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Comparing Thoracic and Intra-Nasal Pressure Transients to Monitor Active Odor Sampling during Odor-Guided Decision Making in the Mouse

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Abstract

Background—Recording of physiological parameters in behaving mice has seen an immense increase over recent years driven by, for example, increased miniaturization of recording devices. One parameter particularly important for odorant-driven behaviors is the breathing frequency, since the latter dictates the rate of odorant delivery to the nasal cavity and the olfactory receptor neurons located therein.

New Method—Typically, breathing patterns are monitored by either measuring the breathing-induced temperature or pressure changes in the nasal cavity. Both require the implantation of a nasal cannula and tethering of the mouse to either a cable or tubing. To avoid these limitations we used an implanted pressure sensor which reads the thoracic pressure and transmits the data telemetrically, thus making it suitable for experiments which require a freely moving animal.

Results—Mice performed a Go/NoGo odorant-driven behavioral task with the implanted pressure sensor, which proved to work reliably to allow recording of breathing signals over several weeks from a given animal.

Comparison to existing Method(s)—We simultaneously recorded the thoracic and nasal pressure changes and found that measuring the thoracic pressure change yielded similar results compared to measurements of nasal pressure changes.

Conclusion—Telemetrically recorded breathing signals are a feasible method to monitor odorant-guided behavioral changes in breathing rates. Its advantages are most significant when recording from a freely moving animal over several weeks. The advantages and disadvantages of different methods to record breathing patterns are discussed.

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Keywords

Breathing frequency; wireless telemetry; nasal pressure; odor decisions

Introduction

Active sampling is a common feature of sensory information processing systems in a variety of modalities (Schroeder et al., 2010). Active gaze control augments visual sampling (Parkhurst and Niebur, 2003), active attentional mechanisms augment auditory processing (Alain et al., 2008), and active air sampling, or sniffing, is a critical component of olfactory information processing in humans (Laing, 1983; Mainland and Sobel, 2006) and other animals (Cenier et al., 2013; Kepecs et al., 2006; Scott, 2006; Uchida et al., 2006; Verhagen et al., 2007). Just as vision is degraded by lack of eye motion (Rolfs, 2009) olfactory perception is degraded by lack of air flow in the nasal cavity (Sela and Sobel, 2010).

Stimulation of olfactory receptor neurons (ORNs) in the nasal cavity is pulsatile, driven by odorants carried by the inhaled air during normal breathing or active sniffing. Binding of odor molecules to odorant receptors triggers a second messenger cascade and ultimately action potentials to be carried to the olfactory bulb (for review see Kleene, 2008). Thus the timing of ORN stimulation and action potential generation will depend on the pulsatile time course of the inhaled air as will the input from ORNs to distal dendrites of mitral/tufted cells located in glomeruli in the olfactory bulb. (Shirley et al., 2010; Verhagen et al., 2007). Corresponding implications for the dynamics of input from mitral/tufted cells (Cury and Uchida, 2010; Shusterman et al., 2011) to piriform cortex follow, where disparate aspects of an odor object are synthesized into a unified odor percept (Gottfried, 2010). Thus, monitoring respiration is essential for a more complete understanding of the responses of mitral/tufted cells during odor sampling (Wesson et al., 2008).

We have recently introduced a novel technical approach to non-invasively monitor breathing and odor-elicited sniffing in mice, utilizing an implanted pressure sensor. The sensor via telemetry continuously monitors the pressure changes in the thoracic cavity of the mouse during respiration and odor sampling. We present a comparison of this approach with data derived from a more established technique using a pressure transducer placed in a nasal cannula previously implanted in the mouse. We also demonstrate that breathing signals recorded using telemetry can be recorded during execution of an odor-guided behavioral task.

Materials and methods

Implantation of thoracic pressure sensor

All mice were handled and surgical procedures were performed in accordance with methods approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

Thoracic pressure sensors (PhysioTel® TA11PA-C10; Data Sciences International, DSI) St. Paul, MN) were implanted in mice using the following procedure derived from work done in both rats and mice (Murphy et al., 1998). For 3 days prior to surgery, mice were provided with a bottle of chocolate Ensure complete liquid diet in addition to their normal rodent chow to allow them to acclimate to the new source of nutrition. One day prior to surgery, the mice were injected with Gentamicin (2 mg/kg, i.m.). The implantable transmitter consisted of a catheter (0.4 mm diameter, 40 mm long) and transducer (10 mm diameter, 14 mm long weighing 1.4 g) which were surgically implanted using aseptic

surgical technique. Mice were anaesthetized with isoflurane. Depth of anesthesia was tested with a toe pinch and respiratory function monitored throughout the procedure and recovery. The abdominal wall was shaved and disinfected with alcohol and Betadine. A 3 cm incision was made along the abdominal midline, and the liver was carefully displaced so that the esophagus was exposed just posterior to its junction with the diaphragm (*esophagicus hiatus*). A small incision was made through the serosal layer of the esophagus and a 24G catheter needle with surrounding sheath (SR-0X2419CA, Terumo, Somerset, NJ) was inserted between the serosal and muscularis layers. The needle was then withdrawn from the sheath and the sheath alone was used to tunnel cranially past the juncture with the diaphragm and into the thoracic cavity. The sheath was withdrawn and the sensor catheter was threaded through the tunnel in the serosal tissue alongside the esophagus. Pressure was monitored continuously using the wireless signal from the transducer, and when maximal pleural pressure changes were attained (approximately 0.25-0.5 cm beyond the *esophagicus hiatus*), the sensor catheter was secured in place at the entry point with a cellulose patch and medical grade tissue adhesive (Surgi-Lock 2oc, Meridian Animal Health, Omaha, NE). The body of the transmitter was then secured to the abdominal wall during closure of the abdominal musculature with sutures and Surgi-Lock. The skin incision was surgically stapled. Recovery from anesthesia occurred in a heated polycarbonate box with soft bedding. The mouse was observed closely until normal locomotion was regained. Mice had normal rodent chow removed and were provided with an unlimited supply of a palatable, liquid diet of chocolate Ensure until recovery was complete. The liquid diet helped to ensure that intestinal blockage was avoided during recovery. Immediately following surgery, the mice were injected with analgesic (0.5-2.0 mg/kg Buprenorphine s.c.). Of the 16 mice implanted with sensors, 14 survived for a month or more for a surgery success rate of 88%.

Implantation of nasal cannula

To monitor intranasal pressure and thus the breathing rate, a 7 mm long 22 gauge stainless steel cannula (OD 0.028", ID 0.0155", part # HTX-22R, Component Supply Co., Fort Meade, FL) was implanted in the nasal cavity. One day prior to surgery, the mice were injected with an antibiotic (2 mg/kg Gentamicin i.m.). Mice were anaesthetized using a ketamine (40 mg/kg), xylazine (10 mg/kg), acepromazine (1.5 mg/kg) solution (i.p.) and depth of anesthesia was measured as described above. Nasal cannulas were implanted in mice using the following procedure derived from work done in mice (Shusterman et al., 2011; Smear et al., 2011). A scalpel was used to make an incision along the midline from the fur transitional area at the tip of the nose to just caudal of the eyes. A small hole was drilled with a carbide bur (FG ½; Henry Schein Dental, Melville, NY) in the bone overlying the nasal cavity and through the underlying nasal epithelium using nasal sutures as landmarks. The nasal cannula was inserted so the bottom of the cannula was level with the interior thickness of the bone and affixed with medical grade adhesive (Surgi-Lock 2oc, Meridian Animal Health, Omaha, NE) and further stabilized with dental cement. After surgery, the mice were injected with analgesic (0.5-2.0 mg/kg Buprenorphine s.c.). Between experimental recordings, the cannula was capped using a piece of 25 gauge tube that was crimped by a 5 mm piece of 22 gauge tube. The distance between the top and bottom of the cap was set such that when the cap was inserted into the cannula, the bottom of the cap would protrude ~200 microns from the bottom of the cannula.

Data acquisition of the telemetric signal

Pleural pressure was measured in the awake mouse using a commercial telemetry system (DSI). The components of the radio-telemetry system have previously been described in detail (Hess et al., 1996; Mills et al., 2000). The transmitter signal was sensed by a receiver platform (RPC-1) and converted to a digitized signal that was then continuously sampled at 500 Hz (filtered 0 – 100 Hz) with the software system Matrix 3643 (DSI).

Data acquisition of the nasal pressure signal

During recording sessions, the nasal cannula was connected to a pressure sensor with polyethylene tubing (801000, A-M Systems, ID 0.015in, OD 0.043 in). The pressure sensor (Honeywell, 24PCEFJ6G) was chosen to have a small pressure range of ± 0.5 psi and minimal internal volume. The signal from the pressure sensor was amplified by a custom amplifier (bandwidth DC - 1 kHz) and recorded with the software system Matrix 3643 (DSI) using a C12V A/D converter and the signal sampled at 500 Hz and filtered at 0 – 100 Hz, similar to the telemetric thoracic pressure sensor signal.

Prior to the sniff recording, the pressure sensor system, which includes the tubing, pressure sensor and associated electronics, was calibrated against a known air flow, measured by the hot wire anemometer (mini CTA 5439, Dantec Dynamics, Denmark) (Fig. 1A). The temporal shift between the flow velocity and pressure signal did not exceed 1 ms.

To estimate the intrinsic time lag between nasal cannula pressure sensor and telemetric pressure sensors, both sensors were calibrated against a pressure source created by a loud speaker (Fig. 1B). The telemetric sensor was found to have a delay of 5 ms compared to the nasal pressure signal. Where appropriate (e.g. in Fig. 5) this delay has been corrected off line.

Processing of breathing signals

Both the telemetry and cannula signals were digitally low-pass Bessel filtered at 15 Hz to eliminate high frequency noise. Data analysis was performed using Origin software (OriginLab Corporation, Northampton, MA). Respiratory inhalation and exhalation peaks were detected using the Origin PickPeak function and inspected visually for accuracy.

Behavioral experiments and recording the telemetry signal in an olfactometer

The olfactometer (Knosys, Lutz, FL) is equipped with an odor port through which the mice sample odors, and a separate water port from which the mice receive a water reward. Odorized air streams from one of eight reservoirs can be presented when the mouse makes a nose poke into the odor port, as detected by an IR beam and detector spanning the opening of the odor port (Fig. 2). Odor selection and delivery are controlled by custom software written in Matlab (Mathworks, Natick, MA). The telemetry signal was continuously recorded during odor-guided behavioral responses in the olfactometer.

For the behavioral experiments the respiration data obtained with the thoracic pressure sensor were analyzed with MATLAB. The data were first band-pass filtered at 3 - 15 Hz and inhalation and exhalation peaks detected using custom software (written in Matlab). After assigning time values to the troughs in the respiration signal, we calculated inter-sniff intervals (ISIs) based on values of adjacent time points.

Results

We set out to compare two methods for recording breathing patterns from freely behaving mice. A method that has recently been introduced (Shusterman et al., 2011; Wesson et al., 2008) records the pressure change in the nasal cavity induced by the in- and exhaled air (Fig. 3A), a downward deflection representing inhalation. Breathing at low frequency was interspersed with bouts of high frequency sniffing as has been reported previously (Wesson et al., 2011). Low frequency breathing was typically monophasic for the thoracic signal and bi-phasic for the nasal cannula recording with the positive pressure change more pronounced. The high frequency bouts of sniffing consist of two phases: inhalation and exhalation. For comparison of the two methods of recording breathing patterns, the thoracic

pressure (Fig. 3A&C) was recorded simultaneously using the implanted pressure sensor (see Materials and methods) in a mouse freely exploring its cage. The telemetry signal (Fig. 3B&D) compared well to the nasal pressure change, with each signal displaying a similar time course, although the thoracic pressure change showed less of an increase in exhalation pressure during high frequency sniffing.

To obtain the sniffing frequency distribution, the timings of the troughs (representing the peak inhalation phase) were determined and sniff frequency histograms were calculated (Fig. 4). Histograms obtained with either recording method were comparable, both showing a bi-modal distribution which could be fitted with two Gaussian functions. Gaussian peaks for breathing at rest centered on breathing frequencies of 2.08 and 2.05 Hz and centered at 5.68 and 5.61 Hz during high frequency sniff bouts for the cannula and the telemetry signals, respectively.

As the change in thoracic pressure leads to a nasal pressure change, we investigated the timing between the two signals. The timing for the peak of each thoracic inhalation cycle was subtracted from the corresponding timing of the peak obtained from the cannula recording and plotted against the breathing frequency of this breathing cycle (Fig. 5). The peak nasal pressure change preceded thoracic breathing change by up to 100 ms at low breathing frequencies but trailed the thoracic pressure change by around 5 ms at sniffing frequencies higher than ~5 Hz.

Changes in breathing frequency were associated with a change in the inhalation pressure waveform. At low breathing frequencies both recording methods typically only showed an inhalation peak during the inhalation phase with the peak amplitude increasing with higher sniffing frequencies (Fig. 6A & C). The cannula exhalation peak also increased with higher frequencies, while the telemetric thoracic signal actually became more negative, suggesting that at higher sniffing frequencies the mouse maintained a lower thoracic pressure for the duration of the sniffing bout (Fig. 6B & D). This can also be seen in Fig. 3B & D as negative shifts of the inhalation peak during high frequency sniffing at $t = 28 - 29$ s.

An example of the use of the telemetry sensor during odor-guided decision-making by a mouse in the olfactometer is shown in Fig. 7. A mouse was trained to distinguish an odorant (i.e., 10^{-4} 1-propanol (vol/vol) dissolved in mineral oil in the odor vial) and clean-air diluted by a factor of 20 supplied through the odor port from the odor of mineral oil and was water-rewarded in the nearby water port only when the correct choice was made (Go/NoGo paradigm). The mouse would initiate the next trial by accessing the odor port, but an odorant would only be presented after a minimal inter-trial period of 2 s following a correct choice and 5 s following an incorrect choice. Time zero in Fig. 7 corresponds to the time of nose insertion into the odor port, to which all trials were aligned. Each dot represents the time of peak inhalation. The vertical line at 0.5 s designates the time for odor onset for the 100 trials shown. All of the trials shown represent correct choices resulting in water reward. Each line in the raster plot contains the following 6 phases of behavior: 1) free moving state (around -1 to -0 s), 2) insertion of nose in the odor port (around 0 to 1.3 s), 3) sampling of odor beginning with odor onset in the odor port (starting at 0.5 and lasting to ~1.3 s), 4) withdrawal of nose from odor port (~1.3 s), 5) moving from odor port to water port (1.3 to 1.5 s), and 6) in water port drinking water reward (1.5 to ~10 s).

The timing of the occurrence of the peak sniffing rate (10-13 Hz) relative to these six phases of behavior in the olfactometer is shown in the plot of time vs. sniff frequency of Fig. 8 with color coding of the sniff frequency per time bin. The data plotted in Fig. 8 are the same data shown in Fig. 7 and make clear that the peak sniffing rate occurs over the time interval of

500 to 1200 msec during which the mouse has its nose in the odor port, presumably engaged in active sniffing to gather information on odor identity.

Discussion

Simultaneous monitoring of several neural and neuromuscular parameters during behavioral tasks performed by mice is becoming increasingly common (Cury and Uchida, 2010; Dombeck et al., 2010; Domnisoru et al., 2013). An important parameter to understand olfactory physiology is the odor sampling rate, which is determined by the inhalation of odorous air and as such is controlled by the breathing and sniffing rates.

In the awake mouse we have now recorded the intranasal pressure using an implanted cannula and external pressure sensor versus an implanted intra-thoracic pressure sensor monitored telemetrically. Comparison of the two signals revealed that they were very similar in terms of monitoring the breathing frequency and thus they are both useful tools. Each method has its own advantages and disadvantages. The nasal cannula signal has superb signal to noise, while the telemetry signal has higher baseline noise and baseline drifts which can be caused by rapid postural changes of the mouse, thus requiring more careful post-experiment analysis. Also, in our hands, not every mouse with an implanted thoracic sensor generated a thoracic pressure signal reliable enough to allow analysis of the breathing signal, probably due to post-surgical repositioning of the catheter along the esophagus. Thus far, we have implanted 16 mice and 14 yielded reliable breathing records. Since the sensor for the telemetry signal is implanted, this signal can be recorded stably for weeks, for some of our mice even months, which is harder to achieve with the implanted cannula, because it is prone to become occluded with time due to scar tissue development or mucous blockage. The latter can cause sudden signal loss during an experiment. Although cannular pressure recordings require a simpler surgical procedure and are lower in cost it does require the animal to be tethered to the pressure sensor with tubing, which can limit both the experimental design and the free movement of the animal. Use of head-fixed awake behaving mice solves the latter problem. A further issue to consider with head fixed mice (and rats) is that their maximal sniffing rate seems to be reduced by up to half compared to freely moving animals (Kepecs et al., 2007; Verhagen et al., 2007; Wesson et al., 2008; Youngentob et al., 1987) thus limiting the regime of sniffing frequencies that one can investigate. An important consideration in choosing a recording method for monitoring breathing rate in the awake behaving rodent is also the precise nature of the experimental question and which behavioral parameters are to be evaluated. For example, if the experimental question is how olfactory sensory neurons or their postsynaptic targets, the mitral cells, are responding to odorous stimulation (Kato et al., 2012), the most direct measurement method would be to record the intranasal pressure change, since this will more closely resemble the stimulation onset of olfactory receptor neurons located in the nasal cavity. Alternatively, if a change in breathing behavior triggered by presentation of novel odorants is of interest, the thoracic pressure signal would be more directly relevant as thoracic pressure changes ultimately cause the intranasal pressure changes.

The frequency and intensity of odorant sampling plays a critical role in shaping the dynamics of responses at all levels of the olfactory system (Phillips et al., 2012; Wachowiak, 2011). The intensity of sniffing affects patterns of airflow in the nasal cavity (Courtillot et al., 2011; Jiang and Zhao, 2010; Zhao and Dalton, 2007) that then influences the deposition of odorant molecules into the mucus layer in which the olfactory cilia are embedded (Cenier et al., 2013). The temporal pattern of odorant deposition into the nasal mucus has dramatic effects on the response dynamics of the olfactory sensory neurons (Ghatpande and Reisert, 2011; Reisert and Zhao, 2011) and in the mitral/tufted cells of the olfactory bulb (Carey and Wachowiak, 2011; Shusterman et al., 2011; Smear et al., 2011; Wachowiak et al., 2013).

The ability of cortical networks to perform pattern completion on noisy odor-elicited inputs is dependent on both experience and patterning of the input from the olfactory bulb (Chapuis and Wilson, 2012). These factors also reinforce the importance of studying odor coding in the awake behaving subject (Blauvelt et al., 2013; Rinberg et al., 2006) or providing natural odor delivery patterns to anesthetized preparations (Cheung et al., 2009). Sniffing measurements in awake behaving rodents have recently shown that modulation of sniffing rate can play an important role in social interactions (Wesson 2013).

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Highlights

- Telemetric pleural pressure sensors reliably record breathing patterns during olfactory tasks.
- Telemetrically recorded signals compare well to breathing-induced pressure changes in the nasal cavity.
- An increase in breathing frequency leads to a phase-shift between the pleural and the nasal pressure signals.

Fig. 1.

The nasal pressure sensor calibration. **A.** The pressure sensor was connected to a cannula, identical to that implanted into the mouse nose by thin polyethylene tubing. The cannula was inserted into a 2.4 mm ID tube. To simultaneously measure flow velocity in the tube, a hot wire anemometer was positioned inside the tube, near the insertion point of the cannula. The airflow inside the tube was driven by the sine-wave movement of a 6" loudspeaker, which was connected to the tube by a plastic funnel and short flexible tubing. The loudspeaker was controlled by a function generator through an audio amplifier. A schematic representation of signals from the hot wire anemometer and the pressure sensors is presented in the top panel. Unlike the pressure sensor, the hot-wire anemometer measures absolute air velocity and produces a rectified signal. The temporal delay (Δt) between signals from the pressure sensor and the anemometer was estimated based on the zero velocity points of the anemometer signal.

For a typical pressure measurement system the pressure signal is usually delayed for some time interval (Δt) relative to the velocity signal due to delays in the pressure sensor. The delay is measured based on zero velocity points. For an optimized pressure measurement system $\Delta t < 1$ ms for relevant frequencies between 1 – 15 Hz. **B.** Measurement of relative time shift between telemetric pressure sensor and cannula pressure sensor. Sensors were connected to a loudspeaker, and the time lag between the pressure signals and the control signals was measured. The relative time shift was estimated as the difference between time lags for both sensors.

Fig. 2. Instrumentation to monitor breathing and sniffing by telemetry from a mouse making odor-guided decisions in an olfactometer. The computer controlling stimulus delivery and water reward is not shown.

Fig. 3.

Comparison of nasal and thoracic pressure changes in a mouse A. The nasal pressure changes recorded via a cannula implanted into the mouse nose. B. Thoracic pressure changes recorded simultaneously with an implanted telemetric pressure sensor. C. and D., same data as in A and B, but on an expanded time base.

Fig. 4. Distribution of breathing frequencies in a freely breathing mouse Breathing frequency histograms obtained from nasal (A) and thoracic (B) breathing signals. Bin width is 0.5 s and solid lines are Gaussian fits.

Fig. 5.
Comparison of the inhalation times for each breathing cycle. The timing of the nasal inhalation was subtracted from the thoracic inhalation and plotted against the breathing frequency.

Fig. 6.

Changes in breathing waveform during higher frequency breathing. A. & C. Both the magnitude of the nasal and the thoracic inhalation phase increased with an increase in breathing frequency. In contrast, the magnitude of the cannula exhalation phase greatly increased with higher breathing frequencies while the thoracic signal shifted to more negative values (D).

Fig. 7.

Raster plot of sniffing (each detected trough indicated by a dot) vs trial number during an odor discrimination task. All trials were aligned with the time at which the mouse nose was inserted into the odor port (0 s). Black vertical line at $t = 0.5$ s represents odor onset. Trials represents sequence of: 1) free moving state (around -1 to -0 s), 2) insertion of nose in the odor port (around 0 to 1.3 s, marked by red dots), 3) sampling of odor beginning with odor onset in the odor port (around 0.5 to 1.3 s), 4) withdrawal of nose from odor port (~ 1.3 s), 5) moving from odor port to water port (1.3 to 1.5 s), and 6) in water port drinking water reward (1.5 to ~ 10 s, marked by blue dots).

Fig. 8.

Raster plot of data in Fig. 7 presented in a 3D graph of time vs. sniff frequency with count of the number of frequency occurrences at any given time pseudo-colored. Time “0” represents insertion of the mouse's nose into the odor port and 0.5 s indicates odor onset. Time bin = 0.5 s, frequency bin = 0.3 Hz.