

The olfactory and hippocampal systems of the mammalian brain produce robust oscillatory activity in the theta (4-12 Hz) frequency band of the local field potential (LFP). These two systems are densely and bidirectionally connected, such that sensory information enters the hippocampus (HPC) from the olfactory bulb (OB) across a single synaptic relay in the entorhinal cortex (EC) (1), and the OB receives direct input from the HPC (2).

OB theta rhythm (2-12 Hz) is well-characterized by respiration and afferent input in anesthetized or awake immobile animals (9-12). It has also been shown that centrifugal input plays some role in shaping the theta rhythm and circuit activity even in anesthetized animals and OB slices (13, 14). Hippocampal theta rhythm is defined somewhat differently and accompanies locomotion and cognitive processing. Two theta sub-bands have been described, with high frequency theta (type 1; 6-12 Hz, atropine resistant) linked to locomotion and low frequency theta (type 2; 4-6 Hz, atropine sensitive) seen in urethane-anesthetized animals and during immobile states and sensory stimulation (15).

The HPC is known to be involved in some sensorimotor processes, in rats including exploratory behavior and sniffing rate changes (3). The EC, HPC and other central areas also modulate sensory activity in the OB (4-6). HPC theta activity (4-12 Hz) is related to sniffing behavior during odor learning (7), and OB theta oscillations, which follow respiration, lock intermittently with those in the HPC (8, 16). This study examines olfactory-hippocampal interactions related to exploratory behavior and odor seeking as sensorimotor processes associated with sniffing.

OB respiratory drive and hippocampal theta rhythm have been shown to be coherent when an animal learns a contingency change in a set of two odors (7). It is noteworthy that sniffing is a natural and ubiquitous behavior that accompanies waking arousal states (17) and natural exploratory behavior. Respiratory studies have suggested that the HPC may also be involved in initiation of changes in respiratory behavior (18). Thus, the olfactory and hippocampal systems are linked not only synaptically but also behaviorally by hippocampal initiation of the change in respiratory behavior and simultaneous change in afferent drive to the OB from the olfactory nerve with changes in sniffing rate.

## Experiments and Results

Four rats were implanted with stainless steel electrodes (100 $\mu$ m diameter) in the OB, anterior pyriform cortex (aPC), lateral EC and dentate gyrus of the hippocampus at the hilus (HPC). The behavioral paradigm was a GO/NO GO (CS+/CS-) task as described in **Figure 1**, and sample data are shown. Note that the activity in each of the brain areas changes with approach of the time for the odor stimulus.

The temporal pattern of theta frequency profiles for the four brain areas are shown in dynamic power spectra (**Figure 2**). Peak theta rhythms in the OB advance from as low as 3-5 Hz at the beginning of trials to 6-10 Hz during the odor identification period. A similar pattern can be seen in the aPC, although at much lower power. The EC often maintains a peak theta rhythm in the 4-7 Hz band, typical of type 2 hippocampal theta rhythm, throughout the entire trial. In some cases there is also significant power in the higher frequency band (type 1 theta), as is seen in this case prior to the sniffing onset. The HPC maintains the slower theta rhythm, and shows an additional higher frequency theta rhythm (7-14 Hz) particularly during the odor identification period.

Dynamic coherence spectra from all six pairs of the four brain areas across the task are shown in **Figure 3**. The OB and aPC show high coherence at the OB sniffing frequency, as indicated by the peak theta rhythm from dynamic power spectra. The OB and EC also show coherence in the theta band at a somewhat lower frequency, close to that of the EC theta rhythm. The OB-PC, OB-EC and EC-HPC coherence bands are all interrupted with the onset of the odor stimulus. The EC-HPC interruption occurs for both the CS+ and CS- conditions and is consistent across animals and most recording sessions in this behavioral paradigm. In a minority of experiments this coherence can be found in parts of the prestimulus region as well. The aPC and EC show high coherence throughout the theta band with a distinct band recognizable at the lower hippocampal theta frequency. The aPC and HPC also show a coherent band in this frequency range, as do the EC and HPC. These last patterns vary across experiments and animals.

OB-HPC coherence during the sniffing period also varies both across and within animals, even when overall performance measures (% correct for a day's session) are consistent. OB-HPC coherence is often insignificant for subsets of trials chosen from throughout the experiment. For this reason we performed a longitudinal analysis of coherence stepped in epochs lasting on the order of 10 minutes (15 correct CS+ trials, which span 30-45 total CS+, CS- and no odor control trials). This analysis of consecutive trial blocks is shown in **Figure 4**. Performance measures (% correct) are calculated for each block of trials spanning the 15 correct CS+ trials (stepped by one trial in this figure), and then either only the correct CS+ trials or all CS+ trials are selected for coherence analysis. The performance statistics are stored and associated with the final CS+ trial of each block.

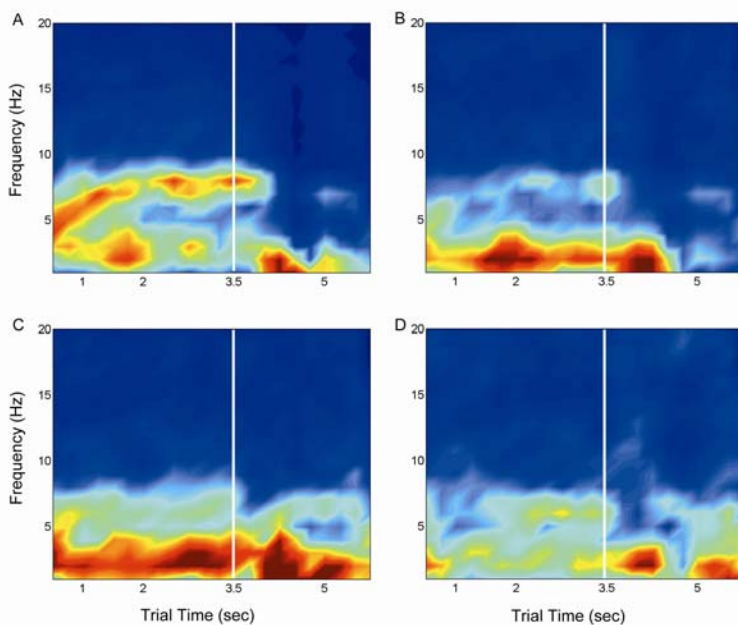
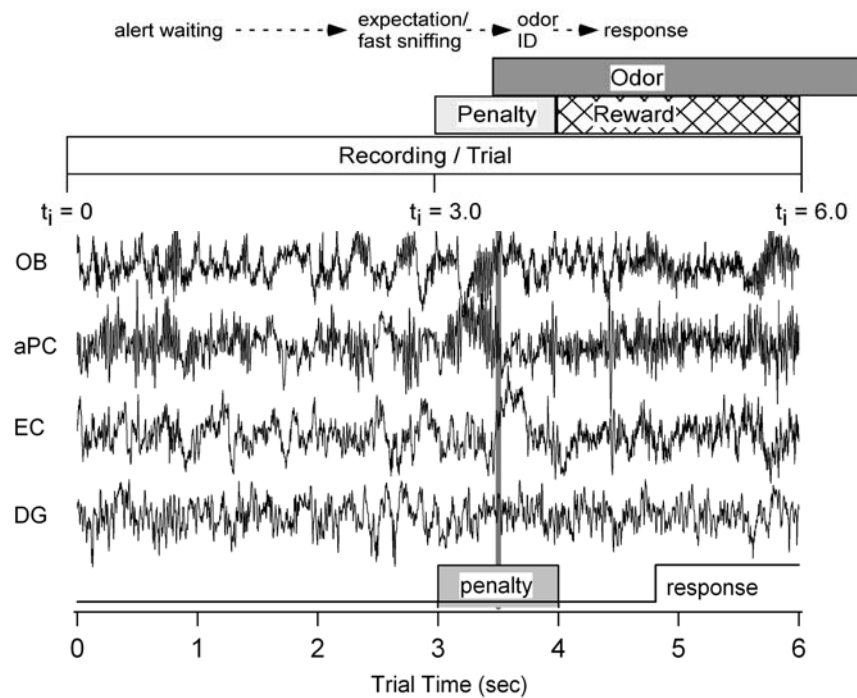
In all tests over all four animals there are trial blocks for which the OB-HPC coherence during the sniffing period (3.25-4.25 sec.) is strong, even when the coherence across a larger set of correct trials selected for similar response times is weak or insignificant. The longitudinal peak frequencies for both the OB and HPC theta band power spectra are shown with the longitudinal coherence spectrum for the odor sniffing period (**Figure 4a**). OB-HPC coherence values vary over the course of a day's experiment (~2 hours), and estimation of the correlation between performance levels and coherence magnitude shows that OB-HPC coherence is positively correlated with performance (**Figure 4b**). This correlation is robust across animals and recording sessions.

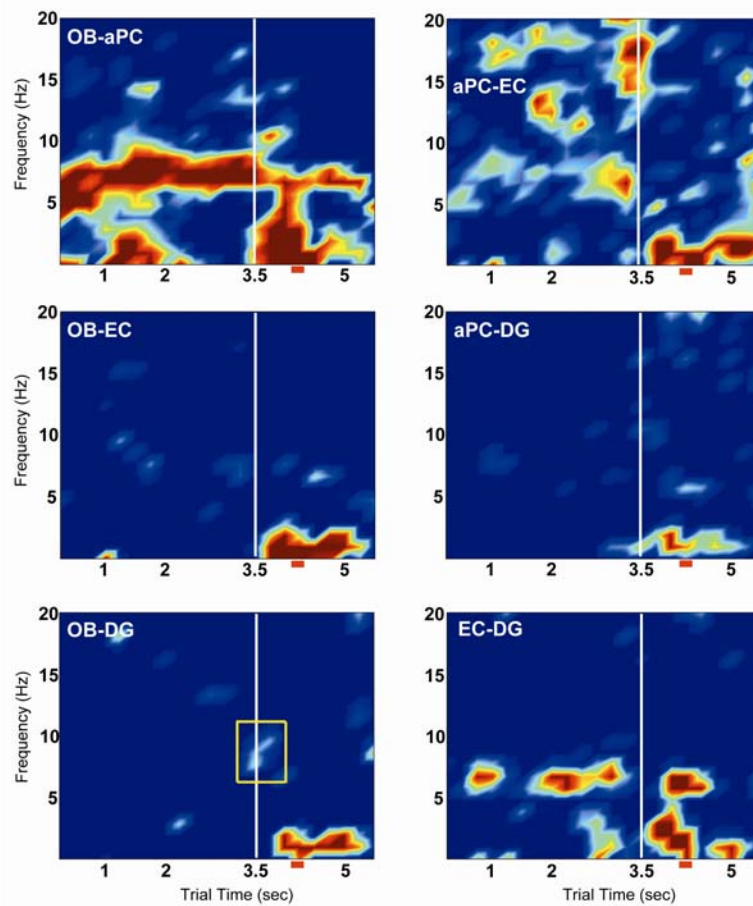
Contingency reversal shows a negative correlation between OB-HPC theta band coherence in the initial CS+ block of reversal learning, as has been shown previously (**Figure 4c,d**) (7). In contrast, the correlation is positive during performance of the two odor task. This suggests that the process of learning a new association may be different from performance of the known task, as the correlation between coherence and performance during reversal learning is negative.

## References

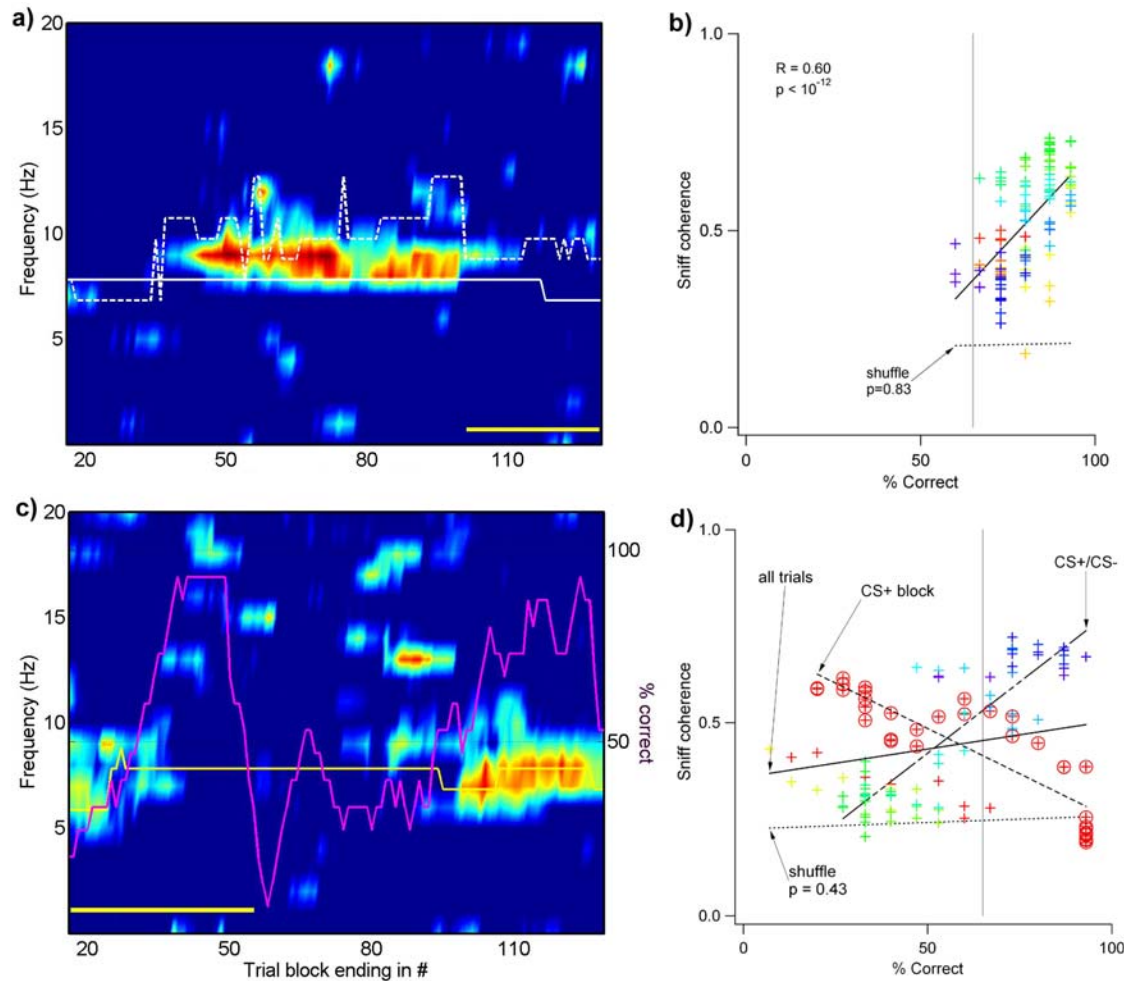
1. Schwerdtfeger, W. K., Buhl, E. H. & Germroth, P. (1990) *J Comp Neurol* **292**, 163-77.
2. van Groen, T. & Wyss, J. M. (1990) *J Comp Neurol* **302**, 515-28.
3. van Lier, H., Coenen, A. M. & Drinkenburg, W. H. (2003) *J Neurosci* **23**, 2459-65.
4. Gray, C. M. & Skinner, J. E. (1988) *Exp Brain Res* **69**, 378-86.
5. Kay, L. M. & Freeman, W. J. (1998) *Behavioral Neuroscience* **112**, 541-553.
6. Kay, L. M. & Laurent, G. (1999) *Nature Neuroscience* **2**, 1003-1009.
7. Macrides, F., Eichenbaum, H. B. & Forbes, W. B. (1982) *J Neurosci* **2**, 1705-17.
8. Vanderwolf, C. H. (1992) *Brain Research* **593**, 197-208.
9. Klingberg, F. & Pickenhain, L. (1965) *Acta Biol. Med. Ger.* **14**, 593-595.
10. Kay, L. M., Lancaster, L. R. & Freeman, W. J. (1996) *International Journal of Neural Systems* **7**, 489-495.
11. Ravel, N., Caille, D. & Pager, J. (1984) *Journal De Physiologie* **79**, A68-A68.
12. Ravel, N. & Pager, J. (1990) *Neurosci Lett* **115**, 213-8.
13. Nickell, W. T. & Shipley, M. T. (1993) *Journal of Neuroscience* **13**, 650-659.
14. Ravel, N., Caille, D. & Pager, J. (1987) *Exp Brain Res* **65**, 623-8.
15. Bland, B. H. & Oddie, S. D. (2001) *Behavioural Brain Research* **127**, 119-136.
16. Forbes, W. B. & Macrides, F. (1984) *Neurobiol Aging* **5**, 7-17.
17. Freeman, W. J., Viana Di Prisco, G., Davis, G. W. & Whitney, T. M. (1983) *J Comp Psychol* **97**, 12-23.
18. Poe, G. R., Kristensen, M. P., Rector, D. M. & Harper, R. M. (1996) *Neuroscience* **72**, 39-48.

## Figures





**Figure 3. Theta band dynamic coherence spectra** for each of the 6 pairings of OB, aPC, EC and HPC. Analysis from 1.024 sec data windows, stepped 0.25 sec across 25 CS+ trials selected for similar response times. Horizontal axis is time through the trial. Onset of the odor stimulus is marked at 3.5 sec. The vertical axis is frequency in the theta and low beta bands (0-20 Hz). Shown are the magnitudes of the t-statistic from the jackknife method with red high and dark blue insignificant. Red bars at approximately 4.2 seconds mark the average time and standard deviation of the bar press response for this set of 25 trials.



**Figure 4. Theta band longitudinal coherence and its relation to performance.** **a)** Longitudinal OB-HPC coherence spectrum from the sniffing period as marked by the horizontal extent of the box in Figure 3 (1.024 sec window centered at 3.75 sec from all CS+ trials). Coherence is displayed as amplitude in color (red high) across the theta band. The dashed line represents peak HPC theta frequency in the range of 7-14 Hz. Solid line: peak OB frequency. **b)** Correlation of performance (% correct in the trial block spanned by the 15 CS+ trials) and OB-HPC coherence in the frequency range spanned by the peak frequencies from the left figure. Coherence is from the time/frequency region indicated by the box in Figure 3 around the sniffing frequency during the odor period. Vertical line: 65% criterion performance level. The colors of the markers represent time through the experiment, with red indicating early trials and dark blue the latest trials. **c)** Contingency reversal learning. OB-HPC coherence over 128 CS+ trials, spanning 200+ total trials. Solid yellow curve is the peak OB frequency. The first block of trials (up to trial 52, marked by the heavy yellow line) contains only CS+ trials; subsequent trials were CS+, CS- and control trials. Performance statistics are overlaid (purple curve). **d)** Positive correlation between performance and coherence in 15 trial blocks at the sniffing frequency for all CS+ trials during learning to criterion and subsequent performance. The initial "CS+ block" of trials shows a strong negative correlation between performance and coherence. Trials spanning the "CS+/CS-" portion of the session show a strong positive correlation between performance and coherence.