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Brainstem-mediated sniffing and respiratory modulation during odor stimulation

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ABSTRACT

The trigeminal and olfactory systems interact during sensory processing of odor. Here, we investigate odor-evoked modulations of brainstem respiratory networks in a decerebrated perfused brainstem preparation of rat with intact olfactory bulbs. Intranasal application of non-trigeminal odors (rose) did not evoke respiratory modulation in absence of cortico-limbic circuits. Conversely, trigeminal odors such as menthol or lavender evoked robust respiratory modulations via direct activation of preserved brainstem circuits. Trigeminal odors consistently triggered short phrenic nerve bursts (fictive sniff), and the strong trigeminal odor menthol also triggered a slowing of phrenic nerve frequency. Phrenic and vagal nerve recordings reveal that fictive sniffs transiently interrupted odor evoked tonic postinspiratory vagal discharge. This motor pattern is significantly different from normal (eupneic) respiratory activity. In conclusion, we show for the first time the direct involvement of brainstem circuits in primary odor processing to evoke protective sniffs and respiratory modulation in the complete absence of forebrain commands.

1. Introduction

The processing of odorants within the main olfactory epithelium involves two primary and anatomically distinct neural systems. The first 'classic' olfactory pathway consists of axonal projections from the olfactory sensory neurons of the nasal epithelium into the glomeruli of the olfactory bulb. Within the glomeruli, the sensory input is forwarded to the mitral and tufted cells, which in turn widely project to forebrain structures such as the piriform cortex, hippocampus and amygdala (Doty, 2001; Firestein, 2001). The second 'naso-trigeminal brainstem' pathway is associated with the trigeminal system (Hummel and Livermore, 2002; Brand, 2006). The anterior ethmoidal and infraorbital nerves, both of which are branches of the ophthalmic division of the trigeminal nerve, also innervate the nasal mucosa. Contrary to the forebrain projection of the olfactory system, the trigeminal sensory fibers project to spinal trigeminal nuclei such as the sub-nucleus caudalis and the sub-nucleus interpolaris (Anton and Peppel, 1991; Anton et al., 1991), which are located in the caudal brainstem.

Most odorants have the ability to co-activate both systems. This was demonstrated by simultaneous electrophysiological recording of olfactory and trigeminal sensory fibers of the nasal epithelium, which revealed a trigeminal response following exposure to a variety of odors that predominantly stimulated olfactory receptors (Beidler and Tucker, 1956). Thus, sensory processing of odors appears to involve the interplay between the top-down cortico-limbic olfactory system and a bottom-up trigeminal brainstem pathway. Both systems contribute to the processing and perception of smell via converging multi-synaptic projections to somatosensory cortico-thalamic, and limbic brain areas.

While, both systems contribute to sensory perception of odorants, the trigeminal pathway may serve additional functions (Hummel and Livermore, 2002). The trigeminal system also safeguards the lower airways (lungs) to prevent inhalation of potentially noxious substances via protective reflexes, including a protective breath-hold or sneeze (Widdicombe, 1986). The latter underpins the tight association of olfaction and breathing. Respiratory modulation of olfactory bulb activity is well established and may involve peripheral sensory feedback from nasal airflow, as well as ascending modulation of olfactory bulb activity via the primary respiratory networks of the brainstem (Buonviso et al., 2006; Kepecs et al., 2006; Wachowiak, 2011).

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The tight interaction between breathing and olfaction is seen in several olfactory behaviors, for example fast sniffing to enhance odor detection during active sensing in the context of exploration or olfactory tasks (Wachowiak, 2011). The general view is that a switch from breathing to sniffing depends on behavioral forebrain (cortical and/or limbic) commands (Kepecs et al., 2006; Wachowiak, 2011), while the primary pattern generator for sniffing resides in close proximity to aspects of the respiratory pattern generator in the medulla oblongata (Moore et al., 2013, 2014). The modulation of brainstem respiratory cell activities during sniffing has been shown (Batsel and Lines, 1973; Du Pon, 1987). The precise source and anatomical pathways of the sniffing command that triggers the changes in respiratory neuron activity, however, are unknown so far; it might arise from either the trigeminal brainstem pathway or descending input from the cortico-limbic olfactory system, or both.

In the present study, we specifically address the potential contribution of the naso-trigeminal brainstem pathway to the mediation of respiratory modulations elicited by odorants; to this end we apply trigeminal and non-trigeminal odorants intra-nasally in an *in situ* perfused, decerebrated, olfactory-bulb-brainstem preparation (Pérez de los Cobos Pallarés et al., 2015). While this experimental approach can be used to study odor processing in the olfactory bulb (Pérez de los Cobos Pallarés et al., 2015), it also provides a unique opportunity for the investigation of trigeminally-mediated odor processing in the brainstem in the absence of confounding influences by the cortico-limbic systems. Previous work demonstrated that stimulation of the trigeminal ethmoidal nerve, or mechanical stimulation of the nasal mucosa (including its irrigation with cold water), reliably triggers cardio-respiratory reflexes such as the diving response in the *in situ* perfused brainstem preparation (Dutschmann and Paton, 2002a,b; Pérez de los Cobos Pallarés et al., 2015). Thus, the trigeminal innervation of the nasal cavity, including the primary sensory relay within the brainstem, remains intact under these experimental conditions. Since specific odorants stimulate the olfactory and the trigeminal system differentially (Doty et al., 1978), we analyzed respiratory responses to odors that are known to trigger a pure olfactory response (e.g. rose odor) vs. irritant odors such as menthol or lavender (linalool) that produce robust co-activation of the trigeminal system. The trigeminal odors menthol and lavender may act via TRP channels (Peier et al., 2002; Elsharif et al., 2015) expressed in the nasal mucosa (see Bessac and Jordt, 2008). For the remainder of the manuscript we refer to these odors as non-trigeminal and trigeminal odors, respectively.

We demonstrate that the brainstem alone can initiate short bursts of phrenic nerve activity that are evocative of fictive sniffing as well as subsequent respiratory depression, in the complete absence of the forebrain.

2. Material and methods

2.1. Ethical approval

All experimental procedures were performed either in accordance with the Australian code of practice for the care and use of animals for scientific purposes or with the stipulations of the German law governing animal welfare (Tierschutzgesetz). The ethics committee of the Florey Institute approved the study design.

2.2. The perfused olfactory bulb brainstem preparation

As described previously (Paton, 1996), juvenile *Sprague* Dawley rats (p17-21) were deeply anesthetized with isoflurane (1-Chloro-2,2,2-trifluoroethyl-difluoromethylether, Isoflurane, Forene®, Abbott GmbH & Co. KG, Wiesbaden, Germany). As soon as the animal failed to respond to a tail pinch, it was transected caudal to the diaphragm and transferred into an ice-cooled chamber filled with ACSF (mM: 1.25$MgSO_4 7HO_2$, 1.25KH_2PO_4, 3 KCL, 125 NaCL, 25 $NaHCO_3$, 2.5 $CaCl_2 \cdot 2H_2O$, D-glucose 10). The animal was decerebrated at the pre-collicular level functionally preserving the brainstem including the periaqueductal gray (Farmer et al., 2014) and the skull was opened. The forebrain was entirely removed by suction, leaving intact solely the olfactory bulb, small adjacent fragments of the piriform cortex and the brainstem (Pérez de los Cobos Pallarés et al., 2015). After removing the lungs, the phrenic nerve was isolated on the right hand side and was cut at the level of the diaphragm to subsequently record respiratory (inspiratory) activity (Paton, 1996). To prevent mechanical and electrical artifacts, the heart was removed after ligation of the aortic arch. Next, the preparation was transferred to the recording chamber, and the descending aorta was cannulated to perfuse (peristaltic pump: Watson-Marlow 520S, Massachusetts, USA) the preparation with ACSF containing 1.25% Ficoll PM70 (Sigma), to provide oncotic pressure during experiments. Flow rates were adjusted according to the age of the animal and perfusion pressure was maintained at 50–70 mmHg (Paton, 1996). The perfusate was continuously gassed with carbogen (95% O_2, 5% CO_2) and warmed to a temperature of 30 °C. The phrenic nerve was aspirated with a suction electrode. While the olfactory bulb was oxygenated via the ophthalmic artery, the brainstem was oxygenated via the basilar artery (for details see Pérez de los Cobos Pallarés et al., 2015). Both arteries were simultaneously perfused via the cannulated aorta.

After a few minutes of perfusion, respiratory movements appeared, and spontaneous rhythmic activity in the phrenic nerve was observed. The neuromuscular blocker vecuronium bromide (Sigma; 0.3 $\mu g\,ml^{-1}$) was added to the perfusate to prevent movement artifacts.

2.3. Odor application

Preparations (n = 12) were stimulated randomly with several non-diluted fragrant oils (menthol, JHP Rödler, Ulm, Germany; lavender and rose, TAOASIS GmbH, Bielefeld, Germany) using a four-channel computer controlled olfactometer, which produced a constant airflow of 70 cc/min (Knosys Olfactometers, Florida). Exposure of the olfactory epithelium to odorants (15–30 s) was achieved via cannulation of the nasal cavities, using a custom-made set of small adaptors for the nostrils (1–3 mm diameter range).

2.4. Recording phrenic nerve activity and field potentials and multi-units in the olfactory bulb

The phrenic nerve activity (PNA) was recorded via suction electrodes (DP-311 Differential Amplifier Warner Instruments, Connecticut, USA), digitized and displayed via a Powerlab 26 T data acquisition device (ADInstruments, Australia) in all experiments. For recording of olfactory bulb activity (n = 12 preparations) we used glass microelectrodes filled with 2 M NaCl. For field potential recordings, the electrode tips were adjusted to an electrical resistance of 0.5–2 MΩ. Recordings were usually performed in the deeper layers of the dorsal olfactory bulb, well below the glomerular layer. Phrenic nerve and olfactory bulb activity were sampled at 1 kHz, amplified and filtered (low pass 10 kHz; high pass 300 Hz).

In another subset of experiments (n = 5 preparations), we recorded vagal nerve activity (VNA) to assess trigeminal odor-evoked respiratory changes in laryngeal motor activity (inspiratory abductor activity and postinspiratory abductor activity, see Dutschmann and Paton, 2002c). Integration of PNA, VNA and olfac-