An arterially perfused nose-olfactory bulb preparation of the rat

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Pérez de los Cobos Pallarés F, Stanić D, Farmer D, Dutschmann M, Egger V. An arterially perfused nose-olfactory bulb preparation of the rat. J Neurophysiol 114: 2033–2042, 2015. First published June 24, 2015; doi:10.1152/jn.01048.2014.—A main feature of the mammalian olfactory bulb network is the presence of various rhythmic activities, in particular, gamma, beta, and theta oscillations, with the latter coupled to the respiratory rhythm. Interactions between those oscillations as well as the spatial distribution of network activation are likely to determine olfactory coding. Here, we describe a novel semi-intact perfused nose-olfactory bulb-brain stem preparation in rats with both a preserved olfactory epithelium and brain stem, which could be particularly suitable for the study of oscillatory activity and spatial odor mapping within the olfactory bulb, in particular, in hitherto inaccessible locations. In the perfused olfactory bulb, we observed robust spontaneous oscillations, mostly in the theta range. Odor application resulted in an increase in oscillatory power in higher frequency ranges, stimulus-locked local field potentials, and excitation or inhibition of individual bulbular neurons, similar to odor responses reported from in vivo recordings. Thus our method constitutes the first viable in situ preparation of a mammalian system that uses airborne odor stimuli and preserves these characteristic features of odor processing. This preparation will allow the use of highly invasive experimental procedures and the application of techniques such as patch-clamp recording, high-resolution imaging, and optogenetics within the entire olfactory bulb.

A main feature of these networks is the presence of oscillatory activity, in both the fast gamma and slow theta range, with the latter known to be coupled to the respiratory rhythm (e.g., Adrian 1950; Bressler 1987; Buonviso et al. 1992; Chaput and Holley 1980; Freeman and Schneider 1982; Macrides and Chorover 1972; Margrie et al. 2001; Onoda and Mori 1980; Rubin and Katz 1999; Sporns and Grinvald 2002). Whereas the origin of theta oscillations is more associated with the glomerular layer network (e.g., Fukunaga et al. 2014; Schoppa and Westbrook 2001; Wachowiak and Shipley 2006), faster oscillations emerge from local dendrodendritic interactions between mitral cells and granule cells in the case of gamma oscillations (Fukunaga et al. 2014; Lagier et al. 2004; Schoppa 2006). Top-down inputs to the external plexiform layer drive beta oscillations (Chabaud et al. 2000; Fourcaud-Trocmé et al. 2014; Neville and Haberly 2003). During odor sensing, slow and fast oscillations can interact within individual mitral cells, a phenomenon called theta-gamma coupling that also occurs in hippocampal networks (Buzsáki and Draguhn 2004; Lisan 2005). This interaction governs the spiking activity of mitral cells and is thought to be crucial for olfactory coding (e.g., Cang and Isaacson 2003; Margrie and Schaefler 2003).

These mechanisms of network odor processing are difficult to investigate in vivo due to movement artifacts, limitation of access to the dorsal side of the olfactory bulb, and interference with anesthesia (e.g., Kato et al. 2012; Li et al. 2012). To address these issues, in situ isolated and perfused whole brain preparations have been used since early in the 1980s, when whole brain preparations of guinea pigs and frogs were developed to study olfactory networks (Alonso et al. 1990; de Curtis et al. 1991; Delaney and Hall 1996; Ishikawa et al. 2007; Llinás et al. 1981; Mühlethaler et al. 1993). Because of ethical issues associated with whole brain approaches in vitro as well as experimental limitations (e.g., tissue hypoxia), such preparations did not become popular. Alternative in vitro techniques such as acute brain slices offer only limited possibilities to investigate oscillatory activity due to the severe reduction of network connectivity and of course do not allow for physiological stimulation. Oscillations in bulb slices are dampened after a few cycles when triggered by electrical stimulation of the sensory afferents (Carlson et al. 2000; Friedman and Strowbridge 2003; Lagier et al. 2004; Schoppa and Westbrook 2001) or require pharmacological stimulation such as perfusion with NMDA to persist (Schoppa and Westbrook 2001).

Here, we present a novel in situ technique that circumvents these problems. Our method is based on established techniques for studying autonomic brain-stem function (Paton 1996). We demonstrate that in this preparation the olfactory bulb can be

IN MAMMALS, ODORANTS INTERACT with receptors on the cilia of olfactory sensory neurons located in the olfactory epithelium. All olfactory sensory neurons that express the same olfactory receptor have axonal projections onto one or several specific glomeruli in the olfactory bulb (Mombaerts et al. 1996). Here, the axons connect directly or disynaptically to glutamatergic mitral and tufted cells (Gire et al. 2012; Najac et al. 2011) for which axons in turn project into higher structures such as the olfactory cortex. The first steps of odor processing in the olfactory bulb are performed within at least two elaborate networks that involve complex synaptic interactions between 1) mitral and tufted cell apical dendritic tufts and diverse subtypes of periglomerular neurons within the glomerular layer and 2) mitral and tufted cell lateral dendrites with inhibitory granule cells within the external plexiform layer (Aungst et al. 2003; Shepherd and Greer 1990).

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reliably perfused via the ophthalmic artery and thus kept viable, allowing for detection of odor responses and oscillatory activity. Potential applications of the preparation include investigations of rhythmic neuronal activity and interactions between odor sensing and respiration. Odor mapping within a large spatial range is also possible due to the accessibility of wide parts of the bulb surface. Since the preparation is decerebrated, there are fewer ethical concerns compared with in vivo or whole brain approaches. This technique thus complements established in vivo and in vitro methods.

METHODS

All experiments were approved in accordance with the stipulations of the German law governing animal welfare (Tierschutzgesetz). Figure 1A shows a schematic representation of the preparation and experimental setup.

The perfused olfactory bulb-brain stem preparation. As described previously (Paton 1996), juvenile Wistar rats (postnatal days 17–21) were deeply anesthetized with isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether; Abbott, Wiesbaden, Germany). As soon as the animal failed to respond to tail pinch, it was transected caudal to the diaphragm and transferred into an ice-cooled chamber filled with artificial cerebrospinal fluid (ACSF; in mM: 1.25 MgSO$_4$·7HO$_2$, 1.25 KH$_2$PO$_4$, 3 KCl, 125 NaCl, 25 NaHCO$_3$, 2.5 CaCl$_2$·2H$_2$O, 10 d-glucose). The lower body was discarded. The animal was decerebrated at the precollicular level, and the skull was opened. The forebrain was entirely removed by suction, leaving intact solely the olfactory bulb. To ensure the anatomic integrity of the olfactory bulb, remaining small adjacent fragments of the piriform cortex and the brain stem were left untouched. After removing both lungs, the dorsal left phrenic nerve was isolated from the pericardial sac and cut at the level of the diaphragm to record subsequently the respiratory (inspiratory) activity (Paton 1996). To prevent mechanical and electrical artifacts, the heart was removed after previous ligation of the major ascending arteries with suture (for details, see Dutschmann et al. 2009). The descending aorta was then isolated from the spinal cord. Next, the preparation was transferred to the recording chamber, and the descending aorta was cannulated (ø 0.8/3 mm; B. Braun Melsungen). The preparation was perfused via the aorta with ACSF containing 1.25% Ficoll PM 70 (sucrose-polymer; Sigma) to provide oncotic...
The perfusate was continuously gassed with carbogen (95% O2-5% CO2) and warmed to a temperature of 30°C. To ensure optimal perfusion of the brain stem and olfactory bulb, major arteries and veins were ligated with suture as they enter or exit the heart. The phrenic nerve was aspirated with a suction electrode. Whereas the olfactory bulb was oxygenated via the ophthalmic artery, the brain stem was oxygenated via the basilar artery, both via cannulation of the aorta (see Fig. 1, A and B). Adjacent fragments of the piriform cortex are likely to have suffered from hypoxia since the branches of the middle brain artery supplying the anterior part of the piriform cortex (the corticostriate artery) were also removed by suction (Scremin 2004). Thus any cortical feedback to the olfactory bulb is highly unlikely to have occurred.

After a few minutes of perfusion, respiratory movements appeared, and spontaneous, rhythmic activity in the phrenic nerve was observed. The neuromuscular blocker vecuronium bromide (0.3 μg/ml; Sigma) was added to the perfusate to prevent movement artifacts. The viability of the preparation was determined by observation of a pattern of phrenic nerve discharge resembling eupnoea (Dutschmann et al. 2000; Paton 1996; see below).

**Odor application.** Preparations were stimulated with several non-diluted fragrant oils (menthol, JHP Rödder, Ulm, Germany; lavender and rose, TAOASIS, Bielefeld, Germany) using a four-channel, computer-controlled olfactometer that produced a constant forward airflow of 70 ml/min during each stimulus delivery only and otherwise applied backward suction with the same (then negative) pressure (KNOSYS Olfactometers). Exposure of the olfactory epithelium to odorants was achieved via cannulation of nasal cavities using a custom-made set of small adaptors for the nostrils (1- to 3-mm diameter range). The duration of odor stimulation was variable (5–40 s).

**Recording phrenic nerve activity, local field potentials, and multunits in the olfactory bulb.** The phrenic nerve activity (PNA) was recorded via suction electrodes with a silver wire (ESW-F20P; Warner Instruments), digitized, and displayed via a PowerLab 26T data acquisition device (ADInstruments). For recording of olfactory bulb activity, we used glass microelectrodes filled with 2 M NaCl. For local field potential (LFP) recordings, the electrode tips were adjusted to an electrical resistance of 0.5–2 MΩ. For multunit recordings, the electrode resistance was 6–10 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 was sampled at 10 kHz, amplified, and band-pass filtered (PNA: low pass 5 kHz, high pass 100 Hz; olfactory bulb activity: low pass 3 kHz, high pass 300 Hz; DP-311 Differential Amplifier; Warner Instruments).

**Data analysis.** From the analysis and offline filtering of raw data, we used LabChart software (version 7.2; ADInstruments). Integration of PNA and olfactory bulb activity was performed with a decay time constant of 100 ms. For Fourier analysis of the frequency domains of spontaneous olfactory bulb oscillations, we used IGOR software (WaveMetrics). From each preparation, LFP data were filtered and analyzed within a randomly selected time window of 50–60 s. If the resulting peaks in the Fourier transform of an interval were at least twofold in amplitude above background level, the power amplitudes were measured and normalized to the largest peak, resulting in a set of main frequencies and their relative powers. These frequency peak amplitudes were measured and normalized to the largest peak, resulting in a set of main frequencies and their relative powers. These frequency peak amplitudes were measured and normalized to the largest peak, resulting in a set of main frequencies and their relative powers. These frequency peak amplitudes were measured and normalized to the largest peak, resulting in a set of main frequencies and their relative powers. These frequency peak amplitudes were measured and normalized to the largest peak, resulting in a set of main frequencies and their relative powers.
data sets from each preparation were then collated and graphed (Fig. 2C). We also performed stationarity analysis to determine the variability of peak frequencies within each experiment using time windows of 20 s, randomly selected from a recording period of 15 min (Fig. 2D).

To perform time-frequency analysis, we also used IGOR software. Sonograms were plotted from filtered raw data using the Gabor method. A spectrum was generated from every 10 data points with a frequency resolution of 10 Hz. The resulting sonogram was optimized using the IGOR Image Processor.

Spike sorting was performed using ADInstruments software (LabChart 7.4, additional module Spike Histogram; Fig. 5). Raw data were filtered using a median filter with a box width of 11 points. All spikes with amplitudes above a threshold of 0.25 mV from baseline were included for analysis. Detected action potentials were associated with putative individual neurons depending on their overall shape parameters, including slope, amplitude, and width.

RESULTS

In the present study, we demonstrate that the olfactory bulb of the decorticated and precollicularly decerebrated preparation (Fig. 1A) can be arterially perfused via the descending aorta and the carotid arteries that branch into the ophthalmic artery (Fig. 1B). The ophthalmic artery provides the main blood supply for the olfactory bulb (Green 1955). Thus the anatomic integrity of olfactory axons innervating the perfused olfactory bulb allows for studies of odor processing in situ.

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**Fig. 3.** Intertrial stability of odor-evoked LFPs in the olfactory bulb in situ. Evoked LFPs on stimulation of the olfactory epithelium with menthol and lavender from 2 different preparations at different time points as indicated on the very left side (in minutes). The upper traces show the ongoing integrated PNA during odor exposure, and the lower traces the simultaneously recorded integrated olfactory bulb field potentials. The duration of odor stimulation is indicated by the horizontal bars above the traces. Note that the duration of stimulation was different across recordings.
General methodology. After the surgical procedures, the olfactory bulb-brain stem preparation was reperfused, and perfusion pressure and flow rates were adjusted to generate an eupnea-like discharge pattern (ramping discharge of 1-s duration) of PNA at a frequency range of 13–20 bursts per minute (average rate 16 ± 3 bursts per minute, n = 9 preparations). This activity indicates the successful resuscitation of the respiratory pattern generator and shows that the brain-stem circuitry is sufficiently perfused (Dutschmann et al. 2000; Paton 1996; Wilson et al. 2001). LFP recordings obtained from deeper layers of the olfactory bulb 10–15 min after successful resuscitation of the brain-stem circuits showed negligible spontaneous oscillatory or tonic activity (Fig. 1C). However, a profound increase in activity levels was observed after 1–2 extended exposures (duration 10–20 s) to olfactometer output containing any type of odor from our odor set (menthol, rose, and lavender) or plain room air.

In some preparations, the initial adjustment of brain-stem perfusion failed, and thus the rhythmic PNA could not be recorded. Stimulus-locked LFPs in the olfactory bulb could still be evoked by exposure to odor in these preparations (n = 12 preparations, data not shown). However, none of these preparations showed the increase in spontaneous activity after sensory stimulation described above. Moreover, none of them showed the spontaneous oscillations in the theta range described below. These observations suggest that the restoration of spontaneous, rhythmic PNA is a key indicator of proper network function also within the perfused olfactory bulb preparation.

Spontaneous oscillatory activity of the olfactory bulb in situ. Spontaneous oscillatory activity in the perfused olfactory bulb in situ was investigated in 10 preparations, with the LFP recordings located at least as deep as the mitral cell layer. We consistently observed ongoing spontaneous oscillations (Fig. 2, A and B). Fourier analysis of the frequency range of oscillatory olfactory bulb activity (Fig. 2, B and C; see METHODS) revealed distinct peaks between 4 and 10 Hz in all of these preparations except for 1; in 3 preparations, there were also pronounced spontaneous oscillations at higher frequencies (20–60 Hz). The stability of theta oscillations was evident from stationarity analysis of the oscillatory activity at different time intervals within the same preparation that showed closely overlapping peaks in the Fourier analysis (Fig. 2D). Changes in perfusate temperature from 30°C up to physiological levels (36°C) or down to 25°C caused increases and decreases in the frequencies of both PNA and olfactory bulb theta activity, respectively (n = 3 preparations, data not shown) but did not suppress oscillatory activity.

Odor-evoked LFPs and oscillatory activity. LFP recordings in the deeper layers of the perfused olfactory bulb revealed robust responses to menthol, lavender, and rose oil applied via a four-channel, computer-controlled olfactometer. In n = 21 preparations, odor application triggered stimulus-locked and reproducible LFPs, as exemplified in Fig. 3. In total, we recorded n = 189 evoked LFP responses (menthol n = 108; lavender n = 41; roses n = 40).

The probability of obtaining an odor-evoked response in a given preparation with functional PNA was 76% (evaluated across n = 10 preparations).

The recordings shown in Fig. 3 also demonstrate that odor responses at a given location were not only reproducible from one recording to the next, but also stable over an extended period of time (40 min). In some preparations, odor responses were detectable for up to 4 h (data not shown).

In accordance with the literature (Buonviso et al. 2003; Martin and Ravel 2014), spectral analysis of LFPs in the perfused olfactory bulb showed a marked increase in power at frequencies between 1 and 100 Hz during odor stimulation compared with baseline (example shown in Fig. 4, A and B), which was observed for each of 12 analyzed odor responses (from n = 10 different preparations).

Odor-evoked single-cell responses. Multiunit activity (MUA) recordings were obtained at the level of the mitral cell layer or below (depth ≥ 500 μm) and thus most likely reflect mitral

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\begin{align*}
\text{LFP} & \quad \text{OB-LFP} \\
\text{JPNA} & \quad \text{PNA} \\
\text{LAVENDER} & \quad \text{ROSE}
\end{align*}
\]

Fig. 4. Odor-evoked responses exhibit oscillatory activity at higher frequencies. A: representative experiment. Odor stimulation triggering responses in the olfactory bulb show oscillatory activity in the beta and gamma frequency ranges. B: sonogram calculated from A. Below, the Fourier analyses for the intervals before stimulation (1), during odor stimulation (2), and afterward (3) are shown.
and/or granule cell activity. Responses to odor stimulation could be reliably detected in most preparations tested \((n = 122\) responses in 10 preparations). The MUA recordings clearly displayed different response types of individual neurons to odor application. Most MUA recordings revealed a strong stimulus-locked excitation of neurons that were silent during baseline \((n = 84\) recordings; see Fig. 5, A and B), whereas other tonically active units showed stimulus-dependent inhibition \((n = 38\) recordings; see Fig. 5C). Both types of responses were observed for all odorants. Spike sorting analysis also revealed OFF responses of individual neurons in one of these recording sites with an onset right after odor application and a duration of a few seconds (Fig. 5B). Other neurons became tonically active after or during odor stimulation (e.g., Fig. 5A).

**Control experiments: trigeminal stimulation and room air stimulation.** Previous studies showed that stimulation of trigeminal afferents (i.e., the ethmoidal nerve) of the nasal mucosa can trigger profound reflex responses such as the

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![Fig. 5. Multunit recordings of evoked olfactory bulb neuron responses. MUA recordings were obtained for various odorants (menthol, lavender, and roses); data shown are from 2 different preparations. Please note the transient response of PNA to the trigeminal odor menthol in A. A and B: evoked excitatory responses of neurons that were silent before stimulation; responses to both odorants in the same location. The recordings show excitation of several individual neurons. In A, a unit with small action potential amplitude remained active after menthol exposure. B: after the end of exposure to lavender, a unit became spontaneously active for \(-5\) s. This could also be explained as an OFF response (see DISCUSSION). Repetitive odor stimulation yielded similar responses at this site \((n = 9)\). C: evoked inhibition of neuronal activity at a different recording site. Note that the dominant tonically firing neurons showed clear odor-evoked inhibition. The low amplitude activity detectable in both PNA and MUA reflects activity from the atria of the heart that was not entirely silenced in this experiment. D: identified different neurons are involved in odor-evoked (ON) and OFF responses. a: Spike sorting analysis (see METHODS) of the odor response shown in A yields 4 different active units. b: Two putative units were involved in the odor response shown in B. After odor stimulation, 2 different neurons were activated for a limited time, corresponding to an OFF response. c: Units that were active during baseline in C (PRE) were inhibited during odor presentation (ROSES). After odor presentation (POST), those units were active again. Two different putative units became active during odor presentation.

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dive response in the perfused brain-stem preparation (Dutschmann and Paton 2002a,b). The integrity of the sensory trigeminal pathways allowed us to conduct control experiments to prove the odor selectivity of the olfactory bulb-brain stem preparation. Irrigation of the nasal mucosa with cold saline in \( n = 7 \) preparations triggered a reflex apnea characteristic of the dive response (Fig. 6A; see Dutschmann and Paton 2002a for details). This stimulus never evoked any response in LFP recordings from the perfused olfactory bulb.

Another important test for odor specificity of the responses was the application of room air via the olfactometer, which never evoked any marked LFP responses in the deeper olfactory bulb, i.e., comparable with the odor responses shown in Fig. 3 (Fig. 6B; \( n = 64 \) tests, \( n = 16 \) preparations). Thus this test also excludes mechanical recording artifacts due to the switching of flow direction through the nostrils. However, the application of room air was observed to cause a slight increase in the power of the spontaneous theta oscillations described above (in 4 out of 5 tested preparations).

**DISCUSSION**

In situ perfused olfactory bulb-brain stem preparation. The olfactory bulb-brain stem preparation is an adaptation of the working heart-brain stem preparation of rat (see Dutschmann et al. 2000; Paton 1996; Paton et al. 1999). This specialized technique was originally designed to study autonomic brainstem function in situ, e.g., autonomic reflexes, mechanisms of sympathetic-respiratory coupling, and hierarchical organization of the central pattern generator for breathing. In the current study, respiratory activity generated by the brain-stem circuits is not coupled to olfactory bulb activity because of the precollicular decerebration. However, it remains an important indicator of the viability of the preparation itself. An active respiratory network implies that the perfusion pressure and flow are sufficient to ensure the oxygenation of the olfactory bulb. This type of monitoring allowed us to maintain a viable olfactory bulb preparation for up to 4 h.

Similar to previous observations in the respiratory brainstem preparation (Dutschmann et al. 2000), we observed that the bulb network required prolonged sensory input to trigger its reactivation. We speculate that this “rebooting” may be necessary because the olfactory network activity collapsed in the absence of rhythmic airflow or other sensory stimulation during the early stages of the preparation. Interestingly, both delivery of air and odorant were sufficient for rebooting, although delivery of air alone did not result in marked LFPs. However, there was a slight increase in theta power during delivery of air. This observation suggests that there is a pathway providing weak input on stimulation with air that contributes to the reactivation of bulb networks, perhaps mediated by mechanoreceptive stimulation of olfactory sensory neurons due to the positive pressure exerted by the airflow ( Grosmaître et al. 2007) or by olfactory stimulation with low concentrations of odorants present in the room air. In any case, we observed that this reactivation is an important prerequisite for proper network function.

Oscillatory activity. In the perfused olfactory bulb preparation, we have consistently observed spontaneous LFP oscillations within a frequency band of \( 4 - 10 \) Hz, corresponding to the theta range. Almost all analyzed recordings showed a major oscillatory component \( \sim 4 \) Hz, whereas a smaller subset of preparations also displayed faster frequency components within beta and gamma bands. Thus the perfused olfactory bulb maintains oscillatory network activity, similar to what has been reported from in vivo recordings (for review, see Kay 2014) and from other semi-intact approaches in other species (Delaney and Hall 1996; Ishikawa et al. 2007).

Hippocampal theta oscillations can synchronize with the olfactory bulb oscillations, most likely via respiration coupling mediated by septal neurons (Tsanov et al. 2014) and could be required for forms of odor learning (e.g., Colgin 2013; Kay 2005; Macrides et al. 1982). Since the air-perfused olfactory bulb preparation lacks central and respiration-coupled inputs, we conclude that the olfactory bulb network itself generates intrinsic theta oscillations that can couple to external inputs (such as respiration and hippocampal activity) as has been also found by others (e.g., Fukunaga et al. 2014; Schoppa and Westbrook 2001; Sobel and Tank 1993). Thus the in situ preparation could be used to verify whether bulbar intrinsic oscillations may contribute to odor-induced bulbar plasticity in the absence of the hippocampus. Moreover, the specific contribution of slow bulbar conductances to the intrinsic theta rhythm generation can now be explored in detail; for example, we have proposed that the activation of \( T \)-type voltage-dependent \( Ca^{2+} \) channels, NMDA receptors, TRPC channels, and their interactions might allow olfactory bulb granule cells to couple to ongoing theta activity (Egger et al. 2003, 2005; Egger 2008; Stroh et al. 2012).

Odor-evoked responses. Despite the lack of information on glomerular mapping and the small odor set in our experiments, there was a high probability for the detection of odor-evoked responses, probably due to the use of undiluted essential oils (corresponding to mixtures of various components), which cause widespread activation of bulbar networks (e.g., Meister and Bonhoeffer 2001). Using multunit recordings from presumptive mitral and granule cells in the perfused olfactory

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**Fig. 6. Representative control experiments for odor specificity. A:** trigeminal stimulation of the nasal mucosa with water did not evoke any LFP in the olfactory bulb but triggered the anticipated protective diving breath hold illustrated by the transient cessation of PNA. **B:** application of room air did not elicit OB-LFP and had no obvious effect on PNA.
bulb, we have demonstrated persistent excitatory as well as inhibitory responses to stimulation with essential oils (see Fig. 5). These basic response types are also known from in vivo recordings of responses to both olfactory stimuli and optogenetically activated glomeruli (e.g., Bathellier et al. 2008; Buonviso et al. 1992; Cang and Isaacson 2003; Davison and Katz 2007; Haddad et al. 2013; Meredith 1986). Some neurons became tonically active following odor stimulation, whereas others showed a bout of increased activity immediately after odor stimulation (Fig. 5B). The latter effect might be explained by mitral cell and/or granule cell OFF responses (Debarbieux et al. 2003; Kato et al. 2012).

In addition, we have consistently observed an increase in oscillatory power in higher frequency bands during LFP odor responses (Fig. 4). This finding is well in line with observations in vivo, where stimulus-locked oscillations in the gamma range have been associated with dendrodendritic interactions between mitral and granule cells during odor processing (e.g., Eckman and Freeman 1990; Fukunaga et al. 2014; Mori et al. 1977; Nusser et al. 2001). The presence of both spontaneous slow and evoked fast oscillations therefore proves that intrinsic bulbar neuronal network interactions within the perfused preparation are preserved. Since beta oscillations are of central origin (Chabaud et al. 2000; Fourcaud-Trocmé et al. 2014; Neville and Haberly 2003), it remains to be elucidated whether any faster oscillations observed here belong to the gamma type.

Taken together, the proofs of principle provided in our study show that the bulbar neuronal networks for initial odor processing are most likely fully functional in situ. Thus the in situ perfused olfactory bulb provides a solid experimental platform for the investigation of fundamental mechanisms of odor processing in the absence of central inputs. This preparation might thus help to dissect out the contribution of purely local odor processing by the olfactory bulb from central influences (e.g., Boyd et al. 2012; Rothermel and Wachowiak 2014) to odor representations.

Perspectives. The observed stability of odor-evoked LFP responses in situ over an extended time is in line with proper, constant oxygenation of the olfactory bulb and thus with sufficient perfusion. Thus the perfused preparation is suitable for the application of pharmacological and other time-consuming experimental paradigms in studies of olfactory processing.

In patch-clamp studies of cardiorespiratory function, various different spatial access routes were realized via a dorsal, lateral, or ventral exposure of the perfused brain stem preparation (Dutschmann and Paton 2003; Moraes et al. 2014b; Paton et al. 1999). Arterially perfused preparations were also demonstrated to facilitate the use of optical technologies such as voltage-sensitive dye imaging (Potts and Paton 2006) and optogenetics (Moraes et al. 2014a). Likewise, the perfused olfactory bulb preparation will allow for highly invasive approaches to investigate the network function of olfactory processing within the entire olfactory bulb. For example, a hemi-head preparation of the mouse was used to explore mirror mapping in the olfactory bulb and olfactory sensory axonal responses to glomerular odor application (Lodovichi et al. 2003; Maritan et al. 2009) but so far without implementation of direct nasal olfactory stimulation.

Moreover, the perfused preparation might also be extended to neonatal rats, since they have a functional olfactory system (Wilson and Sullivan 1994), as well as to older animals (Paton 1996) and thus could be useful for studies of sensory development.

In the current study, slow bulbar oscillations were generated in the absence of respiratory-like airflow. However, a more physiological stimulation technique could be realized via the “sniff playback” device developed by Cheung et al. (2009). In any case, the presence of both spontaneous slow theta oscillations and fast cellular responses in the mitral and granule cell layer of the olfactory bulb will enable investigations of neuronal synchronization to ongoing rhythmic activity, a widely studied topic in various brain areas.

In conclusion, the perfused olfactory bulb preparation is a valuable and complementary tool to decipher mechanisms of spatiotemporal olfactory coding further.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

F.P.d.l.C.P., D.S., D.F., M.D., and V.E. conception and design of research; F.P.d.l.C.P. and M.D. performed experiments; F.P.d.l.C.P., D.S., and V.E. analyzed data; F.P.d.l.C.P., M.D., and V.E. interpreted results of experiments; F.P.d.l.C.P., M.D., and V.E. prepared figures; F.P.d.l.C.P., M.D., and V.E. drafted manuscript; F.P.d.l.C.P., D.F., M.D., and V.E. edited and revised manuscript; F.P.d.l.C.P., M.D., and V.E. approved final version of manuscript.

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