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Minimally invasive highly precise monitoring of respiratory rhythm in the mouse using an epithelial temperature probe

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Abstract

Background—Respiration is one of the essential rhythms of life. The precise measurement of respiratory behavior is of great importance in studies addressing olfactory sensory processing or the coordination of orofacial movements with respiration. An ideal method of measurement should reliably capture the distinct phases of respiration without interfering with behavior.

New Method—This new method involves chronic implantation of a thermistor probe in a previously undescribed hollow space located above the anterior portion of the nasal cavity without penetrating any soft epithelial tissues.

Results—We demonstrate the reliability and precision of the method in head-fixed and freely moving mice by directly comparing recorded signals with simultaneous measurements of chest movements and plethysmographic measurements of respiration.

Comparison with Existing Methods—Current methods have drawbacks in that they are either inaccurate or require invasive placement of temperature or pressure sensors into the sensitive nasal cavity, where they interfere with airflow and cause irritation and damage to the nasal epithelium. Furthermore, surgical placement within the posterior nasal cavity adjacent to the nasal epithelium requires extensive recovery time, which is not necessary with the described method.

Conclusions—Here, we describe a new method for recording the rhythm of respiration in awake mice with high precision, without damaging or irritating the nasal epithelium. This method will be effective for measurement of respiration during experiments requiring free movement, as well as those involving imaging or electrophysiology.

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Introduction

Respiration, a fundamental rhythm of mammalian life, is inextricably linked to olfaction (Shusterman et al., 2011; Kay, 2014) and sensory system activity (Ito et al., 2014; Kleinfeld et al., 2014) in rodents, but poses unique challenges in terms of accurate measurement. Current methods employ the use of temperature or pressure sensors placed within the nasal cavity (Shusterman et al., 2011; Smear et al., 2011; Reisert et al., 2014) or in front of the nares (Ito et al., 2014). However, these methods have significant drawbacks.

Extranasal thermistors are an easy-to-use, non-invasive option for capturing the temperature changes associated with respiration for use in head-fixed mice. However, extranasal thermistors become less reliable during periods of high-frequency sniffing. This is because mice can move their nares and thereby redirect the respiratory airstream, which they often do during sniffing. This causes variability in the phase of the readout and can temporarily result in the loss of signal for one or more cycles. The beginning of inspiration is also difficult to reliably capture, as the probe is continually cooling towards the room temperature. Consequently, extranasal thermistors are most effective when expiration onset during resting breathing (<6 Hz) is of interest, and can be used effectively for creating circular data (Ito et al., 2014).

Pressure sensor coupled chambers for plethysmography offer highly precise measurement of respiration (Lim, 2014), but, by their nature, require rather small recording chambers. This makes them impractical for behavioral experiments that require space for equipment and allow some degree of free movement for the animals. However, simple odor response tests have been successfully conducted in mice (Youngentob, 2005) and rats (Hegoburu et al., 2011) with this approach.

Chronic implantation of sensors allows for the greatest potential mobility for the animal, but current approaches typically penetrate the bone and epithelial tissue overlying the nasal cavity (Shusterman et al., 2011; Smear et al., 2011; Reisert et al., 2014). Pressure changes associated with respiration are measured by placement of a small cannulae into the nasal cavity, allowing for highly accurate readings of respiratory efforts. However, the placement of even very small cannulae into the cavity will cause partial blockage of the narrow airway, leading to irritation and the formation of mucus. Cannulae are prone to blockage by this mucus or blood over time, requiring the blockage to be cleared using high pressure, suction, or the insertion of a wire. These procedures are likely to cause irritation of the highly sensitive soft tissues of the nasal cavity and constitute additional stressors for the mouse. In addition, a pressure transducer or hose needs to be mounted to the cannula during readings, which adds considerable weight and mechanical stress above the nasal bridge.

Intranasal temperature sensors as described in some studies (Uchida and Mainen, 2003; Wesson, 2013) suffer similar drawbacks, as they also penetrate the nasal cavity thus interfering with airflow and causing damage to the epithelium. This approach may still be preferable to pressure cannula since connection to a signal-transducing amplifying system can be accomplished with minimal-weight 2-pin connectors mounted on top of the skull rather than a pressure transducer attached to the cannula on the bridge of the nose. An

additional advantage is that the 2-pin connectors do not restrict the animal's field of vision and are solidly fixed to multiple anchoring screws placed in the skull bone.

Problems with implanted instrumentation penetrating the nasal cavity are exacerbated in mice due to the narrowness of their nasal cavity and the weakness of their neck muscles, which struggle to support heavy monitoring equipment and connectors. Yet, use of mice for such experiments is highly desirable because of readily available genetic tools and disease models in the species. The method of using a non-invasive implant that we present here overcomes many of the pitfalls described above. Animals who underwent this implantation rapidly recovered. There is virtually no waiting time, other than recovery from surgery, before high quality respiratory data can be recorded. This is in stark contrast to some current methods, which require days or weeks of recovery before respiratory signals can be recorded (Wesson, 2013). We demonstrate that this method is highly effective and precise in both head-fixed and freely moving experimental preparations.

Materials and Methods

Animals

Adult male and female C57BL/6J mice were housed with food and water ad libitum in a 12:12-h light/dark cycle. All mice used in this study were raised and all experiments were performed in accordance with procedural guidelines approved by the University of Tennessee Health Science Center Animal Care and Use Committee. Principles of laboratory animal care (NIH publication No. 86-23, rev. 1996) were followed. Animals were monitored for up to 4 days post-surgery to ensure proper recovery before experimentation.

Probe fabrication

Fast time response NTS thermistors (*MEAS-G22K7MCD419*, Measurement Specialties; Hampton, VA) were used as the temperature sensitive element of the probe. Leads were cut to 5cm in length before wires were separated and stripped of insulation on 1mm of the distal end. The stripped ends were then soldered to the pins of a miniature male-female header plug (e.g. Mill-Max Engineering, U.S.A., part#: 861-13-0XX-10-002000) and the soldered connections were insulated with a thin layer of acrylic cement or epoxy. All probes were tested and treated with 90% ethanol for disinfection prior to implantation.

Surgical Procedure

Prior to surgery, mice were weighed and injected with a ketamine/xylazine cocktail to induce anesthesia. Maintenance of anesthesia throughout the surgery was accomplished by supplemental injections of ketamine. An analgesic was subsequently given (0.05ml carprofen solution, s.c.) to facilitate recovery. Once anesthetized, mice were mounted in a surgical stereotaxic frame using 60° non-rupture ear bars (David Kopf Instruments, Tujunga, CA). Body temperature was monitored with an electronic rectal thermometer and maintained at 37–38.0 °C using a feedback controlled heating pad (FHC Inc., Bowdoinham, ME). Eye moisture was maintained by application of sterile lubricating eye ointment (*Systane*, Alcon Inc., Missuaga, Ontario). With care to avoid contact with the eyes, hair was

removed from the scalp and nose with scissors and a depilatory cream and the skin was treated with iodine solution (Xenodine, Veterinary Products Laboratory, Phoenix, AZ).

A sterile scalpel was used to make a midline incision to expose the skull surface from the interparietal skull plate to the anterior edge of the nasal bone. The surface was then cleaned using a dental scraper and sterile cotton swabs. A dental drill (0.7mm round burr) was used to create a hole in the posterior skull for a small machine screw, used to anchor the headcap (*B000FN0J58*, Antrin Miniature Specialties, Fallbrook, CA). Antibiotic ointment was immediately applied to the screw hole to prevent infection and dryness. The dental drill was also used to create the opening for thermistor implantation above the anterior portion of the nasal bone, centered 0.5mm lateral from the midline suture (Nasal fissure +3.1mm). This typically caused minimal to no bleeding and revealed a small cavity overlying highly vascularized soft tissue. To the best of our knowledge, this cavity has not been previously described in the anatomical literature, but exists between the nasal epithelium of the dorsal meatus and the frontonasal bone due to a lack of connective tissues (Fig. 1). The probe was then carefully lowered into this cavity with forceps, while the connector remained attached to a stereotaxic manipulator. We did not find it necessary to fully insert the probe into the cavity; partial insertion (as seen in Fig. 1) yielded no appreciable reduction in signal quality over completely inserted probes (data not shown). Partial insertion thus was preferred to further reduce the risk of damage to the nasal epithelium or narrowing of the airway. A small amount of KwikSil epoxy (World Precision Instruments, Sarasota, FL) was then applied to cover the probe and allowed to dry in order to protect the temperature sensitive element and seal the opening. We found this step to be crucial, as direct contact of the probe with acrylic cement seemed to damage the thermistors. Once dry, skull screws were implanted in their pre-drilled holes and a head fixation bar was attached to the skull bone with super glue. The probe connector was then released from the stereotaxic arm and excess lead wire was coiled around the stem of a cotton swab until the connector piece was able to rest adjacent to the skull screws with an orientation that would not interfere with later head fixation. Acrylic dental cement was then applied to the exposed skull, embedding skull screws, thermistor connector and head fixation bar. Mice were then placed in individual cages under infrared heat lamp until fully awake, and monitored for up to 4 days to ensure proper surgical recovery.

Data Acquisition

Recording sessions began on the fourth day after surgery. Data were stored on a computer hard disk (16 bit A/D converter, sampling rate 2 kHz) using a CED Power1401 and Spike2 software (both Cambridge Electronic Design, UK).

Head-fixed recording—Accuracy of respiratory monitoring by the implanted thermistor was tested by simultaneously recording respiration with a piezoelectric transducer. Mice were placed into the head fixation apparatus and covered by a foam-lined plastic half-tube to prevent injury from excessive movement (Bryant et al., 2009). The implanted thermistor was connected to a custom-built amplifier and the resulting temperature-related voltage changes were digitized at 2 kHz (CED 1401, Cambridge Electronic Design, UK) and stored on hard disk. A piezoelectric strip was placed against the lateral chest wall to detect inflation of the

chest cavity. Movements of the chest wall would bend the piezoelectric strip, which resulted in a measurable electrical current (Fig. 2). A second thermistor connected to a second amplifier was placed in front of the nares for simultaneous comparative recording.

Freely moving recording—Feasibility of recording respiration in freely moving mice with the minimally invasive thermistor was assessed during a recording session in a custom plethysmography chamber (Fig. 3). Prior to recording the chamber was connected to a pressurized air inlet and pressure was calibrated to create 0.5 L/min of continuous airflow through the chamber. The plethysmography chamber ceiling was coupled to the positive inlet of a differential pressure transducer with carrier demodulator (DP45-14 and CD15 respectively, Validyne Engineering, Northridge, CA) to detect changes in chamber pressure. Voltage output from the demodulator was digitized at 2 kHz (CED 1401, Cambridge Electronic Design, UK) and stored on hard disk. Implanted probes were again connected to the amplifier via a thin 1 m long wire. Mice were then allowed to move freely for several minutes while thermistor and plethysmograph data were continuously recorded.

Recording of Sniffing Behavior—Effectiveness for measurement of spontaneous sniffing behavior during odor discrimination was assessed in a novel variant of an odor response task previously described in the literature (Wesson et al., 2008). The odorants selected for exposure were ethyl tiglate (ET), ethyl butyrate (E4), acetophenone (Ac), citronellyl isobutyrate (Cit), ethyl valerate (E5), benzaldehyde (Bz), ethyl hexanoate (E6), and 2-heptanone (2H). Mice were habituated to head fixation on a treadmill in front of an odor port with continuous airflow before exposure to odorants. Once habituated, mice were exposed to the same odorant multiple times for 1 second durations at 1 minute intervals until they no longer responded with fast sniffing. The familiar odor was then alternated with a battery of novel odorants with a range of structural similarities, and respiration responses were simultaneously recorded.

Data Analysis

Data were analyzed in Spike2 (Cambridge Electronic Design, UK) or exported to Matlab (The Mathworks, U.S.A). All recordings were preprocessed in Spike2 by applying a smoothing filter with a time constant of 5ms; this was realized as a 20th order FIR filter with all coefficients equal to 1/20. A DC removal filter was also implemented for both thermistor signals in order to subtract slow drifts in the temperature signal unrelated to respiration phase (see Fig. 4C for example). DC removal was achieved by subtracting the result of the smoothing filter with coefficient of 50ms (a 200th order FIR filter with all coefficients equal to 1/200) from the original signal. Timing of respiratory events in the thermistor signals was compared to corresponding timing of events in the piezoelectric signal. Circular estimates of respiratory phase were created by normalizing each respiratory cycle duration to 2π using the beginning of inspiration in the piezoelectric signal. Relative error of thermistor events was calculated using extranasal thermistor event time minus the corresponding event time in the minimally invasive thermistor. Mean chamber pressure was subtracted from plethysmograph signals using the same DC removal algorithm with a time constant of 500ms so that 0 values correspond to mean chamber pressure. Frequency-dependent effects of respiration on waveform amplitude were not analyzed, as temperature changes can occur

independent of airflow. Furthermore, the drift removal process reduces the amplitude of slower cycles, thus obscuring this relationship in the already tenuous link between nasal temperature and respiratory tidal volume.

Results

Comparison of Head-fixed Recording Methods

We performed a direct comparison of methods by recording respiration in head-fixed mice ($n = 3$) using three different methods simultaneously. The recording of chest expansion via a piezoelectric strip served as the reference as it provided the most direct and precise measurement of inspiration onset. Only periods of stable piezo measurements of chest movements were used for further analysis. The second signal came from a thermistor placed in front of the nares and the third from the minimally invasive implanted thermistor. The onset of downward deflections in the piezo signal was used to identify the beginning of chest expansion which corresponds to the beginning of inspiration. A direct comparison of the piezo and extranasal thermistor traces shows that the extranasal thermistor was less precise at determining the beginning of inspiration due to the constant cooling of the probe by room-temperature air. This led to consistently early estimates for the beginning of inspiration (Fig. 2; delay of $-40.3 \pm 27.4\text{ms}$). By comparison, respiration related temperature changes captured by the implanted thermistor more accurately reflected the correct moment of inspiration, with a delay of $5.4 \pm 8.9\text{ms}$ (Fig. 1C). Frequency-dependent latency changes were subtle, and most apparent at low respiration frequencies (Fig. 2D). Perhaps most importantly however, the response latency was not significantly altered by sniffing behavior, thus making the implanted thermistor appropriate for measurement of sniffing in a head-fixed preparation.

A direct comparison between the two thermistors showed highly synchronous detection of expiration onset, indicating a high degree of precision shared by both methods. This was especially apparent during resting breathing (0.5 to 6Hz; standard deviation 4.6ms), which suggests that extranasal thermistors can be highly effective for acquiring respiration data non-invasively, if expiration is used as the reference point for analysis.

Comparison of freely moving recording methods

Respiration signals from the minimally invasive implanted thermistor were readily recorded in freely moving mice and without movement related artifacts in 3 of the 3 tested mice (see e.g. Fig. 3). Direct comparison with simultaneously recorded plethysmography signals showed that the embedded thermistor signal reliably and accurately reflected respiration at all frequencies in the freely moving mice.

Assessment of sniffing behavior

Sniffing behavior was effectively measured during odor presentation in 3 head-fixed mice. The results obtained were very similar to those of Wesson et al. (Wesson et al., 2008), in several ways (Fig. 4). Mice responded to novel odors with sustained periods of high-frequency sniffing, whereas they responded to familiar odors with only brief, less pronounced increases in respiration frequency. Additionally, odorants that were structurally

similar to a repeated odorant elicited smaller increases in sniff frequency compared to structurally dissimilar odorants (Fig. 4B, E5 and E6 versus Bz). Lastly, mice also demonstrated characteristic dishabituation to a familiar odor following the presentation of the first novel odor (Fig. 4B, sniffing after first E4). Together, these results show that the minimally invasive thermistor implant is as effective a tool for the assessment of sniffing in response to odor as the pressure transducer connected to an intranasal sniff cannula employed by Wesson et al. (Wesson et al., 2008).

Discussion

Current approaches to measuring mouse respiratory movements rely either on measuring temperature changes associated with inhaling cool air and exhaling air warmed up during passage through the airways and lungs, or they use miniature pressure transducers to measure respiration related pressure changes in the nasal cavity via implanted cannulae. Placing a thermal probe directly in front of the nares is the least invasive method, but is only precise when used to detect the onset of expiration during resting breathing (<6Hz). Implanting thermistors into the nasal cavity gives reliable measurements at all frequencies but interferes with the airflow and irritates the sensitive lining of the nasal cavity. Intranasal pressure measurements have the same drawbacks as they too require invasion of the nasal cavity with a small metal or plastic cannula and, in addition, add considerable weight to the nose and partially obstruct the visual field. Another noteworthy approach employs a pressure sensor implanted into the thoracic cavity to detect changes in pressure associated with respiratory movements (Reisert et al., 2014). This method is advantageous in that it does not cause damage to olfactory tissues. However, the temporal precision with which inhalation onset times were detected declined considerably with decreasing respiratory frequency while precision of detection was stable across all frequencies for the minimally invasive thermistor (Fig. 2D). In summary, this novel method of respiration monitoring in mice using minimally invasive thermistor implants is at least as reliable as current methods using implanted sensors, but without the drawbacks, such as obstruction of airflow or damage to the nasal epithelium. Minimally invasive thermistor implants thus allow highly precise respiratory monitoring with reduced pain and stress for the animals and without interfering with natural breathing behavior.

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References

- Bryant JL, Roy S, Heck DH. A technique for stereotaxic recordings of neuronal activity in awake, head-restrained mice. *Journal of neuroscience methods*. 2009; 178:75–79. [PubMed: 19073214]
- Hegoburu C, Shionoya K, Garcia S, Messaoudi B, Thevenet M, Mouly AM. The RUB cage: respiration-ultrasonic vocalizations-behavior acquisition setup for assessing emotional memory in rats. *Front Behav Neurosci*. 2011; 5

- Ito J, Roy S, Liu Y, Cao Y, Fletcher M, Lu L, Boughter JD, Grun S, Heck DH. Whisker barrel cortex delta oscillations and gamma power in the awake mouse are linked to respiration. *Nature communications*. 2014; 5:3572.
- Kay LM. Circuit oscillations in odor perception and memory. *Progress in brain research*. 2014; 208:223–251. [PubMed: 24767485]
- Kleinfeld D, Deschenes M, Wang F, Moore JD. More than a rhythm of life: breathing as a binder of orofacial sensation. *Nature neuroscience*. 2014; 17:647–651. [PubMed: 24762718]
- Lim RZ, Marcus J, Milton Phillipa-Louise, Chan Siow Teng, Tan Jean L. Dickinson Hayley, Murphy Sean V. Jenkin Graham, Wallace Euan M. Measuring Respiratory Function in Mice Using Unrestrained Whole-body Plethysmography. *Journal of Visual Experiments*. 2014
- Reisert J, Golden GJ, Matsumura K, Smear M, Rinberg D, Gelperin A. Comparing thoracic and intranasal pressure transients to monitor active odor sampling during odor-guided decision making in the mouse. *Journal of neuroscience methods*. 2014; 221:8–14. [PubMed: 24056232]
- Shusterman R, Smear MC, Koulakov AA, Rinberg D. Precise olfactory responses tile the sniff cycle. *Nature neuroscience*. 2011; 14:1039–1044. [PubMed: 21765422]
- Smear M, Shusterman R, O'Connor R, Bozza T, Rinberg D. Perception of sniff phase in mouse olfaction. *Nature*. 2011; 479:397–U149. [PubMed: 21993623]
- Uchida N, Mainen ZF. Speed and accuracy of olfactory discrimination in the rat. *Nature neuroscience*. 2003; 6:1224–1229. [PubMed: 14566341]
- Wesson DW. Sniffing Behavior Communicates Social Hierarchy. *Curr Biol*. 2013; 23:575–580. [PubMed: 23477727]
- Wesson DW, Donahou TN, Johnson MO, Wachowiak M. Sniffing behavior of mice during performance in odor-guided tasks. *Chem Senses*. 2008; 33:581–596. [PubMed: 18534995]
- Youngentob SL. A method for the rapid automated assessment of olfactory function. *Chem Senses*. 2005; 30:219–229. [PubMed: 15741600]

Highlights

- A new method of respiratory monitoring is proposed for use in mice.
- This method overcomes weaknesses of previous methods such as invasiveness and inaccuracy.
- This method is effective for measuring breathing even at high sniff frequencies.
- This method is demonstrated to be effective in both head-fixed and freely-moving preparations.
- This method requires less recovery time than other chronic implantation methods.

Figure 1. Surgical placement of thermistor probe

Left panel: Picture of thermistor insertion. Dotted lines were added to highlight the locations of skull sutures used as landmarks for probe placement. Triangle indicates the point from which stereotaxic coordinates were measured; the intersection of the sagittal suture and the posterior edge of the nasal bone. Right panel: (Top left) High contrast image of soft tissues underlying the nasal bone. Poor attachment to the bone creates a space in which a thermistor can be easily implanted. (Right) Anatomy of a coronal section at this location. Illustration based on coronal sections from (Barrios, Nunez et al. 2014).

Figure 2. Comparison of respiration monitoring with minimally invasive and non-invasive thermistors in head-fixed mice

A: Illustration of data sources for comparison (see text for details). B: Example of raw data traces (colors correspond to sensor colors in A) during both fast and slow breathing. Dotted lines indicate times denoted as the beginning of inspiration based on the piezo signal deflection. In the thermistor signals the beginning of inspiration corresponds to the troughs. C: Peri-event time histogram showing the detection time of inspiration in thermistors relative to the piezo. Extranasal thermistors had troughs that often preceded inspiration due to the constant cooling of the probe by room-temperature air, which counteracts the warming due to expiration. D: Scatter plot of detection delay of thermistor signals as a function of respiration frequency based on piezo measurements. Minimally invasive thermistors show only slight frequency-dependent delay during slow respiration. E: Comparison of phase estimates represented as circular data. Circular histograms consist of 360 1-degree bins. Index of straightness (Rayleigh's r) ranges from 0 to 1, for evenly spaced or perfectly aligned data, respectively. These results indicate that the minimally invasive thermistor signal reliably represents the phase of respiration. F: Respiration frequency scatter plot of relative error of detection time for inspiration (green) and expiration (yellow) onset using extranasal and minimally invasive thermistors. Distributions indicate high degree of precision for external thermistors up to 6 Hz respiration (standard deviation ± 4.2 ms) with error increasing to ~ 25 ms for fast sniffing frequencies.

Figure 3. Plethysmographic recording of respiration during free movement

A: Illustration of recording setup. Mice were placed into a small plethysmography chamber while connected to the amplifier for thermistor measurements. B: Diagram for waveform interpretation. Initiation of inspiration appears as troughs in thermistor waveform, which correspond to transitions between above- and below-average pressure values in the plethysmography chamber. C: Example raw data traces of thermistor (blue) and plethysmograph (grey) signals. Thermistor measurements reliably matched plethysmograph measurements across a wide frequency range. Lower traces show the period of spontaneous sniffing marked in the top traces at an expanded timescale. D: Peri-event time histogram showing the detection time of inspiration in the thermistor relative to the plethysmograph. Results are comparable to those in the head-fixed condition.

Figure 4. Measurement of sniffing behavior during odor presentation

A: Photograph of the experimental apparatus showing the mouse on a treadmill with the glass odor port in front of the nares. B: Mean respiration frequency within a 5 second windows over the course of an experiment. Novel odors elicit sustained responses of high-frequency sniffing. Odorants were presented 1 minute apart for 1 second each. Dotted lines are color matched to the odorants listed in corresponding colors on top of the plot. Odor abbreviations listed in methods. C: Expanded time view of sniff rate with raw data for the second novel odorant, Acetophenone. Example 100ms DC removal was performed to aid in visualizing the sniff signal, since sniffing causes an overall cooling of the probe as air is rapidly exchanged through the nose. Black bar corresponds to 1s. D: Histogram of all respiration intervals during the recording session. The distribution is bimodal, corresponding to resting and sniffing respiration modes.