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Olfactory bulb drives respiration-coupled beta oscillations in the rat hippocampus

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Abstract

The synchronization of neuronal oscillations has been suggested as a mechanism to coordinate information flow between distant brain regions. In particular, the olfactory bulb (OB) and the hippocampus (HPC) have been shown to exhibit oscillations in the beta frequency range (10-20 Hz) that are likely to support communication between these

structures. Here we further characterize features of beta oscillations in OB and HPC of rats anesthetized with urethane. We find that beta oscillations simultaneously appear in HPC and OB and phase-lock across structures. Moreover, Granger causality analysis reveals that OB beta activity drives HPC beta. The laminar voltage profile of beta in HPC shows the maximum amplitude in the dentate gyrus, spatially coinciding with olfactory inputs to this region. Finally, we also find that the respiratory cycle and respiration-coupled field potential rhythms (1-2 Hz) - but not theta oscillations (3-5 Hz) - modulate beta amplitude in OB and HPC. In all, our results support the hypothesis that beta activity mediates the communication between olfactory and hippocampal circuits in the rodent brain.

Introduction

The rodent hippocampus (HPC) exhibits local field potential (LFP) oscillations as result of synchronized activity of its neuronal populations (Buzsáki & Draguhn, 2004). The hippocampal subregions display oscillations of different frequencies, reflecting layer-specific synaptic inputs (Scheffer-Teixeira *et al.*, 2012; Lasztóczi & Klausberger, 2014; Schomburg *et al.*, 2014). Sensorial information reaches CA1 and dentate gyrus (DG) by means of inputs from the entorhinal cortex (Steward & Scoville, 1976; Burwell *et al.*, 1995). In particular, the entorhinal cortex receives monosynaptic inputs from the olfactory bulb (OB) and the olfactory cortex (Beckstead, 1978; Wilson & Steward, 1978; Schwerdtfeger *et al.*, 1990). Like the HPC, olfactory brain regions exhibit prominent LFP oscillations (Kay *et al.*, 2009). Interestingly, these oscillations can simultaneously occur in the olfactory system and HPC, thus suggesting that they may be involved in olfacto-hippocampal communication (Macrides *et al.*, 1982; Kay, 2005; Martin *et al.*, 2007; Lockmann *et al.*, 2016).

LFP oscillations in the beta frequency range have been described in the HPC and olfactory areas of anesthetized animals. Adrian first reported that the OB and olfactory cortex of anesthetized hedgehogs and cats could exhibit beta activity. Namely, odor stimulation with amyl acetate during urethane anesthesia induced prominent beta oscillations (Adrian, 1950), though beta also appeared spontaneously in animals under nembutal anesthesia in the absence of odorants (Adrian, 1942). Moreover, a subsequent study by Maclean *et al.* (1952) showed that non-olfactory stimuli - such as salt crystals to the tongue or ear pinching – could induce beta oscillations in the olfactory cortex of nembutal-anesthetized rabbits. Importantly, this latter study demonstrated that beta oscillations occurred in olfactory areas and HPC simultaneously. Beta oscillations were also shown to co-occur in the HPC and OB of urethane-anesthetized rats in response to predator's odors or organic solvents (Heale & Vanderwolf, 1994).

While these early studies in anesthetized animals convincingly demonstrated that beta oscillations may simultaneously occur in olfactory areas and HPC, several features of olfacto-hippocampal beta activity remain unexplored. For instance, none of the previous studies assessed the causal relationship between OB and hippocampal beta oscillations under anesthesia, nor their degree of phase coupling. Additionally, olfacto-hippocampal circuits exhibit three types of low-frequency rhythms: a slow oscillation (SO) coupled to the neocortex, theta oscillations, and a respiration-coupled rhythm (RR) (Wolansky *et al.*, 2006; Viczko *et al.*, 2014; Yanovsky *et al.*, 2014; Lockmann *et al.*, 2016). But whether these low-frequency rhythms modulate the amplitude of beta oscillations remains unknown. Addressing these gaps by modern analysis tools should generate new insights into the network mechanisms underlying beta activity under anesthesia; it should also serve as a ground for future comparisons with beta activity in wake animals (Martin *et al.*, 2007; Gourévitch *et al.*, 2010).

Here we recorded LFPs from OB and HPC of rats **anesthetized with urethane (i.p. injection)**. In both brain areas, spontaneous beta oscillations (10-20 Hz) emerged during the course of anesthesia **while animals breathed room air**. Beta oscillations in OB and HPC occurred simultaneously and had the same peak frequency. Hippocampal beta activity was phase-coupled to OB beta and moreover was driven by it. Voltage depth profile of beta oscillations revealed maximum amplitude in the DG, coinciding with olfactory inputs to the HPC. By simultaneously recording nasal respiration and LFPs, we could demonstrate that respiration phase modulates beta amplitude in OB and HPC. Furthermore, respiration-coupled neural rhythms in OB and HPC also modulated the amplitude of local beta oscillations, while the hippocampal theta rhythm modulated gamma but not beta oscillations. Our results support earlier studies suggesting that beta activity in the rodent brain mediates the communication between olfactory and hippocampal circuits.

Materials and Methods

Ethics statement

All experimental procedures were approved by the Ethical Committee for Animal Experimentation of the Federal University of Rio Grande do Norte (CEUA-UFRN), protocol number 044/2014. The CEUA-UFRN directives are in compliance with the Brazilian federal law for animal experimentation.

Subjects

Experiments were performed in 6 male Wistar rats weighing 300-420 g provided by the animal facilities of the Brain Institute of the Federal University of Rio Grande do Norte. The animals were kept on a 12-h light/dark cycle with food and water available *ad libitum*. Data from these same animals have been analyzed in a previous study (Lockmann *et al.*, 2016).

Surgical procedures

Subjects were anesthetized with intra-peritoneal injections of 500 mg/mL urethane solution (U2500 SIGMA, dissolved in saline). The total dose of 1.5 g/kg was administered by three injections separated by 20 minutes. Rectal temperature was monitored with a thermometer and maintained at 37-38ºC by a thermal pad. Anesthesia induction was certified by the absence of withdrawal reflex to hind paw pinching. Supplemental urethane doses of 0.3 g/kg were administered as needed. Before surgery, 0.5 mL of lidocaine clorhydrate 2% was subcutaneously applied in the scalp for local anesthesia; an intramuscular injection of 0.1 mL of dexamethasone 2.5 mg/mL was also applied to prevent brain swelling. After anesthetic induction, animals were placed in a stereotaxic frame (RWD Life Science) and a scalp incision was made to expose the skull.

Multichannel linear probes (see LFP recordings) were used to record LFPs from the OB (16-channel probe implanted at AP: +8.5 mm, ML: -1.0 mm) and HPC (32-channel probe implanted at AP: -3.5 mm, ML: -2.0 mm). The deepest recording sites for OB and HPC were 1.6 mm and 4.1 mm, respectively. **Bipolar stimulation electrodes were implanted at the lateral olfactory tract (LOT) (AP: +3.0 mm, ML: -3.5 mm, DV: -6.4 mm), and fixed with dental acrylic.** All recordings were performed in the right hemisphere.

Respiration recording

An 18-gauge stainless steel cannula placed in the nasal cavity (accessed through the nasal bone) was used to record nasal air pressure. The cannula was connected to a pressure sensor (Honeywell 24PCAFA6G), and the output signal was recorded through auxiliary channels of our recording system (RZ2, Tucker Davis Technologies). Downward and upward deflections in air pressure recordings correspond respectively to inspiratory and expiratory phases of the respiratory cycle.

LFP recordings

For simultaneous multisite recordings along the dorsoventral hippocampal axis, we used 32-channel silicon probes (Neuronexus, A1x32-5mm-100-413). Recording sites were 100-µm spaced and had 28 µm of bare diameter. Signals were amplified and digitized at 24,414 Hz (PZ2 and RZ2, Tucker-Davis Technologies). No digital filters were applied during the recording. All recordings were referenced to a stainless-steel screw placed in the interparietal bone. LFPs were obtained by offline low-pass filtering (<500 Hz) and down-sampling to 1000 Hz.

Electrical micro-stimulation

Bipolar stimulation electrodes consisted of a twisted pair of Teflon-coated stainless-steel wire (7914 AM-Systems) with 1-mm distance between the tips. Electrical stimuli were generated by a current isolator (ISO-Flex, A.M.P.I.), which was set to deliver 100-µs constant **monophasic** current pulses with various amplitudes. **The positive and negative poles of the stimulator were connected, respectively, to the deepest and the most superficial wire of the bipolar electrode**. The stimulus amplitude was set as the minimum current to evoke a visible deflection in the raw signal (**amplitude** range: 100-250 µA). The stimuli were triggered by TTL pulses (RZ2, Tucker-Davis Technologies) at 0.05 Hz. The electrical stimulation protocol consisted of a train of 10 stimuli to the LOT **(see Surgical procedures for stereotaxic coordinates). Of note, monophasic electrical stimulation at this amplitude and quantity has been shown to not cause tissue damage (Piallat *et al.*, 2009).** Evoked potentials were obtained by averaging 100-ms LFP segments locked to stimulus onset. The hilus of the DG was identified as the channel with the highest positive component of the LOT-evoked potential. The hippocampal fissure was identified as the channel with the maximum theta power (Brankack *et al.*, 1993).

Perfusion and Histology

After the recordings, the animals were transcardially perfused with 0.9% sodium chloride followed by 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde. 120-µm coronal sections were made in a vibrating tissue slicer (EMS) and mounted on glass slides. Electrode tracks were visualized and photographed in a microscope equipped with epifluorescence (Zeiss); see Lockmann *et al.* (2016) for histological sections.

Data analysis

Spectral Analysis

Recorded data were analyzed offline using built-in and custom-written MATLAB codes (Mathworks). Power spectra were calculated by means of Welch's periodogram (built-in MATLAB *pwelch* function). Coherence spectra of signal pairs were computed using magnitude-squared coherence (built-in MATLAB *mscohere* function). Both power and coherence spectra calculations were carried out in 100-s data segments using 10-s Hamming windows with 95% overlap. For group results, we considered the coherence value at the beta peak frequency.

Time-frequency power decomposition was calculated by means of the built-in MATLAB *spectrogram* function, using 10-s sliding Hamming windows with 95% overlap. The mean beta band power shown in Figure 1 was computed by averaging the power in the 10-20 Hz frequency band of the spectrogram.

Autoregressive power spectrum and Granger causality analysis

Granger causality analysis measures whether the past of a time series X is useful to forecast the present of a time series Y, and is based on autoregressive modeling. In our dataset, Granger causality spectra provided frequency-specific causal effects between OB and hippocampal LFPs. To compute

autoregressive and Granger spectra, we used the MVGC MATLAB toolbox (Barnett & Seth, 2014; available online at http://users.sussex.ac.uk/~lionelb/MVGC/). The algorithm was applied to 50-second LFP periods, down-sampled from 1000 Hz to 100 Hz. **The results we report in Figure 3 were obtained with a fixed model order of 50, which was above the Akaike Information Criterion and provided a good frequency resolution. Similar results were obtained with different orders (25, 50, 100, 150 and 200).**

Laminar profile

The laminar profile is a plot of the average amplitude of an oscillation as a function of recording depth. For estimating the laminar profile of beta oscillations, we selected for each animal a 100-s period with high beta power. LFPs were filtered between 10 and 20 Hz using the *eegfilt* function (Delorme & Makeig, 2004). For each channel, the beta-triggered LFP average was obtained as follows: we first localized the timestamps associated with the peaks of the beta oscillation (built-in MATLAB *findpeaks* function); we then extracted non-overlapping 250-ms filtered LFP epochs centered on these timestamps; finally, the average beta trace was obtained by computing the mean over all 250-ms epochs. **Current source density (CSD) was estimated as the negative of the second spatial derivative of the average beta trace. This method has been firstly described by Nicholson & Freeman (1975) and applied to hippocampal recordings by Brankack *et al.* (1993). The CSD at time t and position z is defined as**

$$\boldsymbol{CSD(z,t)} \approx \frac{-X(z+h,t)+2X(z,t)-X(z-h,t)}{h^2}$$

where $\mathbf{X}(z,t)$ is the average beta trace computed for the probe contact located at z and h is the distance between contacts (100 µm). Note that the CSD is not estimated for the first and last contacts.

Cross-frequency coupling analysis

To assess cross-frequency coupling in OB and hippocampal LFPs, we used the framework previously described by Tort *et al.* (2010). Phase-amplitude plots were computed using 20° phase bins, using either respiration, HRR or theta phase. The mean amplitude in each phase bin was normalized by the sum across bins, so that amplitude values in each plot sum to 1. This procedure corrects for differences in absolute beta amplitude among animals.

The phase-amplitude modulation index (MI) quantifies the deviation of the empirical phase-amplitude distribution from the uniform distribution, which corresponds to the lack of coupling. The comodulation maps were obtained by computing the MI between multiple band-filtered frequency pairs.

Statistics

Group data are expressed as mean ± standard error mean (SEM). Statistical differences were assessed by paired t-test (built-in MATLAB *ttest* function). Correlation coefficient shown in Figure 1 was computed using MATLAB *corrcoeff* built-in function.

Results

Hippocampal beta oscillations phase-couple to and are driven by OB beta

We analyzed data from six urethane-anesthetized rats freely breathing room air. Data consisted of recordings of nasal respiration along with LFPs from the OB and HPC. In both regions, LFPs were recorded through multisite linear probes (see Materials and Methods). The dataset was part of a previously published study, in which we described different types of hippocampal slow rhythms (<5 Hz) under urethane anesthesia (Lockmann *et al.*, 2016). Here we characterized a faster rhythm in the 10-20 Hz frequency band, which we refer to as beta to

be consistent with the terminology used in previous studies (Vanderwolf & Zibrowski, 2001; Kay *et al.*, 2009). The following results were obtained from LFP periods in which the HPC exhibited prominent beta activity; for each region, the channel with the highest beta power was selected.

Figure 1A shows time-frequency power analysis of OB and HPC LFPs in an example epoch of beta activity - evident as a temporary power increase in the 10-20 Hz frequency band. Raw LFPs during such epochs displayed large voltage oscillations in the beta frequency range in OB and HPC (Fig. 1B). The time courses of beta band power in both brain regions were strikingly similar (Fig. 1A, lower panel). Consistently, the scatterplot of beta power in OB vs HPC confirmed a positive correlation between the two variables (mean correlation coefficient: 0.805 ± 0.052, n = 6 rats; Fig. 1C,D). Moreover, beta activity in both regions had the same peak frequency (Fig. 2A), ranging from 11.99 to 14.39 Hz (13.16 ± 0.39, n = 6 rats). Additionally, phase coherence spectrum between OB and HPC LFPs peaked at the same beta frequency as in the power spectrum (mean coherence: 0.63 ± 0.06; Fig. 2B,C), thus showing that OB and HPC beta oscillations are phase-locked.

We next investigated whether beta activity originates in the OB or the HPC using Granger causality analysis. To avoid causality bias due to power differences, we selected 100-s LFP epochs in which OB and HPC had similar levels of beta power. Figure 3A shows power spectra for such periods computed by autoregressive (AR) modeling. As in the Fourier spectral analysis in Figure 2A, beta in the AR spectra had the same peak frequency in OB and HPC (Fig 3A). In agreement with our selection criteria, mean AR beta power between regions showed no significant difference (Fig. 3B; n = 6 rats, $t(5) = 0.1662$, $p = 0.87$, paired t-test). Interestingly, directionality analysis revealed that OB caused beta oscillations in HPC: OB-to-HPC Granger causality spectrum had a much greater peak in the beta frequency range than

HPC-to-OB causality (Fig. 3C). Across animals, Granger causality in the beta range was significantly higher for OB-to-HPC compared to HPC-to-OB directionality (n = 6 rats, $t(5) = 3.2817$, $p = 0.02$, paired t-test; Fig. 3D).

In all, these results show that HPC and OB beta oscillations occur simultaneously, have the same peak frequency, and phase-lock. Moreover, HPC beta is driven by OB beta.

Laminar profile of hippocampal beta oscillations

The HPC receives layer-specific synaptic inputs which give rise to distinct oscillatory patterns (Scheffer-Teixeira *et al.*, 2012; Lasztóczi & Klausberger, 2014; Schomburg *et al.*, 2014). The results reported in the previous section considered only one of the 32 channels of the linear probe implanted in the HPC (the one with highest beta power). We next proceeded to analyze all probe channels in order to estimate the voltage-depth profile of beta oscillations along the hippocampal dorsoventral axis.

The OB and the olfactory cortex project to the entorhinal cortex, which is the major source of olfactory information to the HPC via the perforant pathway. Accordingly, electrical stimulation of the lateral olfactory tract (LOT) - the main axonal bundle leaving the OB - evokes a potential in HPC with the maximum amplitude at the hilus of DG (Wilson & Steward, 1978; Yanovsky *et al.*, 2014). As expected, upon LOT stimulation we found a well-defined region of high-amplitude positive evoked potentials, anatomically corresponding to the DG hilus (Fig. 4A left). We then computed the distribution of beta power across the dorsoventral axis of the HPC. Interestingly, we found that beta oscillations have a laminar profile similar to LOT evoked potentials, also exhibiting maximum power at the DG hilus (Figs. 4A right and 4D left).

To confirm that hippocampal LOT evoked potentials and beta oscillations reflected local synaptic currents - and not volume conduction from other areas -, we carried out current source density analysis (CSD, see Materials and Methods) **in the same LFP segments as analyzed in Figures 1 and 2**. CSD revealed that beta oscillations and LOT evoked potentials have current sinks and sources in the DG (Fig. 4B). Consistently, we also found that LFP beta phase reverses from the hippocampal fissure to the DG hilus (Fig. 4C), and that OB-HPC beta coherence is lowest at the fissure (Fig. 4D right).

These results suggest that beta propagates from OB to downstream areas through the LOT, and probably reaches the HPC by means of entorhinal inputs to the DG.

Nasal respiration and respiration-coupled LFP rhythms modulate the amplitude of beta oscillations

The phase of low-frequency LFP oscillations can modulate the amplitude of faster oscillations (Colgin *et al.*, 2009; Tort *et al.*, 2009). During urethane anesthesia, the rat HPC exhibits three different rhythms with peak frequencies lower than 5 Hz: theta (~ 4 Hz), slow oscillations (SO, ~0.7 Hz) coupled to the neocortical up-and-down states, and a rhythm coupled to nasal respiration (HRR, ~ 1.2 Hz) (Wolansky *et al.*, 2006; Lockmann & Belchior, 2014; Yanovsky *et al.*, 2014; Lockmann *et al.*, 2016). In our dataset, beta oscillations did not co-occur with SO, but co-occurred with HRR and theta.

The hippocampal beta described here and the previously described HRR (Yanovsky *et al.*, 2014; Lockmann *et al.*, 2016) have common features: each rhythm couples to a similar rhythm in OB and has the maximum amplitude in the DG hilus. In 5 of our 6 animals, we found epochs in which beta oscillations co-existed with respiration-coupled LFP rhythms in OB-RR and HRR (Fig 5A-C). We then investigated whether the phase of the respiratory cycle

modulates the amplitude of beta oscillations during these epochs. Noteworthy, beta activity was not continuous; rather, it emerged at regular intervals coinciding with the troughs of the nasal respiration signal. Phase-amplitude plots revealed that indeed beta amplitude coupled to a preferred phase of the respiratory cycle, both in OB and HPC (Fig. 5C left). To assess the coupling between respiration phase and other LFP frequencies, we computed comodulation maps – bidimensional representations of the modulation index (MI) for different pairs of phase and amplitude frequencies (Tort *et al.*, 2010). The comodulation maps showed that respiration phase preferentially modulates the amplitude of beta oscillations in OB and HPC (Fig. 5C middle). To assess the statistical significance of actual coupling levels, we compared them with a probability distribution of MIs obtained from 200 surrogate LFP segments (see Materials and Methods). Figure 5C shows the surrogate probability distribution of MIs along with the actual MI values; notice that the latter are greater than the chance distribution for the example LFPs. Beta-respiration coupling was significant in all animals (Fig. 5D; z-scored MI > 1.96). **Moreover, when computing average LFPs triggered by the beta peaks, we observed clear beta oscillations coupled to respiratory cycles in the average trace (not shown), thus ruling out the possibility of spurious coupling due to sharp deflections of the LFP signal (Kramer *et al.*, 2008).**

Finally, we analyzed phase-amplitude coupling during periods in which HRR, theta and beta oscillations simultaneously occurred, found in 4 of 6 animals. Consistent with previous reports (Yanovsky *et al.*, 2014; Lockmann *et al.*, 2016), theta was the predominant oscillation in CA1, while HRR dominated in DG (Fig. 6A top). Comodulation maps and phase-amplitude plots computed for CA1 and DG LFPs revealed that theta preferentially modulated the amplitude of gamma in CA1, while in DG HRR preferentially modulated the amplitude of beta oscillations (Fig. 6A-C).

These results suggest that the network mechanisms underlying beta and HRR are related and likely to differ from the mechanisms underlying theta and gamma.

Discussion

In this study, we characterized LFP beta oscillations (10-20 Hz) in the HPC of urethane-anesthetized rats. We showed that HPC beta phase-locks to co-occurring OB beta and moreover is driven by it. Laminar analysis of voltage recorded from different channels in linear probes revealed that beta oscillations have the maximum amplitude at the DG hilus, the same region where inputs carrying olfactory information arrive in the HPC. Furthermore, we found that the respiratory cycle itself and respiration-coupled LFP rhythms – but not theta – modulate the amplitude of beta oscillations.

Early studies in anesthetized animals have described oscillations in the 12-30 Hz range - by then not called as beta -, which were prominent in the olfactory system and could reach the HPC. Namely, Adrian (1942, 1950) reported 15-20 Hz waves in the OB and olfactory cortex of anesthetized hedgehogs, while Maclean *et al.* (1952) was the first to show 12-20 Hz oscillations in the HPC of anesthetized rabbits, which co-occurred with a similar oscillation in the olfactory cortex. Heale & Vanderwolf (1994) also described simultaneous 15-30 Hz "fast waves" in the DG and OB of anesthetized rats. While these papers demonstrated the co-occurrence of beta activity in the olfacto-hippocampal circuit, they have not explored their features in further details due to technological limitations at the time. Our results complement these early studies by showing that simultaneous OB and HPC beta oscillations in urethane-anesthetized rats have the same peak frequency and phase-couple (Figs. 1 and 2), and also that OB drives beta activity in the HPC (Fig. 3). Of note, previous studies in freely behaving rats have reported similar results to ours. For instance, Martin et al. (2007) showed OB-HPC

coherence in the beta frequency range during odorant sampling in go/no-go tasks. Moreover, OB beta was shown to drive HPC beta oscillations in a similar task (Gourévitch *et al.*, 2010). **In contrast to our results, Wilson & Yan (2010) reported that the HPC would drive OB beta oscillations. However, differently from us, they seem not to have analyzed LFP periods of prominent beta activity; for instance, beta oscillations were not evident in the raw signal and beta coherence in their data was rather low.**

The OB and dorsal hippocampus communicate to each other by means of feedback and feedforward polysynaptic projections (Steward & Scoville, 1976; Beckstead, 1978; de Olmos *et al.*, 1978; Wilson & Steward, 1978; Schwerdtfeger *et al.*, 1990). Projections from the OB reach the entorhinal cortex, the main gateway to the hippocampus, both directly and after a relay in the piriform cortex (Wilson & Steward, 1978). Similarly, the hippocampus projects back to the entorhinal cortex, which in turn sends connections to olfactory structures (Insausti *et al.*, 1997). Although the dorsal hippocampus and the OB do not communicate through monosynaptic pathways, the dorsal hippocampus drives theta oscillations in OB (Nguyen Chi *et al.*, 2016), and the OB drives beta oscillations in the dorsal hippocampus (present results and Gourévitch *et al.*, 2010). Interestingly, Neville & Haberly (2003) demonstrated that OB beta depends on feedback projections from the olfactory cortex. **Therefore, rhythmicity in one area can influence the other area through polysynaptic pathways. Of note, monosynaptic feedback projections were shown to connect the ventral hippocampus to the OB (van Groen & Wyss, 1990; Gulyás *et al.*, 1998), but whether oscillations in these regions are functionally coupled remains to be investigated.**

While in non-anesthetized studies beta oscillations were elicited by odorant sampling (Martin *et al.*, 2007; Gourévitch *et al.*, 2010), they are not strictly evoked by odorants. For example, the beta bursts observed by Adrian (1942) could occur in the absence of odorant stimulation, but, importantly, not in the absence of rhythmic nasal airflow. In fact, tracheal respiration abolished beta oscillations, which were restored by intranasal puffs of clean air. The subsequent paper of Maclean *et al.* (1952) also reported the emergence of beta oscillations during non-olfactory stimulation such as ear pinching and salt crystals delivered to the tongue. Supporting these results, we found that HPC beta activity occurs in the absence of odorant stimulation and is strongly modulated by the phase of nasal respiration (Fig. 5). Indeed, in vitro experiments of Grosmaitre *et al.* (2007) showed that olfactory sensory neurons in the nasal cavity respond to mechanical stimuli, thus suggesting that nasal airflow alone may be sufficient to activate downstream olfactory regions. Consistently, we have previously demonstrated that rhythmic nasal airflow entrains an LFP oscillation coupled to breathing cycles in the olfactory bulb (RR) and HPC (HRR) (Lockmann *et al.*, 2016), and here we further showed that HRR phase modulates the amplitude of HPC beta (Fig. 6).

Beta oscillations in freely behaving rats have been studied in the context of olfactory coding and learning. Noxious odorants, like toluene, evoke strong beta oscillations in the olfactory system and HPC, which are more prominent in DG than CA1 (Vanderwolf, 1992; Chapman *et al.*, 1998). During olfactory go/no-go tasks, the sampling of some odorants increases beta power in OB and HPC, and the learning of odor discrimination is associated to higher OB-HPC coherence in the beta frequency range (Martin *et al.*, 2007). **On the other hand, the latter study also showed that rats learn to discriminate odors irrespectively of the power of beta oscillations. Since different odorants induce beta oscillations of variable power (Martin *et al.*, 2007), and some non-odorant stimuli can also induce prominent beta (Maclean *et al.*, 1952), whether beta oscillations are necessarily related to olfactory**

coding seems controversial. Furthermore, it has been shown that HPC beta power increases during exploration of novel objects (França *et al.*, 2014) or novel environments (Berke *et al.*, 2008), but not when the same objects or environments became familiar. This indicates that HPC beta is not only related to the coding of sensory features but also to novelty detection.

An intriguing question is what network mechanisms determine the emergence of beta activity in the olfacto-hippocampal circuit. Interestingly, Heale & Vanderwolf (1995) showed that atropine and scopolamine abolish DG beta oscillations evoked by toluene, thus suggesting that acetylcholine may be involved in the gating of OB-HPC beta coupling. In turn, this finding may be related to the observed beta activity during novel experiences (Berke *et al.*, 2008; França *et al.*, 2014), and to our present results showing co-occurrence of beta activity along with urethane-induced theta oscillations, a brain state known to depend on cholinergic transmission (Kramis *et al.*, 1975). Moreover, we did not observe beta oscillations during SO periods, a brain state previously shown to be interrupted by cholinergic activation (Steriade *et al.*, 1993; Steriade, 2004).

Previous studies on beta oscillations in HPC have recorded from different subregions (Heale & Vanderwolf, 1994; Chapman *et al.*, 1998; Martin *et al.*, 2007; Berke *et al.*, 2008; França *et al.*, 2014; of note, the seminal work by Adrian, 1942, 1950 and Maclean *et al.*, 1952 did not report the recorded subregion). Vanderwolf (1992) was the only one to compare beta power in single wire recordings from different HPC subregions of freely behaving rats, and found that beta was much more prominent in the DG than in CA1. In our study, we performed multisite recordings to obtain a detailed picture of the variations of beta amplitude along the HPC dorsoventral axis. In agreement with Vanderwolf (1992), we found that beta oscillations have highest amplitude and phase reverse in the DG (**note that volume conduction from other brain regions would likely affect all electrodes of the linear probe, and not only the DG**

electrodes). We also demonstrated through CSD analysis that local synaptic currents are likely to generate beta oscillations in the DG. Importantly, **current sinks/sources** were induced in the same site by electrical stimulation of the LOT, further confirming that beta is conveyed to downstream regions by axons leaving the OB (Fig. 4). **Altogether, these results strongly suggest that hippocampal beta oscillations are not volume conducted from other brain regions.**

Respiration and respiration-coupled rhythms have been previously shown to modulate the amplitude of beta oscillations in the olfactory system. Adrian (1942) observed that beta oscillations in OB and pyriform cortex appeared as bursts at each inspiration. More recently, Buonviso *et al.* (2003) and **Cenier *et al.*, 2009** reported that the respiration-coupled LFP rhythm (RR) in the OB modulates the amplitude of local beta oscillations. During urethane anesthesia, the rat HPC exhibits different low-frequency oscillations (< 5 Hz), including SO, theta, and a respiration-coupled rhythm (HRR) coherent with the RR in OB (Lockmann *et al.*, 2016). Consistent with Buonviso *et al.* (2003), here we found that HRR modulates beta amplitude in HPC. In addition, we showed that neither theta oscillations nor SO modulates beta (in fact, SO and beta do not co-exist).

In all, our results indicate that related network mechanisms generate beta and respiration-coupled LFP oscillations in the olfactory regions of the rodent brain. In the HPC, both HRR (Nguyen Chi *et al.*, 2016) and beta (present results) are driven by coherent oscillations at the same frequencies in OB. We speculate that (H)RR-beta coupling across the olfacto-hippocampal circuit may mediate the interaction between sensory and memory networks.

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Author Contributions

A.L.V.L. designed research, performed research, analyzed data and wrote the paper

D.A.L. performed research, contributed unpublished reagents/analytic tools

A.B.L.T. designed research, analyzed data and wrote the paper

Data Accessibility Statement

All data created during this research are available upon request.

Abbreviations

AR: auto-regressive

CA1: region 1 of Cornu Ammonis

CSD: current source density

DG: dentate gyrus

HPC: hippocampus

HRR: hippocampal respiration-coupled rhythm

LFP: local field potential

LOT: lateral olfactory tract

MI: modulation index

OB: olfactory bulb

RR: respiration-coupled rhythm

SEM: standard error of the mean

SO: slow oscillation

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Figure Legends

Figure 1. Beta oscillations occur simultaneously in olfactory bulb (OB) and hippocampus (HPC). (A) Example time-frequency power analysis of LFPs recorded from OB and HPC in a representative rat. White arrows indicate an epoch of prominent beta oscillations. Bottom traces show mean beta band (10-20 Hz) power. Beta power increases/decreases simultaneously in OB and HPC. Red dashed lines indicate the 100-second epoch analyzed in C. (B) Raw LFP traces showing concomitant beta oscillatory activity in OB and HPC. (C) Linear correlation between OB and HPC beta band power for the example in A (power computed in fifty non-overlapping 2-second windows). (D) Boxplot distribution of correlation coefficients across animals (n = 6).

Figure 2. OB and HPC beta oscillations are phase-locked. (A) Example OB and HPC power spectra during a 60-second epoch of prominent beta activity. Beta peak frequency is the same in OB and HPC. Inset shows beta peak frequency in OB and HPC across animals (n = 6). (B) OB-HPC phase coherence during the same period, revealing a peak within the beta band.

Inset shows OB and HPC LFPs. (C) Boxplot distribution of beta coherence across animals (epoch length: 60 seconds).

Figure 3. OB causes HPC beta oscillations. (A) Mean OB and HPC power spectra estimated by autoregressive modeling (AR; see Material and Methods). (B) Mean beta AR power peak across animals (n = 6). OB and HPC beta AR power did not differ in the LFP epochs used for computing Granger causality (epoch length: 100 seconds of prominent beta activity). (C) Mean Granger causality spectra. Notice prominent peak within the beta band only for the OB-to-HPC causality. (D) Mean beta causality (n = 6 rats) * $p<0.05$, paired t-test. Error bars and shaded areas represent ± SEM.

Figure 4. HPC beta oscillations have maximum amplitude in the dentate gyrus (DG). (A) Evoked potentials in the hippocampal dorsoventral axis to lateral olfactory tract (LOT) stimulation in a representative animal (left). LOT-evoked potentials have maximum amplitude in the DG hilus. Black arrow indicates stimulus artifact. Laminar profile of beta oscillations shows that HPC beta also has maximum amplitude in DG hilus (right). (B) Current source density (CSD) analysis shows sinks and sources in the DG following LOT stimulation (left) and during beta activity (right). (C) Raw (black) and filtered (10-20 Hz, light gray) LFPs recorded from the hippocampal fissure and hilus. Notice higher amplitude and phase-reversal of beta oscillations in the hilus. (D) Mean amplitude of LOT-evoked potentials, beta oscillations and OB-HPC coherence along the hippocampal dorsoventral axis (n = 6**; same data as in Figures 1 and 2**). Shades represent ± SEM.

Figure 5. Respiration phase modulates the amplitude of beta oscillations in OB and HPC. (A) Example traces of simultaneous recordings of air pressure in the nasal cavity along with OB and HPC LFPs, during epochs in which respiration-coupled LFP rhythms coexisted with beta oscillations. Raw signals are depicted in black, and band-pass filtered LFPs in blue (respiration frequency range, 0.7-1.6 Hz) or gray (beta range, 10-20 Hz). RR, respiration rhythm; HRR, hippocampal respiration rhythm. (B) Power spectra of the raw signals depicted in A. Note that OB and HPC spectra have peaks at the same frequency as respiration and at the beta range. (C) Left panel shows example phase-amplitude plots (beta amplitude vs. respiration phase). Middle panel shows example comodulograms. Amplitude and phase frequencies were obtained from the LFP and respiration signals, respectively. Power spectrum of the respiration signal is shown on top of the comodulograms. Dashed line indicates respiration peak frequency. Respiration phase modulates beta amplitude in OB and HPC. MI, modulation index. Right panel shows the probability distribution of 200 surrogate MI values. Dashed line indicates the actual MIs (z-score indicated above). (D) Z-scored OB and HPC MIs for each rat (n = 5). Red line indicates the 1.96 z-score. One animal did not exhibit respiration-coupled rhythms simultaneously with beta and was excluded from this analysis.

Figure 6. Hippocampal respiration-coupled LFP rhythm (HRR), but not theta, modulates beta amplitude. (A) Example comodulograms (LFP phase vs. LFP amplitude) computed for CA1 and DG LFPs in an epoch of simultaneous HRR, theta and beta. Power spectra of CA1 and DG LFPs as well as of nasal signal are depicted on top. HRR dominates the DG spectrum, while theta dominates in CA1. (B) Phase-amplitude plots for DG and CA1 subregions. MI, modulation index. (C) Z-scored MIs across amplitude frequencies (n = 4 rats) computed for CA1 theta phase (red) and DG HRR phase (black). Shades represent ± SEM. Note that HRR preferentially modulates beta amplitude in DG, while in CA1 theta preferentially modulates gamma amplitude. Two rats did not simultaneously exhibit theta, HRR and beta, and were excluded from this analysis.