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Respiratory Airflow Pattern at the Rat's Snout and an Hypothesis Regarding Its Role in Olfaction

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WILSON, D. A. AND R. M. SULLIVAN. *Respiratory airflow pattern at the rat's snout and an hypothesis regarding its role in olfaction.* PHYSIOL BEHAV **66**(1) 41–44, 1999.—Respiratory airflow outside the external nares of the rat was mapped by monitoring temperature fluctuations with a thermistor and simultaneous piezoelectric monitoring of respiration-associated chestwall movement. The results demonstrated that both exhalation and inhalation airflow were directed laterally. Relatively little air exchange occurred anterior to the nares. These results suggest that the two nares of the rat take independent, bilateral samples of the odor environment. Combined with recent descriptions of laterally specific, spatial receptive fields in piriform cortical neurons, an hypothesis is outlined describing a mechanism of odor orientation in the rat involving comparisons of timing or intensity of bilateral odor stimulation. © 1999 Elsevier Science Inc.

Respiration Odor orientation Piriform cortex Odor plume Chemotaxis

ODORS play a critical role in modulating or directing many animal behaviors. Orientation to odor cues, navigation through odor plumes, and tracking odor trails have been described in many species. Recent work, especially in invertebrates, has begun to identify mechanisms of odor-guided movement. Orientation within an odor plume or tracking an odor trail requires comparisons of odor intensity that allow the animal to maintain itself within the plume or on the trail, and move appropriately through the plume.

Two basic strategies exist for orienting in chemical gradients: 1) sequential sampling of concentration and comparison with a memory of the previous concentration (14), and 2) comparisons of simultaneous samples at two disparate locations on the body (1,6,11). Bilateral sampling is utilized by some invertebrates with bilateral olfactory antennae. It has been hypothesized that odor tracking by these animals occurs through comparisons between intensity or timing of odor stimulation of the two antennae (1). Removal of one antenna (5,10) or artificial crossing of the antennae (10) results in impaired odor tracking ability. The forked tongue of some species of snakes may also be used in a similar fashion [(17); although see (7)].

Mammals, like the rat, can also orient to odor plumes and follow odor trails (2,15). The use of simultaneous bilateral comparisons of odor stimulation for odor tracking in the rat, however, has traditionally appeared unlikely given the relatively small internaral distance.

On the other hand, the bilateral nasal passages in the rat are relatively well isolated, each containing an independent olfactory receptor sheet with receptor neurons that project exclusively ipsilaterally to the main olfactory bulb. One of the sites of bilateral convergence in the olfactory pathway is the piriform (olfactory) cortex. Recently, spatial receptive field characteristics have been described in neurons in the piriform cortex of rats (18). Thus, neurons have been described that respond only to odors delivered to the ipsilateral receptor sheet (ipsilateral naris), only to the contralateral receptor sheet, to either side, or only to bilaterally presented odors.

Although several hypotheses exist regarding the role of bilateral convergence in the olfactory system (3,18), the discovery of spatial receptive field properties raises the possibility that the piriform cortex participates in odor localization through comparisons of bilateral input. Such odor localization could only occur, however, if there was at least partial nonoverlap in the air sampled by the two nares. The present report, therefore, mapped spatial patterns of respiratory airflow at the rat naris to determine the amount of overlap that occurs during bilateral odor sampling. The results suggest that in-

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spiratory and expiratory airflow is directed laterally from the nares, functionally increasing internaral distance.

METHODS

Adult male Long–Evans hooded rats (200–500 g) were used as subjects. Animals were housed in polypropylene cages lined with wood chips. Food and water were available ad lib. Lights were maintained on a 12:12 light:dark cycle, with testing occurring during the light portion of the cycle.

Animals were anesthetized with urethane (1.5 g/kg) and placed in a stereotaxic apparatus with the dorsal plane of the snout (eyes to tip of nose) horizontal. Chest wall movements were monitored with a piezoelectric device strapped around the chest immediately caudal to the front legs. Airflow at the external nares was inferred from fluctuations in the resistance of a thermistor probe during relative heating by warm exhaled air and relative cooling by inhaled air. Thermistors are commonly used to monitor respiratory airflow in a variety of animals (4). The thermistor (0.75 mm diameter, 5 mm length) was mounted on a stereotaxic manipulator for precise localization around the naris. Outputs from the piezoelectric and thermistor probes were amplified, digitized (200–500 Hz) with a 1401plus A/D converter (Cambridge Electronic Design, Inc.) and analyzed with Spike2 software for the Macintosh (Cambridge Electronic Design, Inc.). Piezoelectric output was also passed through a window discriminator to provide a pulse output synchronous with onset of inhalation. Synch pulses were used for waveform averaging over multiple respiratory cycles.

The thermistor was moved with the stereotaxic manipulator in either 0.5- or 1.0-mm increments in three dimensions around the snout to qualitatively map respiratory airflow changes relative to the naris. Sampling at each site lasted for at least 20 s to allow waveform averaging (Spike2 software) over at least 40 respiratory cycles at each spatial location. Two-dimensional plots (2D space and space vs. time) of the data from averaged waveforms were constructed using algorithms provided with Transform imaging software (Fortner Research).

At the end of the recording, two of the animals were perfused with 4% paraformaldehyde, their skulls were decalcified, and horizontal sections (240 μ) were taken of the snout.

RESULTS

Respiratory airflow patterns were mapped in five animals, with similar results obtained in all animals as judged by visual inspection of plotted data. As shown in Fig. 1, movement of the chest wall during respiration coincided with changes in output of the thermistor located near the naris. The rat naris is composed of a medial, roughly spherial hole and a lateral groove (Fig. 1). Thermistor measurements anterior to the hole indicated relatively little airflow over the respiratory cycle. On the contrary, large inhalatory and exhalatory air currents were detected lateral to the naris, near the end of the lateral goove.

A two-dimensional plot of mean peak-to-peak thermistor change over the respiratory cycle (exhalation and inhalation combined) was created for a horizontal plane level with the dorsal edge of the naris (Fig. 2). As shown in Fig. 2B, peak airflow (thermister change) occurred in a caudal laterally directed plume. Relatively little anterior directed airflow was detected. This corresponds with the medial lateral orientation of the nasal passage interior to the naris (Fig. 2A). Although the measurements shown in Fig. 2 are for the horizontal plane at the dorsal edge of the naris, similar results were obtained in

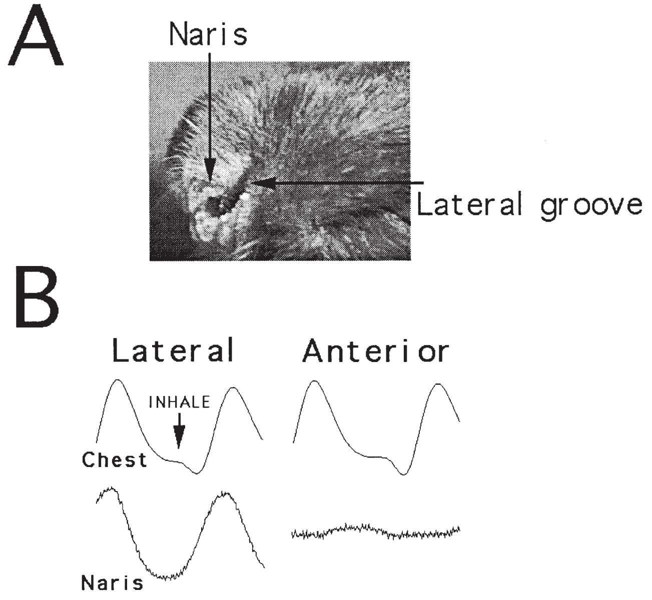

FIG. 1. (A) Lateral view of the rat snout showing external naris and lateral groove. (B) Examples of raw data obtained simultaneously with a piezoelectric monitor of chest wall movement (top) and a thermistor monitor of airflow at the naris (bottom). Inhalation is down and exhalation is up on all records. Both inhalation and exhalation produce large air currents (thermistor output) lateral to the naris while there is relatively no airflow anterior to the naris.

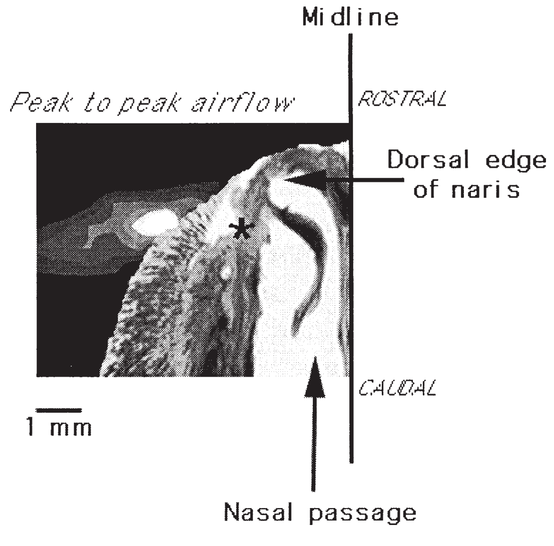

FIG. 2. Peak-to-peak airflow (thermistor output) as a function of location around the rat naris plotted as a gray scale image (white: peak flow; black: no flow) next to a horizontal section through the rat nose. Maximal airflow is directed laterally, with the greatest flow occurring along the lateral groove of the naris (asterisk), and little detectable air current directed to the anterior. The horizontal section through the rat nose shows a medial lateral bend in the nasal passage interior to the naris. Similar patterns were observed in all animals analyzed.

horizontal planes at all dorsal–ventral sites examined (1–2 mm dorsal and 1–2 mm ventral to the dorsal edge of the naris).

Mapping of respiratory airflow into and out of models of the human naris have demonstrated spatially discrete inspiratory and expiratory airflows (8). To determine if inspiration and expiration were similarly spatially displaced in the rat, thermister output was mapped in a two-dimensional plot of location along the lateral side of the snout versus time during the respiratory cycle. As shown in Fig. 3, while both exhalation and inhalation were directed laterally, inhalation airflow is relatively more diffuse, producing an inspiratory air plume wider than the narrow expiratory jet. Airflow at the onset of inhalation occurred slightly anterior to airflow during the peak of inhalation.

DISCUSSION

The internal nasal air passage in the rat curves medially from the external naris into the vestibulum nasi, and from there travels caudally eventually passing over the turbinates of the olfactory receptor sheet [Fig. 2; (9)]. This medial–lateral bend immediately interior to the naris, as well as the external caudal lateral groove of the naris (Fig. 1), may combine to produce the laterally directed respiratory airflow patterns described here. As reported for respiratory airflow in models of human nose (8), inspiratory airflow was relatively more diffuse than expiratory airflow, although in the rat both were directed laterally. One consequence of the diffuse inhalatory airflow is a reduction in rebreathing of exhaled gas (8). More detailed analyses, perhaps utilizing dynamic fluid models (8), will be required for quantitative descriptions of respiratory airflow in the rat. In addition, given that the present experiments were performed under urethane anethesia, which modifies respiratory pattern (e.g., reduces respiration rate), studies in awake animals at varied respiratory rates (e.g., sniffing) will be valuable.

The laterally directed respiratory airflow patterns described here, combined with the previous demonstration of spatial receptive field properties of piriform cortex neurons,

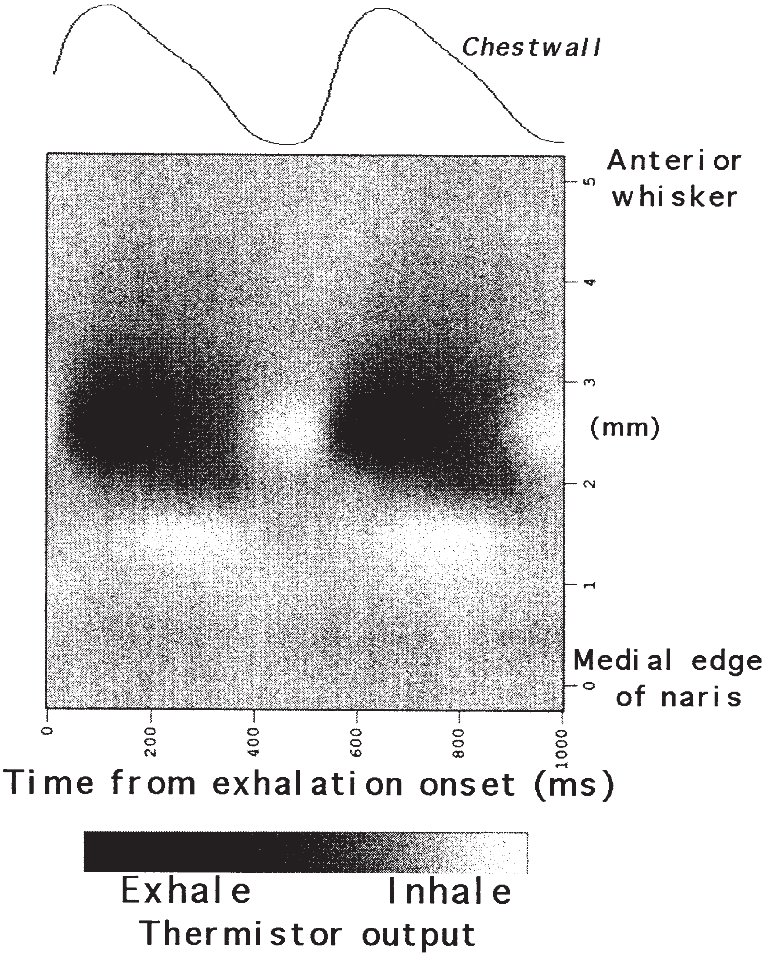

FIG. 3. Airflow (thermistor output) as a function of time over two respiratory cycles and location along the lateral surface of the rat's snout. Thermistor measurements were made within 0.5 mm of the lateral surface of the snout in 0.5 mm increments and converted to gray scale values (white: cooling/inspiration; black: warming/expiration). Both inhalation and exhalation are directed laterally, although inhalation formed a wider sweep than exhalation.

suggests that the rat olfactory system may be capable of bilateral comparisons of odor stimulation. Lateral inhalation may provide sufficient isolation of the two nares to allow differential bilateral input, similar to that observed in invertebrate antennal systems (5,10). Spatial receptive fields in the piriform cortex could be used to compare either odor concentration at the two nares, or potentially, time delays between stimulation of the nares. Inhibitory commissural connections between the two olfactory bulbs may accentuate these bilateral differences (13). Time delays between input to the two antennae in lobsters has been shown to be a more sensitive indicant of odor source direction than differences in odor concentration when combined with knowledge of the wind/water flow direction (1).

Furthermore, exploration in rats is associated with active sniffing. Active sniffing, which occurs at 5–10 Hz in the rat (19), could be used to increase the frequency of odor sampling, allowing greater sensitivity to, or enhancement of, timing differences. Whisking (rapid anterior–posterior sweeping of facial vibrissae) that is associated with sniffing in the rat could serve two roles. First, whisking could diffuse boundaries within patchy odor plumes, mixing the to-be-inhaled air, thus facilitating odor acquisition by the nares. Second, whisking could facilitate associations between odors and features of the substrate or local environment with which those odors are paired.

Bilateral comparisons of odor stimulation may be one source of information guiding nocturnal mammals such as the rat to odor sources. Bilateral comparisons of odor stimulation may not occur in humans, given that the nares and airflow patterns are directed in an anterior, overlapping pattern (8). In fact, humans cannot spatially discriminate between odorants delivered unilaterally to the left or right naris in the absence of concomitant trigeminal activation (12,16).

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