Directional Coupling From the Olfactory Bulb to the Hippocampus During a Go/No-Go Odor Discrimination Task

Boris Gourévitch,1,2 Leslie M. Kay,3,4 and Claire Martin2,4,5,6

1Centre National de la Recherche Scientifique Unité Mixte de Recherche 8195, Centre de Neurosciences Paris-Sud, Orsay, France; 2Université Paris-Sud, Orsay, France; 3Department of Psychology, The University of Chicago, Chicago, Illinois; 4Institute for Mind and Biology, The University of Chicago, Chicago, Illinois; 5Centre National de la Recherche Scientifique Unité Mixte de Recherche 8165, Laboratoire Imagerie et Modélisation en Neurobiologie et Cancérologie, Orsay, France; and 6Université Paris Diderot, Paris, France

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Gourévitch B, Kay LM, Martin C. Directional coupling from the olfactory bulb to the hippocampus during a go/no-go odor discrimination task. J Neurophysiol 103: 2633–2641, 2010. First published February 17, 2010; doi:10.1152/jn.01075.2009. The hippocampus and olfactory regions are anatomically close, and both play a major role in memory formation. However, the way they interact during odor processing is still unclear. In both areas, strong oscillations of the local field potential (LFP) can be recorded, and are modulated by behavior. In particular, in the olfactory system, the beta rhythm (15–35 Hz) is associated with cognitive processing of an olfactory stimulus. Using LFP recordings in the olfactory bulb and dorsal and ventral hippocampus during performance of an olfactory go/no-go task in rats, we previously showed that beta oscillations are also present in the hippocampus, coherent with those in the olfactory bulb, during odor sampling. In this study, we provide further insight into information transfer in the olfacto-hippocampal network by using directional coherence (DCOH estimate), a method based on the temporal relation between two or more signals in the frequency domain. In the beta band (6–12 Hz), coherence between the olfactory bulb (OB) and the hippocampus (HPC) is weak and can be both in the feedback and feedforward directions. However, at this frequency, modulation of the coupling between the dorsal and ventral hippocampus is seen during stimulus expectation versus odor processing. In the beta frequency band (15–35 Hz), analysis showed a strong unidirectional coupling from the OB to dorsal and ventral HPC, indicating that, during odor processing, beta oscillations in the hippocampus are driven by the olfactory bulb.

INTRODUCTION

Sensory processing involves numerous and widespread brain areas. This raises the question of how perceived information is transmitted, stored, and efficiently retrieved from diffuse networks. Neuronal oscillations have been proposed as a solution for rapid information transfer between distant neuronal groups because they could represent a framework for long range synchronization between distributed cortical regions (Tallon-Baudry and Bertrand 1999; Uhlhaas et al. 2009; Varela et al. 2001). Oscillations have been correlated with a variety of perceptual and cognitive functions (Buzsáki 2006) and present numerous advantages for cortical computation (Cardin et al. 2009; Fries 2009; Singer 1993). Among the various cortical rhythms, beta frequency oscillations (15–35 Hz) seem to be more adapted for establishment of synchrony over larger distance than faster oscillations such as the gamma rhythm (60–90 Hz) (Kopell et al. 2000; von Stein and Sarnthein 2000). Indeed, beta oscillations have been observed in large scale networks during visual short-term memory maintenance and object processing (Sehatpour et al. 2008; Tallon-Baudry et al. 2001), motor maintenance behavior (Brovelli et al. 2004), odor-guided movement initiation (Hermer-Vazquez et al. 2007), and during behavioral expression of conditioned odor aversion (Chapuis et al. 2009).

Both the olfactory system and the hippocampus (HPC) share strong oscillatory events. Earlier studies have shown that the HPC is an olfactory receptive area. Beta band (15–35 Hz) oscillatory activities can be elicited in the dentate gyrus (DG) in response to olfactory stimulation (Heale and Vanderwolf 1999). Theta oscillations (theta I; 6–12 Hz) link the OB and HPC in some circumstances, with strong coherence between HPC theta oscillations and respiratory-linked theta activity in the OB during odor sampling in a go/no-go odor discrimination task (Kay 2005). Anatomically, the olfactory bulb (OB) is closely connected to the HPC via its direct projection to the entorhinal cortex (EC) (Vanderwolf 1992). In turn, the HPC sends feedback projections to the OB through the EC but also directly with specific cells of the ventral HPC projecting to the OB (Gulyás et al. 1998; van Groen and Wyss 1990). Linked with strong intrahippocampal connections (Amaral and Witter 1989; Moser and Moser 1998), the dorsal (septal) and ventral (temporal) hippocampal subfields are anatomically distinct and are characterized by functional dissociations such as during fear conditioning (Yoon and Otto 2007).

Interactions between sensory cortex and the HPC have been proposed to correlate with acquisition and retrieval of memory (Buzsáki 1996). Previous studies have shed light on oscillatory bursts at beta frequency (15–35 Hz), particularly prominent in the OB and piriform cortex (PC) and specifically elicited by learned odors. The correlation of this rhythm with behavior argues for its involvement in odor recognition and memory (Martin et al. 2004). Beta oscillations were hypothesized to be the signature of a functional network involved in learning because they disappeared after isolation of the OB from feedback projections (Martin et al. 2006). Although all the actors of this network are not identified, the hippocampus exhibits beta oscillations during odor discrimination that are coherent with OB oscillations (Martin et al. 2007). However, we lack information regarding the directionality of functional connectivity among recording sites in the context of odor discrimination learning.
In this study, we re-examine these data, extending our analysis by the use of directed coherence (DCOH) (Saito and Harashima 1981). DCOH describes not only the correlation of local field potential (LFP) signals recorded in distinct structures but also the direction of information flow between these structures. This method has been widely used, especially by Takigawa and colleagues, for studies of connections between cerebral hemispheres (Wang et al. 1992), links between the frontal and the occipital cortices for alpha rhythm (Wang and Yunokuchi 2002), and functional interactions between cortical areas for rats under methamphetamine (Takigawa et al. 2000). DCOH was recently used to study the interconnections between different areas of the human auditory cortex (Goure´vitch et al. 2008; Guéguin et al. 2006).

With this method, we are able to show that beta activity recorded during odor sampling in the HPC is driven by input from the OB. In addition, modulation of directional theta band coupling is shown between the two hippocampal subfields rather than between OB and HPC.

METHODS

Electrophysiological recording and behavior

This study is based on the reexamination of electrophysiological activity and experimental procedures that have been described in detail in Martin et al. (2007). The main points are summarized here.

Four adult male Sprague-Dawley rats (375–425 g; Harlan, Indianapolis, IN) were implanted with stainless steel formvar-coated recording electrodes (100 μm diam, 100–500 kΩ, California Fine Wire). After recovery, rats were trained in a go/no-go task to discriminate sets of two odors. All procedures were carried out with approval from and oversight by the University of Chicago Institutional Animal Care and Use Committee, according to guidelines set by the Association for Assessment and Accreditation of Laboratory Animal Care.

Electrodes were positioned stereotaxically in the olfactory bulb (OB) (8.5 mm anterior to bregma, 1.5 mm lateral, depth ~4 mm), the dorsal part of the hippocampus (DH) at the level of the dentate gyrus (4.8 mm posterior to bregma, 2 mm lateral, depth ~3 mm), and in the ventral part of the hippocampus (VH) (5.8 mm posterior to bregma, 2 mm lateral, 20 deg from vertical, depth ~9 mm). The reference and ground electrodes were connected to skull screws located above the posterior portion of the contralateral cortical hemisphere. Electrodes were inserted into a connector (Ginder Scientific) and fixed onto the rat’s head with dental acrylic. Two weeks of recovery separated surgery from recording sessions.

For each rat, electrophysiological measures were recorded during every session throughout training. Neural data from the different electrodes along with behavioral event markers were recorded with a Neuralynx Cheetah 32 system. Signals were sampled at 2,016 Hz and amplified (x2,000), and analog filters were set at 1–475 Hz. A unity gain preamplifier headstage (NB Labs, Denison, TX) was used for signal conditioning.

Experiments were conducted in an operant chamber (MedAssociates, St. Albans, VT), and behavioral events and responses of the animal were controlled and monitored (Coulbourn Graphic State, Allentown, PA). The behavioral events occurred as follows: illumination of the house light indicated that the odor port was active. The rat initiated the odor delivery, which was triggered by detection of a nose poke in the odor port. One of two possible odors, assigned to valence CS+ or CS−, was pseudorandomly delivered for 1.5 s. One second after the end of the odor pulse, the response lever extended from the wall. If the odor was the CS+, pressing the lever delivered a sucrose pellet (45 mg; Research Diets and Bioserve). Pressing the lever for the CS− odor switched off the light and doubled the intertrial interval from 7 to 14 s. The rats learned to press the lever (go response) following sampling of the CS+ odor and to avoid pressing the lever (no-go response) following sampling of the CS− odor.

Behavioral response latency was quantified for each trial as the time elapsed between the nose poke and the lever press. The response to CS− was classified as a correct no-go response when the rat did not press the lever for ≥8.8 s (the time from the beginning of the odor pulse until the lever retracted for both the CS+ and CS− trials). A correct go response to CS+ consisted of a lever press before the same delay. Each session lasted ~1 h, corresponding to 100–150 trials. Performance was considered to be at criterion if a rat showed ≥90% correct choices on two consecutive blocks of 20 trials, including both CS+ and CS− trials (pseudorandomly interleaved). Once the criterion was reached, the odor pair was changed in the next session. For the study of electrophysiological data in relation to behavior, we assign for each animal a beginner level (1st 20 presentations of each odor/valence), a criterion level (1st 40 trials with animals at the criterion), and a postcriterion block to be the 40 trials following the criterion level. These levels may belong to the same session or not.

Four odor-contingency pairs were presented successively to the animals. First, they were trained on octanol CS+/propanol CS− (OP) and then transferred to heptanol CS+/hexanol CS− (HH) and geraniol CS+/citrall CS− (GC). Finally, the GC pair was reversed to citral CS+/geraniol CS− (GCrev). We previously showed that there was no beta band coherence increase between OB and HPC for the first odor pair (Martin et al. 2007); consequently, this odor pair was not included here.

Data analysis

LFP signals were analyzed off-line with Matlab 7 (Mathworks). Analysis was focused on a 4 s interval surrounding the odor onset. Data were inspected to discard trials containing movement artifacts (~5% of trials); easily recognizable by a simple visual examination of raw signals. Spectral analysis was done on the raw analog filtered 1- to 475-Hz signal.

Power analysis

For a given animal and session, the time-frequency power spectral density (PSD) was estimated for each trial by short-time Fourier transform of LFPs using a Hamming window of 1,008 points, an overlap of 90%, and zero-padding of 4,032 points, which leads to a frequency resolution of 0.5 Hz at the sampling frequency 2,016 Hz. To emphasize significant spectral changes in the time-frequency PSD after the odor onset, a 2.5% lower level and a 97.5% upper level were estimated for each frequency from the distribution of time-frequency PSD values based on all trials and all values within the preodor interval (~3 to −1 s relative to the odor onset). For a given time and frequency point, we define decrease and increase spectrottemporal significance histograms as the percentage of trials whose time-frequency PSD value is below the 2.5% level or above the 97.5% level, respectively.

Functional coupling

Directional flows between LFPs recorded in two regions are estimated by DCOH (Saito and Harashima 1981). The seminal work and previous papers (Goure´vitch et al. 2006, 2008) detail the mathematical basis of DCOH. Briefly, DCOH is a spectral indicator (value between 0 and 1) that describes the frequency-specific correlation of two signals according to their temporal relationship. It estimates the fraction of the PSD of a signal Y explained by the spectral content of a signal X. Mathematically, DCOH is a spectral transform of an autoregressive model with exogenous inputs (ARX), which linearly relate the current output Y(t) to a finite number of past inputs Y(t − k) (autoregressive part) and X(t − k) (exogenous part). In other words,
we study how a LFP signal can be explained by a linear combination of its own past values and past values of another given signal. Computation details for ARX models are given in Wang and Yuno-kuchi (2002). The order for the ARX model is often determined by information theory-based calculations (Akaike’s or Bayesian criterion; Akaike 1969; Schwarz 1978). In this study, because precise spectral intervals are investigated, the model order is chosen so that the ARX power spectra of X and Y are the most accurate to reflect peaks in X and Y PSD estimated by FFT. After low-pass filtering LFPs below 252 Hz, an order of 30 was finally chosen. DCOH was estimated with a frequency resolution of 1 Hz in the interval 0–50 Hz before and during odor stimulation (respectively −3 to −1 and 0–2 s intervals) and averaged over trials.

Computations of averaged DCOH on 200 randomized Gaussian signals of the odor period length (2 s) indicated that the baseline for DCOH for any frequency is 0.2 instead of 0 in theory. This bias cannot be avoided because it mainly stems from the short signal length: if the length of the same randomized Gaussian signals is 20 s, the bias is only 0.05. In any case, this bias level was not removed from DCOH values to keep them between 0 and 1 but is shown on each figure of raw DCOH values. This bias does not have any influence on our results because we considered comparisons of DCOH between preodor and odor periods.

Statistical analysis

The correlation coefficient is used to address the comparison of averaged PSD and DCOH coefficients over trials or over sessions. Significance of a correlation coefficient r between n paired observations (null hypothesis: $r = 0$) is tested using the Fisher’s z transform of $r$ (Dunn and Clark 1969):

$$z = \frac{1}{2} \log\left(\frac{1 + r}{1 - r}\right)$$

for $n > 30$, using correlation coefficient table of critical values otherwise.

For a given behavioral condition, significance of coupling relations between two structures was assessed as follows: for all trials, DCOH values for each frequency were compared between 0 and 2 s (i.e., after odor onset) and −3 and −1 s (i.e., before odor onset) using two-sided Wilcoxon tests (Wilcoxon 1945) at risk 0.1% (5% with Bonferroni correction for the 51 tests between 0 and 50 Hz).

All other tests in the study (comparison of DCOH or PSD between conditions or behavioral responses) involved non-Gaussian independent unpaired datasets and therefore were performed using two-sided Mann-Whitney tests at risk 5% (Mann and Whitney 1947).

RESULTS

We recorded LFPs from the OB, DH, and VH while rats performed a two odor go/no-go discrimination task on successive odor pairs. All the rats reached criterion performance for the three pairs of odors in $72 \pm 41$ trials for HH, $128 \pm 84$ trials for GC, and $423 \pm 53$ trials for GCre

As shown in the previous paper (Martin et al. 2007), in the absence of odor stimulation, the LFP is dominated by slow oscillations (respiratory/theta band, 2–12 Hz) both in the OB and the hippocampus. During odor sniffing, the theta band was restricted to the higher frequency range (6–12 Hz). When the rat reached criterion performance for discrimination of a given odor pair, oscillations in the beta frequency band (15–35 Hz) increased during odor sampling in the OB. Simultaneously, beta activity was also visible in the DH and VH with smaller amplitude (Fig. 1). No significant decrease of oscillatory activity is reported in this frequency range (see Supplementary Fig. S1). Our previous analysis showed increased coherence in the 15–35 Hz frequency band between the OB and both hippocampal subfields during odor sampling (Martin et al. 2007). To further elucidate the characteristics of this coupling, we report here directional coherence analysis, a measure that

1. The online version of this article contains supplemental data.
estimates the directional flow of the signal within the areas recorded.

**DCOH in the olfacto-hippocampal network**

DCOH analysis was conducted on all rats and sessions for the three odor-reward pairs after the initial odor set. Figure 2 shows an example for one session and one animal of the DCOH spectrum (between 0 and 50 Hz) averaged over trials. Significant changes elicited by odor sampling are observed in the beta (15–35 Hz) frequency band (Wilcoxon tests, *P* < 0.001; see METHODS). We observe a marked increase in DCOH after odor onset (0–2 s, thick line) compared with the preodor period (−3 to −1 s, dotted line) for the OB to DH and OB to VH spectra. In the theta frequency band, peaks of DCOH can also be observed for the DH to OB and the DH to VH spectra. However, contrary to the beta band, it is not always modified by the odor, and the peak is also often present before the stimulus onset (no significant difference before vs. after odor onset). For the rest of the paper, we focus exclusively on modification occurring in beta (15–35 Hz) and theta (6–12 Hz) frequency bands.

The difference between the odor and preodor periods was computed for every trial. The result averaged over each single session (average 54 ± 14 trials per session; range, 19–82) is shown for heptanol trials (CS+) in Fig. 3. Similar plots for the other odors are available in Supplementary Material (Supplementary Figs. S2 and S3). Significant flows found over animals, odors, and sessions are summarized in global propagation schemes in Fig. 4.

In the beta band (15–35 Hz), directional coupling from OB to DH and from OB to VH is significant for all the rats and odor pairs (HH, GC, and GC reversal) in at least one session, whereas significant opposite flows (from hippocampus to OB) are very rare (see summary in Fig. 4A). The strongest DCOH increase is found for the HH odor pair, which is the pair associated with the first rule transfer. No significant flow is observed in the beta band between the two hippocampal subfields.

Concerning the theta frequency band (6–12 Hz), a few significant DCOH increases can be seen during the odor period, but the results are not as clear as for the beta band. Coupling is significant for each odor pair from OB to VH but not for every session and animal (Fig. 4B). Contrary to the beta band results, opposite flows can be found between OB and both DH and VH (for example, OB→VH and VH→OB for HH).

Examination of the reversal does not show specific effects, and opposite flows can also be found (OB→DH and DH→OB for GC reversal).

One consistent feature in the theta band is that, when the DCOH is averaged over rats and sessions for all odors, a significant decrease is found between DH and VH in both directions for the CS+ condition and for the flow DH→VH for the CS− condition (Figs. 3 and 4B; minus signs in Supplementary Fig. S4). This decrease in coherence means that coupling between ventral and dorsal hippocampus at theta frequency is higher during the preodor than the odor period.

As shown in the example DCOH spectrum (Fig. 2), when they occur, peaks in the theta band are often present both before and after odor onset. Comparison of DCOH absolute values between beta and theta frequency bands before odor onset show that average DCOH is always higher for theta compared with beta frequencies (Wilcoxon tests, *P* < 0.05) for preodor −3 to −1 s (Supplementary Fig. S4).

One can observe that uncoupling is more robust from DH to VH than in the opposite direction. Interestingly, even though uncoupling is significant for both odors (CS+ and CS−) in the direction DH→VH, it is never seen for CS− from VH to DH (Fig. 4; Supplementary Fig. S4). This effect is reinforced by the reversal: sampling of geraniol leads to VH→DH theta flow decrease when rewarded positively but not after the reversal when it is the CS−.

Finally, beta oscillation power in the OB is related to olfacto-hippocampal coupling. Examination of the relationship...
between coupling strength and beta band power shows significant positive correlations for both CS\textsubscript{H11001}/H11001 and CS\textsubscript{H11002}/H11002 between the OB and the two hippocampal subfields (Fig. 5). This effect cannot be caused by oscillation power strength directly because the DCOH estimate is normalized and is invariant with respect to multiplication because of the use of ARX models. The correlation is seen on average values for a session and does not seem relative to single trials within a session. The correlation between DCOH and power values over trials shows that, for all animals, sessions, and conditions (176 correlations), only eight isolated values were significant (\(P<0.05\)), which is fewer than would be expected solely caused by type I error.

We next examined whether coupling strength is also related to behavioral output.

Influence of behavioral task and learning on beta frequency coupling

We first examined whether the behavioral accuracy of animals within single trials was related to variation of DCOH. The go/no-go task gives the opportunity to compare correct and incorrect behavioral output on a trial by trial basis. In cases where more than five trials for both correct and incorrect responses were available in the same session (Fig. 6, top), we compared correct and incorrect go-no responses for the CS\textsubscript{H11002} odor. Only four isolated sessions showed a significant difference in coupling strength dependent on whether the behavioral output was correct or not, and the differences were not significant across the several cases.
Because variation of DCOH is not directly related to the accuracy within single trials, to study the possible impact of expertise, we examined coupling using groups of trials belonging to the same learning level. All the animals reached criterion in one to three sessions for odor pairs HH and GC and in two to six sessions for the reversal (Fig. 6, bottom). We previously showed that, in the OB, beta power was related to behavior in that the maximum power was reached when animals attained the criterion level for the discrimination, followed by a decrease when animals were overtrained (Martin et al. 2007). Thus beta band averaged DCOH values were plotted using the same groupings of trials as in our previous study (beginner, criterion, and postcriterion; Fig. 7). First, examination of the influence of odor contingency on olfacto-hippocampal coupling for each level shows few differences between CS\textsubscript{H11001}/H11001 and CS\textsubscript{H11002}/H11002 trials (see signs in Fig. 7). For both OB→DH and OB→VH coupling, when a difference is significant between CS\textsubscript{H11001}/H11001 and CS\textsubscript{H11002}/H11002, it is in favor of CS\textsubscript{H11001}. In addition, in no case is a difference significant at the beginner level. Thus Fig. 7 shows that there is no clear cut relationship between behavioral level and beta band coupling strength, as was the case with beta power (Martin et al. 2007). In addition, the data do not show specificity related to the reversal of geraniol-citral odor set.

DISCUSSION

The goal of this study was to provide a deeper understanding of the functional relationship between the OB and the HPC for odor processing and learning. We evaluated the pattern of activity propagation within signals recorded from the olfacto-hippocampal network, using the DCOH estimate, an analysis method based on the temporal relation between two signals in the frequency domain. DCOH can be very powerful in affording insight into network processing, because it gives information not only on coupling between signals recorded from different brain areas but also about the direction of apparent information propagation. In this study, we examined apparent activity flow during olfactory discrimination tasks known to involve the hippocampus (olfactory rule learning and reversal) (Bunsey and Eichenbaum 1996; Knafo et al. 2005; Wiener et al. 1989).

The main result is that odor sampling elicits unidirectional coupling in the beta band (15–35 Hz), in the bottom-up direction, from the OB to dorsal (dentate gyrus region) and ventral HPC signals. We found no flow in the opposite direction (DH or VH toward OB) or between the two hippocampal subfields. In the beta band, DCOH correlated with oscillation power but is not significantly impacted by performance, and no difference in this pattern was found for the odor contingency...
reversal. Examination of the theta band (6–12 Hz) did not show such a robust effect; apparent information transfer between OB and hippocampal subfields could be found in both directions with no clear pattern. However, the one reproducible effect in this frequency band is a decorrelation between the two HPC subfield signals during odor sampling.

**Choice and interpretation of the DCOH**

The most widely used methods for modeling information flow between two or more signals are based on multivariate autoregressive (ARX) models inspired by the Granger causality. They use a model of prediction of $X(t)$ from a linear combination of all available past information for $X(t)$ and $Y(t)$ (Ashley et al. 1980; Granger 1969; Sims 1972). The main methods are the Geweke measures (Geweke 1982, 1984), the directed coherence (Saito and Harashima 1981), the directed transfer function (Kaminski and Blinowska 1991; Korzeniewska et al. 2003), and the partial directed coherence (PDC) (Sameshima 1999). Even though the PDC seems to be the best theoretical framework for causality estimation between a multivariate set of signals, simulations show that, when a common source of noise exists between signals, this method leads to biased estimates of the frequency and the strength of the causality link (see model 2 in Gouërétvitch et al. 2006). Indeed, there is a risk that flows found between two structures might actually come from a common source located in another region and propagating to both structures by simple volume conduction effects. DCOH takes into account a common source of noise between the two signals $X$ and $Y$ and reduces this possibility.

The linearity of the model may also be questioned. In our study, it is likely that distance and cytoarchitectonic differences between the OB, DH, and VH induce nonlinearities in transfer functions between signals from the structures. Some propagation flows might be missed by methods based on ARX models; however, these models are robust to several weak nonlinearities (Gouërétvitch et al. 2006).

When using information flow models, it must be acknowledged that what the model identifies as information flow is only a measure of the apparent flow between measured signals. We use the measurements to infer flow of information between the neural structures that produce these signals. Thus the following are inferences based on the information flow estimated from the LFP signals in OB, VH, and DH.

**Directional coupling between OB and HPC**

The hippocampus plays a major role in forming the representation of relations among odor memories (Eichenbaum 1998), and even though this structure does not seem to have a critical role in simple odor discrimination, odor learning is accompanied by enhanced CA1 hippocampal neuron excitability just before and during rule learning (Zelcer et al. 2006). However, the complex relation between sensory cortices and the HPC is not yet entirely clear (Buzsaki 1996), and one question is whether LFP oscillations can inform our understanding of interactions and plasticity occurring along the OB–HPC axis. Numerous studies in different systems have used coherence as an indication of structures’ coupling, but very few examine the direction of that coupling, which carries crucial insight into modes of information transfer among brain areas.

We show that beta oscillations can support information transfer from the OB to the hippocampus during odor sampling in this task. Our hypothesis is that, with learning, a functional link is created between the OB and HPC, and beta oscillations may represent an efficient carrier for odor information to the HPC. This hypothesis is supported by a study in which electrical stimulation was applied at various frequencies between 2 and 35 Hz in the piriform cortex (PC) (Chapman et al. 1998). It was found that optimal responses in the dentate gyrus (DH in this study) were obtained for frequencies of stimulation within the beta band (14–18 Hz), pointing to a role for beta-frequency activity in the gating of olfactory input to the hippocampus. The same authors showed that high-amplitude coherent beta oscillations spread in a caudal direction from the olfactory bulb to the entorhinal cortex (EC) and dentate gyrus after presentation of high concentrations of toluene (Chapman et al. 1998). A rostro-caudal propagation of oscillations at 35–36 Hz after the presentation of urine odor has also been shown along olfactory structures (OB, PC, and EC), but the hippocampus was not examined (Boeijjinga and Lopes da Silva 1989). However, this study was in cats, and the frequency may correspond to the low gamma frequency associated with attentive behavior (Kay 2003).

Although data mentioned previously were obtained for spontaneous odor presentation, one study examined coupling between the OB and limbic areas during learning of an operant task where rats were trained to respond to reinforced odors (Kay and Freeman 1998). During expectation, before odor onset, input from the EC to OB was observed in the beta frequency band, whereas the flow was reversed during odor sampling from the OB to EC and dentate gyrus and occurred at the gamma frequency (35–120 Hz). Even though bidirectional coupling was shown along the olfactory areas axis, no consistent OB-HPC coherence increase was found at the beta frequency. This difference may be explained by the fact that, in that study, only one odor discrimination was performed by the animals. In this data set, results from the first odor pair used for task learning actually failed to show a beta band coherence increase (data not shown, cf. Martin et al. 2007).

One question that can be raised concerning go/no-go conditioning is about the strategy adopted by rodents to solve the task: do they learn the two odors equally or is the knowledge of one association sufficient to solve the task? Beta oscillations have always been observed for both CS+ and CS− (Martin et al. 2004), and we confirm this observation with OB-HPC coupling. Whether processing of the two odors relies on different cortical circuits is thus far not known, but these data confirm that beta activity is associated with processing in both reward contingency conditions. In addition, even though DCOH is globally related to OB beta power, no clear relation was found between coupling strength and criterion attainment for each odor set discrimination (Fig. 7). Accordingly, coupling is not predictive of performance (Fig. 6); instead, it may be a more global mechanism elicited by odor sampling but not regulated by the expertise of animals for a given odor pair discrimination once they have learned the generic behavior.

In the previous study (Martin et al. 2007), we found a learning-dependent ordinary coherence increase at the beta frequency for DH-VH in two rats. No corresponding DCOH increase was found here between DH and VH (Fig. 4; Supplementary Fig. S4). A possible explanation is that DCOH does not perfectly factorize the ordinary coherence along the direction, mostly because the estimation methods are completely different (see comparisons of ordinary and directed coherence in Takigawa et al. 1996 and
had to keep track of the timing of the intertrial interval. Such common sources of oscillating activity have been shown to induce misleading connectivity schemes (Albo et al. 2004) and are theoretically ruled out by the DCOH model used in this study, which takes account of a common source. If this is true, it reinforces the suitability of DCOH for studying interactions among groups of cerebral regions.

Even though the ventral HPC has a direct projection to the OB (van Groen and Wyss 1990), our study showed no directed coherence in the feedback direction from HPC to OB. Thus the results exclude a direct influence of the HPC on OB beta oscillations but do not exclude such influence on other parts of the system, possibly mediated through the entorhinal cortex. For instance, the PC, strongly connected with the entorhinal cortex (Haberly 2001), is likely to be modified by higher brain areas during olfactory learning (Cohen et al. 2008).

Beta activity in the olfactory system has been hypothesized to be a network phenomenon and is likely to reflect coordinated activity of distant cerebral areas involved in the expression of a learned behavior (Chapuis et al. 2009; Martin et al. 2006). Multiple brain regions involved in olfactory processing such as orbitofrontal cortex or amygdala have been shown to exhibit beta oscillations in different types of odor learning in rodents (Chapuis et al. 2009) and humans (Jung et al. 2006). Removal of centrifugal projections to the OB abolished beta oscillations in both OB and PC, showing the importance of long range interactions for the expression of this rhythm (Martin et al. 2006). Consistently, slow oscillations in the beta frequency band are more suited for long range transmission than faster oscillations (Kopell et al. 2000), such as gamma oscillations in the OB. In fact, in a two-alternative choice paradigm, gamma oscillations whose power was increased during odor discrimination were restricted to the OB (Beshel et al. 2007). It is possible that beta or gamma oscillations are favored according to the behavioral context (go/no-go or 2-alternative choice), in parallel with a change in the functional system.

**Theta activity**

In the OB, oscillatory activity associated with respiratory activity has a frequency range (2–12 Hz) that overlaps with the well-characterized theta rhythm in the hippocampus (4–12 Hz). This frequency has been proposed as a good candidate for olfacto-hippocampal coupling (Kay 2005; Kay et al. 2009). However, in this study, we did not observe specific features of OB-HPC coupling at the theta frequency band as in other studies that showed that the coherence between hippocampal theta and sniffing was increased with performance improvement (Kay 2005) or at the beginning of reversal of a learned odor-contingency pair (Macrides et al. 1982). In our study, we did not find specific effects in DCOH related to reversal, and we confirmed our previous observations of an absence of clear theta coherence in our behavioral task (Martin et al. 2007). These discrepancies between studies may come from the difference in the protocols used. Indeed, Macrides et al. (1982) focused on the nasal airflow, a measure that can exhibit slight differences from theta recorded through neuronal activity. For the other study (Kay 2005), the difference may rely on the attentional demand, because the rats had to keep track of the timing of the intertrial interval.

Surprisingly, in these data, the strongest effect observed at theta frequency occurs between the two hippocampal subfields rather than between OB and HPC: a decorrelation between DH and VH. This decorrelation is observed because we compare the odor period relative to the preodor period, which means that DH-VH theta coupling is stronger during the preparatory period (before the rat decides to sample the odor) than during the stimulus period. Contrary to beta oscillations that emerge only during odor processing, theta oscillations are constantly present in the signal and represent the more salient rhythm during ongoing activity. Decorrelation could be interpreted as functional dissociation between the two hippocampal subfields. Several studies suggest that dorsal and ventral hippocampus can play functionally distinct roles in memory (Moser and Moser 1998) or at different stages of memory formation (Yoon and Otto 2007). Because differences in intra-hippocampal coupling are observed between CS+ and CS−, it is likely that emotional or reward processing brain areas are part of these networks (Schoenbaum et al. 2000).

**Conclusion**

DCOH provides a valuable tool to study directional network interactions for long range oscillations. We showed that, during learning tasks, olfactory information, as represented by directed coherence of the LFP signal, is transferred from the first sensory relay to the hippocampus through beta oscillatory rhythm rather than through theta rhythm. To further understand the network underlying olfactory learning, which has been shown to extend far beyond OB and HPC, this type of analysis should be extended to larger sets of brain regions involved in olfactory processing, including, for instance, piriform, entorhinal, or orbitofrontal cortices.

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**References**


