiratory output.

4) Respiratory Monitoring Software: The software was written in Symantec C++ for Macintosh (version 7.03; Symantec; Cupertino, CA). The system provides pull-down menus and windows for the interactive entry of subject and experimental information. Experimental parameters such as the quantity of air or odorant stimuli to be delivered and the frequency of odorant delivery can be entered for each recording run or left at default values. The motor testing window is used to control the movement of the syringes for testing or for filling with air or odorant. The software updates the menu bar displaying a series of indicators for the completion of each breath during the control respiratory run and the delivery of the stimulus during the odorant test run.

Fig. 2 illustrates the respiratory parameters utilized by the CRO to time the stimulus delivery. The respiratory flowrate is monitored by means of serially transmitted samples sent to the monitoring computer for analysis. To avoid reaching ADC limits, the maximum output used in calculations was 1600 steps in ASCII format rather than 2048 steps. Any values in excess of 1600 were set to 1600 in the software. Due to the use of a nonrebreathing valve, only the inspiratory values are analyzed. During expiration, the serial sampling continues until the commencement of the next inspiration is detected. This is determined once the sampled respiratory value exceeds the baseline maximum range (see Fig. 2). Each sampled flowrate value is subjected to a series of comparisons to determine the respiratory parameters of each breath: peak flowrate, respiratory rate, the timing of the respiratory parameters, and the volume of the inspiratory breath. The monitoring software stores to file all the respiratory information, and calculates averages for the respiratory parameters which are updated at the conclusion of each breath throughout the recording period. The detection of peak inspiratory flowrate is used to calculate (predict) the timing of the air or odorant delivery synchronous with each breath.

The average time of peak inspiratory flow is initially calculated in a control period for a minimum of ten breaths, and then continuously updated throughout the recording run. Stimulus delivery time is calculated as: Average time of peak inspiration minus 30% average time of peak inspiration. At the calculated stimulus delivery time (based on the average previous breath values), a signal is sent to the microprocessor to drive one of the syringe stepper motors for stimulus delivery. The software writes to a separate file the timing of the stimulus delivery for use in the associated electrophysiological analysis. Fig. 3 provides a software flowchart of the program logic resulting in measurement of these respiratory and delivery parameters.

B. Testing Procedures

Extensive testing was undertaken to assess the reliability and accuracy of the software and hardware in meeting the requirements of the system.
1) **Software Testing:** During initial testing, artificially generated signals (analog simulation signals of varying voltages and frequencies) were used to simulate a breath profile and to determine the precision of the calculated or derived “respiratory” values computed by the software. This tested the effect of changes in signal amplitude and changes in the baseline offset on the calculated maximum and minimum respiratory parameters. These tests were repeated using actual respiratory signals, with subjects varying their respiratory flows (from shallow, fast breaths to deep, slow breaths). This series of tests was designed to demonstrate that the software accurately handled any variations in real respiratory inputs.

2) **Hardware Testing:** Extensive testing was conducted on the syringe system performance in combination with the serial interface device and the monitoring hardware and software. Tests were conducted to measure the relationship between the number of motor steps and the corresponding syringe volume, the frequency of the motor drive and the syringe flows, and the timing of the motor drive injection in relation to the respiratory signal. Tests were replicated with both syringes to assess their similarity. The relationship of the syringe delivery volume (measured in mL) to the number of motor steps was studied with the stepper motor driven at different frequencies, with four trials averaged for each designated number of motor steps to give a mean performance value for each syringe and stepper motor. The frequency was controlled using a signal generator with a pulse width of 10 μs (5 V max). Internal delays (determined by empirical testing) had to be incorporated into the software to allow the necessary time for the signal to pass from the monitoring computer to the signal device and back in order to prevent the stepper motors from moving too fast and stalling.

Pressure changes caused by the injection of the syringe were measured to determine the nature of the odorant/air delivery caused by the stepper motor-syringe movement, using a semiconductor pressure transducer attached to the end of a 1-m length of the PFA tubing connected to the delivery syringe, and displayed on a digital storage oscilloscope.

C. Validation of the Prototype

The delivery system was extensively tested during a series of three 5-min recording periods for each of the 50 subjects (23 males and 27 females, mean age 25 years, age range 15–50 years). The test odorant used was n-butanol, diluted from the original concentration of 774 ppm (supplied and calibrated by BOC Gases; Melbourne, Vic. Australia). The dead-space of the facemask (calculated by water displacement to be approximately 50 mL) maximally diluted the 1 mL of delivered odorant within the facemask by a factor of 50. When diluted in the facemask, the concentrations used in testing ranged from 1.1 ppm to 3.87 ppm. Only one odorant concentration was used in any one recording session. All subjects were tested for n-butanol sensitivity using the Sniffin’ Sticks test of Olfactory Performance Threshold Test (Burghart; Wedel, Germany). This test uses 16 concentrations of n-butanol in a forced-choice triangle, ascending and descending staircase method. The test was performed following completion of all electrophysiological recording sessions. All subjects were found to have a normal range of sensitivity to n-butanol [17], with threshold levels at or near the concentrations used in the testing procedure.

The same procedure was used for each of the test sessions. Subjects were seated with the facemask of the CRO fixed into place and asked to quietly breathe through the nose (with mouth closed) for the duration of the control and test recording periods. The variable gain LED display is monitored and the gain set for a series of initial breaths. Subjects quickly became accustomed to the respiratory apparatus. The gain can then be further adjusted throughout the recording periods if there are respiratory changes evident as the subjects become more relaxed. Following a control respiratory monitoring period of ten breaths, 1 mL of either control medical air or test odorant (n-butanol) was injected into the facemask through the delivery tubing. These were in a pseudo-random order coinciding with the inspiratory phase of each breath in the natural breathing cycle, at an air to odorant ratio of 3:1. The software prevents any instances of consecutive odorant stimuli over the 5-min recording period. Each recording period was followed by a rest period of approximately 3–5 min during which the respiratory apparatus was removed. Each recording period required approximately 7–8 min, with the duration of the control period depending on minor variations in the respiratory cycle of the subject. During expiration, the software prompted the subjects to indicate, using a two-button response box, whether they had perceived an odorant in the previous inspiration (“yes” or “no” responses). These behavioral responses from the large subject group were collated to assist in determining the accuracy of odorant detection.

III. RESULTS

The syringe flowrate/motor frequency tests conducted using Syringe B (the odorant delivery syringe) revealed the performance limits of the delivery system. The maximum motor driving frequency at which the delivery flowrate was most reliably achieved with an optimal delivery time of 500 ms was found to be 300 steps/s. The time taken to delivery 1 mL was too great at less than 300 Hz, and motor performance was unreliable at frequencies greater than 300 Hz, with the motor seen to stall or oscillate.

The pressure changes due to the injection by the syringe were recorded at the tubing outlets using the semiconductor pressure transducer. These changes revealed a gas pressure “on/off” profile which was seen to approximate a rectangular wave at the start, end of, and synchronous with, the stepper motor controlled movement of the syringe. Fig. 4 illustrates the approximate rectangular wave signal recorded during these tests.

Respiratory and motor drive signals were sampled during a respiratory recording to further demonstrate the timing of the syringe stimulus delivery during inspiration. The square wave “on/off” signal for the syringe was sampled from the serial device signal which drives the motors. Figs. 5 and 6 show actual respiratory profiles of a subject and the motor drive profile for air syringe delivery (approximately 1 mL), sampled during the same respiratory recording period. These figures also show the effect of the variable gain, as Fig. 5 reveals a saturated respiratory signal, and Fig. 6 shows the respiratory profile of a different breath with the gain adjusted to prevent saturation of the signal.
Fig. 4. Rectangular wave pressure signal (bottom) and stepper motor drive signal (top) recorded during tests of the syringe output pressure changes at the distal end of 1 m of PFA tubing (1-mm ID). The pressure trace was obtained using a semiconductor pressure transducer (MPX10; Motorola; Oak Hill, TX), and the traces recorded on a digital storage oscilloscope (model 2211; Tektronix; Marlow, U.K.; 50 mV/division, 0.1 s/division).

Fig. 5. The relationship between the syringe delivery timing and the respiratory profile showing the on/off profile of the stepper motor drive (top trace) and the inspiratory phase of the respiratory cycle (bottom trace), revealing a saturated respiratory signal.

Fig. 6. The relationship between the syringe delivery timing and the respiratory profile showing the on/off profile of the stepper motor drive (top trace) and the inspiratory phase of the respiratory cycle (bottom trace). The respiratory values were obtained during the same recording run as shown in Fig. 5. The effect on the signal of using the variable gain control to modify the respiratory input signal is clearly illustrated.

IV. DISCUSSION

The testing procedures focused on the accuracy and precision of timing and delivery control by the system. The assumption that the delivery of odorant would occur during the breath in which it was injected was supported by the evidence of the pressure changes associated with timing of the delivery of air or odorant. The gas pressure “on/off” profile due to the stimulus injection by the syringe approximated a rectangular wave, consistent with previous recommendations for recording and reporting chemosensory ERPs [1]. This showed that the delivered aliquot of odorant was not subject to a slow rise time or a continuing “leakage” or tail-off by residual pressure in the syringe or sample delivery line. This step function performance of the syringes and drive system suggests that a pulse delivery of gas would be delivered out of the system, provided the tubing is full of odorant to begin with. However, this does not eliminate any diffusion of the gas which may occur out of the open-ended delivery tubes. Analysis of the behavioral responses during the repeated recording periods by the subject group did not reveal any conscious awareness of odorant during air delivery breaths; however, the low odorant concentrations used in the study may have contributed to this lack of awareness even if leakages were occurring. It is expected that if very strong concentrations of odorant were delivered, any leakage or diffusion could become more obvious to the subjects. At the low concentrations used in these tests, it is unlikely that the minuscule amounts leaking out would be detected by the subjects. During inspiration, leakage from the delivery tubing is not a problem, as the delivery is occurring at the same time. During expiration, when an accumulation of unwanted stimulus material could occur in the mask, the mask is being flushed by expiratory flow from the subject. The subjects’ behavioral data indicated that they only detected the odorant following odorant delivery breaths (but not following

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<td>SUMMARY OF CRO RESPIRATORY DETAILS (IN SECONDS) FOR THE TEST SUBJECT GROUP (n = 50), FOR A TOTAL OF 150 RECORDING PERIODS (THREE PER SUBJECT) WITH A MEAN OF 58 AIR AND 15 ODORANT DELIVERIES FOR EACH 300-S RECORDING PERIOD</td>
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0.998, demonstrating the similarity in performance between the stepper motors. The linear relationship of number of steps to volume delivered was clearly demonstrated for both syringes.

The respiratory details of the subject group for the 150 recording periods are presented in Table I. The repeated testing of this large group revealed natural variations in real respiratory inputs, however the introduction of the variable gain control provided a simple device to lessen the affect of any individual variations.
air delivery) in the recording session. This suggests that, in the current paradigm, there were no problems associated with the odorant remaining at a detectable level in the facemask during control air breaths. Future analysis of the gas in the facemask (by gas chromatography) may determine if any leakage is happening. These points notwithstanding, the system fulfilled the performance requirements.

The software testing confirmed the accuracy of the software to follow variations in real respiratory inputs for different subjects, and for the same subject over time, provided the varying flow rates were within the range of the serial-sampling device. These tests showed the effect of variations in amplitude of the input analog signal and demonstrated the affectiveness of the system in monitoring the natural variations in respiratory flow amplitudes, utilizing the adjustable gain.

The delivery of the odorant or control air to the subject through tubing inserted into the facemask and located close to the external nares can also be affected by subject variations; however, the tubing can be adjusted once the facemask is in place, thus reducing the effect of these variations.

These repeated tests of the system incorporated sessions in which the CRO was integrated with electrophysiological recording systems. The system proved capable of providing the timing and delivery details which would be necessary for the integration of this system with brain recording techniques. In subsequent experiments, the system has been modified to provide trigger pulses suitable for event-marking of several EEG recording systems. The performance of the odorant delivery system suggests its potential to be used to investigate information about the underlying brain activity. The same system has now been in use for several years, using a removable syringe version, with ten different odorants. The total number of recordings to date is over 250, each involving three or four recording periods [18]–[21]. The CRO has been successfully integrated with the Swinburne University of Technology’s steady state probe topography (SSPT) recording system, a NeuroScan EEG system and an EGI/EEG system. While there have been minor problems, expected when a prototype is in constant use and subject to modification, the system has fulfilled its promise and continues to provide a very suitable method of delivering odorants, with accurate measurement of the timing and quantities of stimuli delivered.

In conclusion, throughout these preliminary test recordings the CRO system demonstrated its potential to be used to deliver olfactory stimuli synchronous to natural respiration for integration with neurophysiological recording techniques.

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REFERENCES


Dr. Owen is a founding member of the Australasian Association for ChemoSensory Science (AACSS) and a member of AChemS, ECRO, IOP, and the Sensometric Society.
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He has many years experience in veterinary physiology research, followed by years in industry as product development and technical manager in the area of medical electronics. In 1991, he took up a Senior Lecturer position in the School of Biophysical Sciences and Electrical Engineering at Swinburne University of Technology, Victoria, Australia. In 1999 he established the Sensory Neuroscience Laboratory within this school. In his position as Director of this research group, he is involved in developing physiological monitoring and delivery techniques in basic and applied smell, taste, somatosensory, and visual research projects.

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