An Olfacto-Hippocampal Network Is Dynamically Involved in Odor-Discrimination Learning

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Martin C, Beshel J, Kay LM. An olfacto-hippocampal network is dynamically involved in odor-discrimination learning. J Neurophysiol 98: 2196–2205, 2007. First published August 15, 2007; doi:10.1152/jn.00524.2007. Several studies have shown that memory consolidation relies partly on interactions between sensory and limbic areas. The functional loop formed by the olfactory system and the hippocampus represents an experimentally tractable model that can provide insight into this question. It had been shown previously that odor-learning associated beta band oscillations (15–30 Hz) of the local field potential in the rat olfactory system are enhanced with criterion performance, but it was unknown if these involve networks beyond the olfactory system. We recorded local field potentials from the olfactory bulb (OB) and dorsal and ventral hippocampus during acquisition of odor discriminations in a go/no-go task. These regions showed increased beta oscillation power during odor sampling, accompanied by a coherence increase in this frequency band between the OB and both hippocampal subfields. This coherence between the OB and the hippocampus increased with the onset of the first rule transfer to a new odor set and remained high for all learning phases and subsequent odor sets. However, coherence between the two hippocampal fields reset to baseline levels with each new odor set and increased again with criterion performance. These data support hippocampal involvement in the network underlying odor-discrimination learning and also suggest that cooperation between the dorsal and ventral hippocampus varies with learning progress. Oscillatory activity in the beta range may thus provide a mechanism by which these areas are linked during memory consolidation, similar to proposed roles of beta oscillations in other systems with long-range connections.

INTRODUCTION

Contemporary theories propose that long-term memory consolidation is facilitated by temporary bidirectional interactions between sensory cortices and hippocampal regions (Buzsaki 1996; Eichenbaum 2000; Nadel and Moscovitch 1997; Squire and Alvarez 1995; Willigen et al. 2004). Because the two systems share strong bidirectional connections, the loop formed by the olfactory system and the hippocampus (HPC) represents a very good model to assess functional connections between the two levels during learning. Anatomically, only two synapses separate the olfactory bulb (OB), the first relay of odor processing, from the dentate gyrus (DG) of the hippocampus via the lateral entorhinal cortex and the lateral perforant path (Vanderwolf 1992). The OB also reaches the entorhinal cortex via the piriform cortex (PC), which has been shown to be involved in storage and retrieval of olfactory memory traces (Barkai and Saar 2001; Haberly 2001). In turn, the HPC sends projections to the OB through the entorhinal cortex (EC) and also directly from the ventral HPC (CA1 area) to the OB granule cell layer (Gulyas et al. 1998; van Groen and Wyss 1990). Connected through 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, mostly studied in relation to fear conditioning (Richmond et al. 1999; Yoon and Otto 2007).

One mechanism capable of producing functional long-range interactions across such a network is synchronous oscillatory activity as it may create a functional link between two remote neuronal populations or brain areas and thus produce a temporal window for transient communication (Fries 2005). In addition, because large-scale population cooperativity implies coincident neuronal activity arriving at downstream targets, oscillatory synchrony could produce the kind of firing precision necessary to enhance synaptic efficiency leading to network plasticity (Schaefer et al. 2006; Singer 1993). An increasing number of studies in various sensory systems have found a clear relationship between neural population activity and perception (Kay 2003; Liu and Newsome 2006; Mehring et al. 2003; Pesaran et al. 2002) and memory storage (Herrmann et al. 2004; Tallon-Baudry and Bertrand 1999).

Both the OB and HPC show prominent oscillatory modes that share common properties. In the OB, oscillatory activity associated with respiratory drive and afferent input has a frequency range (2–12 Hz) that overlaps with the theta rhythm in the hippocampus (4–12 Hz) (Kay 2005; Macrides et al. 1982). Although in this frequency band hippocampal and OB oscillations are usually uncorrelated, a previous study reported that the olfactory system and the HPC were linked in the high theta frequency band (6–12 Hz) associated with performance accuracy in an olfactory sensorimotor discrimination task (Kay 2005). Sniffing has also been shown to be associated with hippocampal theta rhythm during odor contingency reversal learning in a go/no-go task (Macrides et al. 1982). Other rhythms, such as beta (15–35 Hz) and gamma (40–100 Hz) oscillations have been recorded in both structures and have also been found to be related to behavior (Csecsvari et al. 2003; Kay 2003; Kay and Freeman 1998; Vanderwolf 2001). In the OB and piriform cortex, acquisition of odor discrimination in a go/no-go task leads to the emergence of powerful odor-induced beta oscillations (15–40 Hz) (Martin et al. 2004; Ravel et al. 2003). Intact feedback projections to the OB are necessary for the expression of this activity (Martin et al. 2006), which suggests a long-range cooperative network. Our hypothesis is

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that beta oscillations link the different elements of a network involved in acquisition of a relation between an odor and a behavioral significance.

In the present study, we describe beta band coupling between the hippocampus and the OB associated with odor-guided associative learning. We recorded the local field potential (LFP) simultaneously in the OB and the dorsal and ventral hippocampus of rats engaged in an olfactory go/no-go task involving appetitive reinforcement. To describe dynamics of functional connectivity between the OB and the HPC, we analyzed characteristics of the oscillatory activity and its interregional coherence during learning and criterion performance of odor pair discriminations. We found an increase of beta band power in both the dorsal and ventral hippocampi during odor sampling, an increase in coherence with the OB specifically during odor sampling, and an increase in intrahippocampal coherence related to criterion performance.

METHODS

Four adult male Sprague-Dawley rats (375–425 g; purchased from Harlan, Indianapolis, IN) were implanted with electrodes after a short shaping period (learning to obtain a reward without any odor experience). After recovery they were then trained in a go/no-go task (GNG) to discriminate sets of two odors successively. For the training, animals were dieted to and maintained at 85% of their ad libitum weight. 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 Association for Assessment and Accreditation of Laboratory Animal Care.

Electrophysiological recording

Animals were implanted with stainless steel formvar-coated recording electrodes (100 μm diam, 100–500 kΩ; California Fine Wire; inter-tip vertical distance ~0.5–1.0 mm) under pentobarbital anesthesia. Electrodes were positioned stereotaxically in the lateral olfactory tract (LOT) (2.7 mm anterior to bregma, 1.6 mm lateral from midline, 14 deg from vertical, depth ~8 mm), OB (8.5 mm anterior to bregma, 1.5 mm lateral, depth ~4 mm), the dorsal part of the HPC at the level of the DG (dHPC; 4.8 mm posterior to bregma, 2 mm lateral, depth ~3 mm), and in the ventral part of the HPC (vHPC; 5.8 mm posterior to bregma, 2 mm lateral, 20º from vertical, depth ~9 mm). The depth of placements was positioned at the level of the principal cell layers in the OB and HPC using multunit activity and the evoked field potential induced in response to electrical stimulation of the LOT (10 V; 0.02- to 0.08-ms duration; 0.5-Hz stimulation rate).

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 threaded round nine-hole 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 every session throughout training (except for presurgical initial shaping). Neural data from the different electrodes along with behavioral event markers were recorded with a Neuralynx Cheetah 32 channel system. Signals were sampled at 2,016 Hz, amplified (×2,000), and analog filters were set at 1–475 Hz. A unity gain preamplifier headstage (NB Labs, Denison, TX) was used for signal conditioning.

Verification of electrode placements

After experiments were complete, rats were injected with a lethal dose of urethane, and electrode placements were marked by passing current through the tip of the stainless steel wires. Electrode tips were marked using the Prussian Blue reaction, as rats were perfused intracardially with a 10% formalin solution with 4% potassium ferrocyanide. Brains were extracted from the skull, sectioned coronally, and stained with Neutral Red. As illustrated in Fig. 1, recording electrode placements were at the level of the mitral cell layer in the OB, at the vicinity of the hilus in the dorsal HPC, and in the CA1 layer in the ventral HPC. However, in the dHPC, considering the relatively large distance covered by LFP recording we cannot exclude some influence from CA1.

Behavior

Experiments were conducted in a 30 × 25 × 50-cm operant chamber (MedAssociates, St. Albans, VT) within a Faraday cage. The front wall of the chamber contained an odor port, under which protruded a pellet delivery tube. A retractable lever was located on the right side of the port. A light was positioned on the top of the back wall. Behavioral events and responses of the animal were controlled and monitored by a separate computer (Coulbourn Graphic State Allentown, PA), and event markers were transmitted directly to the Neuralynx recording system via the digital IO line. Odors were generated in glass test tubes by bubbling air (100 ml/min) through a column of pure odorant solutions and injecting the odorized air into a carrier air stream (400 ml/min) via a computer-controlled olfactometer. For all odorants, odorized air was diluted to ~20% of saturated vapor with a continuous plain air stream. Because the odorants were of various volatilities, producing saturated vapor concentrations related to the vapor pressure, the absolute airborne concentrations of odorants varied.

Illumination of the 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 then pseudorandomly delivered for 1.5 s. One second after the end of the odor pulse, the lever extended from the wall. If the odor was CS +, pressing the lever delivered a sucrose pellet (45 mg; Research Diets and Bioserve). Pressing the lever for odor CS − 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 odor CS + and to avoid pressing the lever (no-go response) following sampling of odor CS − (Fig. 2, A and B).

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
picturing the time course of a single trial (see description in METHODS). B: example of a latency curve for 1 rat, during the acquisition of geraniol(+) /H11002 citral(−) (blocks 1–10; each block is 20 trials) and the reversal (block 11 to response to CS which time the trial ended and the lights extinguished. A correct go pulse until the lever retracted for both the CS+ and CS− trials), at which time the trial ended and the lights extinguished. A correct go response to CS+ consisted of a lever press before the 8.8-s delay. Each session lasted 1 h, corresponding to between 100 and 150 trials. For each session, performance was analyzed in blocks of 20 trials separately for the two odors. Performance was considered to be at “criterion” if a rat showed ≥90% correct choices on two consecutive blocks, including both CS+ and CS− trials (pseudorandomly interleaved). After criterion was reached, an additional session was performed with the same odor, and then the odors were changed. For analysis of electrophysiological data, 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.

odors used were octanol-propanol (Fisher Scientific, Fair Lawn, NJ; pair 1), hexanol-heptanol (ICN Biomedicals, Aurora, OH; pair 2), geraniol (Fisher Scientific, Fair Lawn NJ)-citral (Fluka Chemika; pair 3). After pair 3 was completed, we reversed the valence attached to the odors, so that geraniol CS+ became CS− and inversely for citral (reversal).

**Data analysis**

LFP signals were analyzed off-line with IGOR Pro 5.03 (WaveMetrics, Portland, OR). Analysis was focused on a 4-s interval surrounding the odor onset. Data were inspected to discard trials containing movement artifacts (~5% of trials); these are 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.** Auto-spectra were estimated by first applying a 1,024-point Hamming taper to each data window, then taking the fast Fourier transform (FFT) and computing the spectrum. Half-overlapping 1,024 point windows were used to obtain the averaged power spectrum for the 2-s preodor and odor period time segments. Total power was obtained by integrating the band between 15 and 35 Hz. Power spectra were averaged for the two periods across the 20 (beginner level) or 40 (criterion and postcriterion levels) consecutive trials corresponding to a given performance level. Dynamic power spectra centered on odor delivery were estimated for each 1,024 point window stepped by 250 ms.

**COHERENCE ANALYSIS.** Coherence spectra between the pairs of sites were calculated as the normalized cross-spectral density of two waves using a FFT window of 1,024 samples as has been reported elsewhere (Kay and Freeman 1998; Lowry and Kay 2007). Because coherence is the cross-spectrum normalized by the autospectra of the two signals, it is not affected by the absolute power of these signals. Coherence is then a measure for the interdependence of two signals in the frequency domain with values between zero and one. A value of zero means that the two signals are independent at the considered frequency, whereas a value of 1 means that the signals are identical in frequency and have a constant phase relationship. Enhancement of coherence signifies an increase of frequency similarity and phase consistency between oscillatory signals in two brain regions.

Statistical analyses for beta frequency (15–35 Hz) coherence were done with a FFT window of 1,024 samples. Averaged coherence estimates were obtained by averaging the values in the band between 15 and 35 Hz. Then the values were averaged for the preodor and the odor period across the 20 (beginner level) or 40 (criterion and postcriterion levels) consecutive trials for a given odor-contingency pair corresponding to the given level.

**Statistical analyses**

For behavioral results, we assessed whether the time necessary for the animals to learn the discrimination was different according to odor pair. The number of trials necessary to reach criterion was compared across pairs using a t-test.

Statistical analysis of power and coherence changes were done by two different methods. As the distribution of power values is not normal, statistical comparisons were performed with nonparametric tests. First the difference between values of preodor and odor time windows was tested for each reward condition, training level, and odor using a Wicoxon signed-rank test. Then the difference between beginner versus criterion and criterion versus postcriterion were assessed separately using the power ratio (odor period/preodor period). All pairwise differences were tested using the Mann–Whitney U test for unmatched samples.

Coherence values were normally distributed (Kolmogoroff-Smirnoff Normality test, P > 0.9), so repeated-measures ANOVAs were performed to assess significant differences during the odor
sampling period versus the preodor period (significance level set at \(P < 0.05\)). Bonferroni post hoc tests were used to assess pairwise comparisons. Two independent factors were tested: the level of training (beginner, criterion, postcriterion) and the odor-contingency value (CS+ and CS−).

RESULTS

LFPs were recorded in the OB (ventral portion near the mitral cell layer), dorsal HPC (dHPC, near the hilus of the DG), and ventral HPC (vHPC; in CA1: Fig. 1) while rats performed a two odor GNG discrimination task. After the task was learned with a first odor pair, it was then transferred successively to two other pairs, and the final pair was then reversed in contingency.

Acquisition of the first pair of odors took significantly longer than the others, with an average of 348 ± 72 trials for pair 1 [octanol(+)/propanol(−)], compared with 72 ± 41 and 128 ± 84 trials for pair 2 [hexanol(+)/heptanol(−)] and pair 3 [geraniol(+)/citral(−)], respectively [pairs 1 and 2, \(t(5) = -6.472 P < 0.005\); pairs 1–3, \(t(5) = -3.625 P < 0.05\); pairs 2 and 3, \(t(6) = 1.21 P > 0.1\)]. However, the number of trials to reach criterion for the reversal of pair 3 (423 ± 53 trials) was not different from acquisition of pair 1 \([t(5) = 1.619 P > 0.1\), geraniol(+)/citral(−) vs. citral(+)/geraniol(−) \(t(6) = -5.980 P = 0.001\); Fig. 2, B and C].

To compare changes in the LFP signal associated with odor-discrimination learning, we defined three behavioral levels for every odor-contingency pair: beginner, the first 20 presentations of each odor in the pair (CS+ and CS−); criterion, the 40 trials of each odor starting with the first 90% correct no-go block; and postcriterion, the 40 trials of each odor just after the criterion level.

Modifications of beta band activity during odor sampling

As shown in Fig. 3, during spontaneous activity outside of the odor sampling periods, we observed typical OB LFP activity, i.e., bursts of gamma activity (60–100 Hz) superimposed on slow waves related to respiration. In the HPC, the power spectrum was dominated by the theta frequency (4–12 Hz; Fig. 3, A and B). As soon as the behavioral performance of the animals improved for each odor set, beta oscillations were evident in the OB raw signal during odor sampling (Fig. 3A). Hippocampal signals also contained observable beta oscillatory activity in the same time period with smaller amplitudes than in the OB (Fig. 3A).

To characterize changes in spectral properties, we computed for each animal, recording site and odor-contingency pair, time-frequency plots averaged across 40 trials (20 trials for the beginner level) for the time period surrounding odor onset (−2 s to 2 s relative to odor onset). These show that when power and/or coherence increased, this phenomenon was limited to the beta band (15–35 Hz) and was elicited only during the odor sampling period. Figure 3B illustrates, for one animal, the time frequency representations of the LFP signal surrounding odor sampling at the criterion level. What we observed in the OB, an increase of beta power together with a decrease in gamma power (60–100 Hz) during odor sampling (see Fig. 3A; gamma bursts are indicated with arrows), is consistent with what has already been described (Martin et al. 2004). Figure 4 shows the power spectra averaged for all the animals in the three recorded regions. During odor sampling, beta band power also increased in both the dorsal and ventral HPC. Although the oscillation was lower in amplitude, the frequency band was the same (15–35 Hz). All of the following analyses focus exclusively on the beta band oscillatory activity.

High beta power in the OB is transiently centered on discrimination acquisition

To analyze beta band (15–35 Hz) activity elicited by odor sampling, we compared the 2-s odor period starting with the
odor onset (odor period) with the 2 s just prior to odor onset (preodor period). Analysis performed animal by animal showed that for all behavioral levels (beginner, criterion, post-criterion), during odor sampling there was a significant power increase in the beta frequency band relative to the preodor period (Wilcoxon signed-rank test \( P < 0.001 \)), except for one animal in criterion and postcriterion levels for the first odor pair (octanol-propanol \( P < 0.20 \)).

To compare beta power values across odors, behavioral levels, and rats, we assessed the ratio of power between the odor and preodor periods. In the OB, odor-elicited beta power showed a strong correlation with learning and, aside from variations in amplitude with different odor sets, showed an increase from beginner to criterion levels (Fig. 5; Mann-Whitney U test, \( P < 0.001 \)), except for one animal in criterion and postcriterion levels for the first odor pair (octanol-propanol \( P = 0.20 \)).

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For odor pair 3 (geraniol(+)citral(−)), there was also a decrease in beta power from criterion to postcriterion level (Fig. 3; Mann-Whitney U test, \( P = 0.019 \); pair 2: \( P < 0.001 \); pair 3: n.s.; reversal: \( P < 0.001 \)).

This learning-related increase and then decrease in beta power was not associated with the number of odor presentations or trials. Because criterion performance was achieved in different numbers of trials for each odor-pair, learning level trial blocks (except for the beginner block) did not correspond to the same absolute place in the total number of presentations. This was verified during the reversal task, as after the contingencies were reversed, the same odor set required a larger number of trials before the beta power increase and before the animals reached criterion performance.

**Beta power increases during odor sampling in the HPC**

During odor sampling, the HPC also showed an increase in beta band power although it did not span as large a range as in the OB (Figs. 4 and 6). For all the animals and odor sets, both in the dorsal and ventral HPC, beta power was significantly
signed-rank test: dHPC than the vHPC or equal in the two subfields (Wilcoxon
through beta power during the preperiod was higher in the
power between the odor and preodor periods for each. Even
between the dorsal and ventral HPC, we used the ratio of
band power values (Fig. 6). To compare beta power values
the dorsal and ventral subfields show similar evolutions in beta
associated with learning levels as in the OB. Within the HPC,
did not follow the repeatable series of increases and decreases
range for a coherence increase.

Coherence increases between OB and HPC subfields in the
beta band during odor sampling

Coherence measures allow us to estimate changes in coop-
erativity between brain areas independent of amplitude or
power differences. Figure 7 (top and middle) displays the
coherence values between OB and HPC subfields from the
odor and the preodor periods during criterion level averaged
over all rats. This confirms the specificity of the beta frequency
range for a coherence increase.

Figure 8 focuses on beta band coherence values for beginner
and criterion levels. Contrary to what was observed for other
odor pairs, the first odor set [pair 1, octanol(+)/propanol(−)]
did not show higher beta band coherence during odor sampling
compared with the preodor period for either OB-dHPC
\[ F_{\text{pre/odor}}(1,476) = 1.547 P = 0.21 \text{ n.s.} \] or OB-vHPC
\[ F_{\text{pre/odor}}(1,476) = 1.953 P = 0.16 \text{ n.s.} \] (Fig. 8, top and middle).
For the other two odor pairs and for the reversal, there was a
significant increase in coherence from the preodor to the odor
sampling period for every learning level (ANOVA repeated
measures, \( P < 0.01 \)). Transfer to a new odor set did not
produce a reset of coherence as for beta power in the OB.
However, there was an increase in coherence between beginner
and criterion levels for OB-dHPC for odor pair 2 [with no
interaction with preodor/odor period increase \( F_{\text{level}}(1,396) = 5.153 \, P < 0.05 \) and after reversal for both OB-dHPC \( F_{\text{pre/odor*level}}(1,476) = 3.692, \, P < 0.05 \) and OB-vHPC \( F_{\text{pre/odor*level}}(1,476) = 6.935, \, P < 0.01 \). In addition, in the reversal task there was a significant difference of coherence between CS\(+/-\) and CS\(-/+\) with a significant interaction with the preodor/odor period increase for OB-vHPC: \( F_{\text{pre/odor*odor}}(1,476) = 4.816, \, P < 0.05 \); but not for OB-dHPC \( F_{\text{pre/odor*odor}}(1,476) = 0.269, \, P = 0.6 \, \text{n.s.}, \, F_{\text{odor}}(1,476) = 8.203 \, P < 0.005 \). The coherence increase was not strictly related to the high power of the beta band signal because, for example, the power increase for pair 1 reached about the same level as for pair 3 and did not show a significant increase in coherence.

Because the respiration cycle influences odor processing in the olfactory system (Kepecs et al. 2006), we also examined theta band (4–12 Hz) coherence. In the current study, we only observed a trend toward OB-dHPC coupling in the theta band for CS\(+\) of the first odor set for some rats [see Fig. 7 octanol\(+\) trace]. We did not find consistent fluctuations in coherence correlated with performance (Kay 2005) or with the reversal process (Macrides et al. 1982).

**Coherence between dHPC and vHPC**

Coherence between the two hippocampal subfields showed differences from the OB-HPC patterns. As expected and as can be seen in Fig. 7 (bottom), coherence values in the preodor period were already higher than OB-HPC pairs. Also notice the very high values in theta band coherence for both preodor and odor periods.

Focusing on the beta band (Fig. 8, bottom) one notable feature is that for all four odor-contingency pairs, coherence between the two hippocampal subfields increased from the beginner level to the criterion level [with a significant interaction with preodor/odor period increase except for pair 1; pair 1, \( F_{\text{level}}(1,476) = 9.305 \, P < 0.05 \); pair 2, \( F_{\text{pre/odor*level}}(1,396) = 5.968 \, P < 0.05 \); pair 3, \( F_{\text{pre/odor*level}}(1,476) = 4.183 \, P < 0.05 \); reversal, \( F_{\text{pre/odor*level}}(1,476) = 3.773 \, P = 0.05 \). Thus we conducted separate analyses for beginner and expert levels to assess the coherence increase during odor sampling relative to the preodor period. Indeed, the odor-evoked coherence increase was never significant in the beginner level \( (P > 0.1) \). Beginning with the second learned pair at criterion, odor sampling produced an increase in beta band coherence compared with
DISCUSSION

Our results suggest a functional coupling between the OB and the dorsal and ventral parts of the HPC, related to odor-discrimination learning. LFPs were recorded in these structures in awake behaving rats learning to perform successive odor discriminations in a GNG task. We confirm the close relation between beta activity in the OB, the first relay of olfactory processing, and learning (Figs. 3 Figs. 4 Figs. 5) (Martin et al. 2004; Ravel et al. 2003), and for the first time, we show an increase in beta band (15–35 Hz) oscillatory power in the dorsal and ventral HPC and an increase in coherence between the OB and HPC and between the two subfields of the HPC in the same frequency band during the learning task. All of these effects occur specifically during odor sampling.

Beta oscillation power in the OB and HPC

Beta frequency (15–35 Hz) activity has previously been observed in the olfacto-limbic circuit during presentations of behaviorally significant odors (Zibrowski and Vanderwolf 1997), following repeated presentations of the same odor (Gray and Skinner 1988; Lowry and Kay 2007; Vanderwolf and Zibrowski 2001) or for odors experimentally associated with a reward (Martin et al. 2004). In the present report, for every new odor-contingency pair, the same transient increase in beta power occurs during the build-up of every new association and diminishes once this representation is formed confirms that these oscillations are related to associative odor-discrimination learning in the GNG task and are not just part of a simple familiarization or sensitization process or due to vapor phase concentration (Lowry and Kay 2007).

The involvement of the HPC was different from that of the OB. Beta oscillation power within HPC subfields did not show significant changes with respect to learning level, as beta band power was stable from beginner to criterion levels for most of the odors (Fig. 6). This discounts the hypothesis that the beta increase in the HPC is the result of drive or conduction from the OB. We infer that modulation of HPC beta oscillation power may be driven by the task context or by odor sampling rather than by the learning process itself.

Beta band coherence between the OB and HPC

One of the most important results of the present study concerns the beta band (15–35 Hz) coherence increase between the OB and the two hippocampal subfields that occurs precisely during odor sampling (Figs. 7 and 8). As suggested by computational models of the HPC (Kopell et al. 2000; Schaefer et al. 2006; Traub et al. 2004), slower frequencies (in the beta frequency range or less) are more efficient than faster frequencies for inducing plasticity related phenomena, and large-scale oscillatory coupling is an advantage in binding together widespread areas of the brain (Fries 2005). Experimental data have confirmed these effects, in cats (Roelfsema et al. 1997) and humans (Tallon-Baudry et al. 2001), as slow activity is related to long-range synchronization. Thus under the assumption that beta oscillations serve to functionally connect widely separated
Hippocampal involvement in the network underlying olfactory learning has been suggested by studies showing odor-specific responses of neurons in the dorsal HPC (Wiebe and Staubli 1999; Wood et al. 1999). Its implication in olfaction was also assessed by Chaillan et al. (1999), who showed that olfactory learning was accompanied by potentiation of a polysynaptic response to electrical stimulation of the LOT in the DG of behaving rats.

In this study, contrary to beta power evolution in the OB, we did not see a reliable and repeated increase in OB-HPC coherence that followed the learning process. Also, for the first odor set, there was not a significant increase in OB-HPC beta band coherence (Figs. 7 and 8). However, early in the first transfer to a new odor set coherence between the OB and the two HPC subfields increased and remained high, without resetting at the beginning of each new odor set (Fig. 8), although within the HPC circuit, coherence increased from beginner to criterion levels (see following text). We cannot exclude the possibility that the lack of coherence in the first odor set is dependent on the odors used. However, beta power is significantly increased during odor sampling in the first set in both the OB and HPC without a related increase in coherence, which argues against this interpretation. These results may be explained by the observation that the HPC is more involved in the transfer of a rule to an olfactory stimulus set than in performing an odor discrimination per se. This is consistent with lesion studies (Bunsey and Eichenbaum 1996). One common property of hippocampal-dependent tasks is the requirement of learning “flexibility,” to employ knowledge obtained in one set of circumstances to solve new problems (Cohen and Eichenbaum 1991; Eichenbaum 2001; Eichenbaum et al. 1999; Squire et al. 1993). In our study, for the first odor pair, the rules of the GNG task are learned at the same time as the odor discrimination itself, and acquisition of the following sets requires rule transfer, which very likely involves different networks. Our hypothesis is that a functional link between sensory areas and the HPC is required for the correct behavioral utilization of sensory cues in the specific context where flexibility is needed but not for the sensory process itself.

This hypothesis is supported by other studies. First, it has been shown that hippocampal lesions do not impair rats’ ability to perform an odor-discrimination task, but they cannot express flexible forms of memory, like transitivity (Bunsey and Eichenbaum 1996). Another argument comes from studies of olfactory learning-related cellular modifications (Hess et al. 1995; Knafo et al. 2005), which are detected in the HPC only after acquisition of rule learning. In particular, upregulation of the L1 cell adhesion molecule implicated in synaptic plasticity has been shown to be predictive of rule learning in the piriform cortex but occurs only after completion of rule learning in the HPC (Knafo et al. 2005). That may also explain why no consistent OB-HPC coherence increase was found in the beta band in previous studies (Kay 2005; Kay and Freeman 1998). In those studies, a single odor pair was used; this did not require a rule transfer.

In our analysis of the HPC theta rhythm and oscillations associated with respiration in the same frequency band in the OB, we did not find that coherence magnitude was either positively correlated with performance of a known odor dis-
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