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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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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.

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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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1. 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

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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.

2. Materials and methods

2.1. 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 anesthetized 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%.

2.2. 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 anesthetized 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 μm from the bottom of the cannula.

2.3. Data acquisition of the telemetric signal

Pleural pressure was measured in the awake mouse using a commercial telemetry system (DSI). The components of the radiotelemetry 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).

2.4. 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,

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