TITLE: Sleep-like states modulate functional connectivity in the rat olfactory system

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RUNNING HEAD: Olfactory cortex functional connectivity during sleep

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ABSTRACT

The present study was an examination of state-dependent functional connectivity during spontaneous activity between the piriform cortex and its upstream and downstream connections. Rats were anaesthetized with urethane and allowed to spontaneously cycle between fast-wave and slow-wave states similar to fast-wave and slow-wave sleep states. Local field potential recordings were made from the olfactory bulb, piriform cortex, dorsal hippocampus, amygdala and primary visual cortex. The results demonstrate that during slow-wave sleep-like states, when the piriform cortex shows reduced sensitivity to odor input via the olfactory bulb, there is enhanced coherence with other forebrain structures. Granger causality analyses suggest that the link between piriform cortical and hippocampal activity during slow-wave state is in the direction of the hippocampus to the piriform cortex rather than the reverse. The results suggest that slow-wave sleep-like states may provide an opportunity for the transfer and/or consolidation of information related to odor memories, specifically at a time when the piriform cortex is less sensitive to sensory input.

KEYWORDS:

Piriform cortex, memory, odor, consolidation, perceptual learning, slow-wave sleep
INTRODUCTION

It is increasingly apparent that sleep plays an important role in memory (Deregnaucourt et al. 2005; Diekelmann and Born 2010; Maquet 2001; Shank and Margoliash 2009; Stickgold and Walker 2007; Walker 2009) by allowing replay and/or transfer of recently learned information within and between circuits. Thus, single-unit and ensemble activity during sleep states is shaped by, and may recapitulate activity that occurred during waking (Louie and Wilson 2001; O’Neill et al. 2008; Pavlides and Winson 1989; Peyrache et al. 2009; Skaggs and McNaughton 1996; Sutherland and McNaughton 2000). Furthermore, activity in disparate brain regions, e.g., the hippocampal formation and sensory neocortex, can become temporally synchronized during sleep states, potentially facilitating synaptic plasticity and/or transfer of information between those regions (Ji and Wilson 2007; Molle et al. 2006; Peyrache et al. 2009; Sutherland and McNaughton 2000). Finally, the structure of sleep, and time spent in rapid eye movement (REM) and slow-wave non-REM states can be modified by activity during waking (Datta 2000; Eschenko et al. 2008; Leconte et al. 1974; Magloire and Cattarelli 2009), potentially providing enhanced opportunities for memory associated events or other adjustments in synaptic strength to occur following training (Huber et al. 2004; Karni et al. 1994; Massimini et al. 2009; Yotsumoto et al. 2009).

The olfactory system maybe an ideal model system for studying the interplay between sleep and memory. Odor-specific spatiotemporal patterns emerge in the olfactory bulb due to combinatorial activation of olfactory sensory neurons expressing one of a large set of different odorant receptors, and precise spatial organization of the projections from those sensory neurons into the olfactory bulb (Buck 1996). The piriform cortex serves an experience-dependent pattern recognition role, capable of pattern completion and pattern separation of the olfactory bulb input (Barnes et al. 2008; Haberly 2001). Familiarity with odors can enhance both cortical ensemble decorrelation and behavioral discriminability of the familiar odor from similar odors, i.e., perceptual learning (Kadohisa and Wilson 2006; Li et al. 2008). Disruption of either the cortical afferent input patterns (Chaudhury et al. 2009; Mandairon et al. 2006) or synaptic plasticity within the piriform cortex (Linster et al. 2009; Wilson 2001), can prevent this perceptual learning. Furthermore, the piriform cortex (and olfactory bulb) receive
extensive inputs regarding behavioral state and odor associations, greatly expanding the
nature of the information pattern to be stored. Thus, the piriform cortex, two synapses
from the nose, learns input patterns and uses those patterns to shape odor perception.

In waking animals, functional connectivity between the olfactory system and
other forebrain areas is strongly influenced by behavioral state (Chabaud et al. 2000;
Fontanini and Bower 2005), attention and learning (Gourevitch et al. 2010; Martin et al.
2007; Plailly et al. 2008) and demands of ongoing behaviors (Hermer-Vazquez et al.
2007). Recent work has demonstrated that during periods of slow-wave sleep-like state in
urethane anesthetized animals, the piriform cortex shows reduced reactivity to odor input
(Murakami et al. 2005; Wilson 2010). The present work examined whether during this
apparent off-line slow-wave activity (SWA), cortical functional connectivity shifts
toward other forebrain regions, potentially promoting linking of olfactory information
with context or meaning. The present results suggest strong, state-dependent changes in
functional connectivity of the piriform cortex with other regions during fast- and slow-
wave sleep-like states.

MATERIAL AND METHODS

Subjects

Mature male Long Evans hooded rats, obtained from Charles River Laboratories
were used as subjects. Animals were provided ad lib food and water and recorded from
during the light portion of the circadian cycle. All experiments were conducted in
accordance with the guidelines of the National Institutes of Health and were approved by
the Institutional Animal Care and Use Committee of the Nathan Kline Institute.

Local field potentials and coherence

Animals were anesthetized with urethane (1.5 g/kg) and placed in a stereotaxic
frame for recording. Local field potential were recorded with tungsten microelectrodes
(50-500 kOhms). Potentials were recorded relative to skull reference. Signals were
amplified (200x) and band-pass filtered (0.3Hz to 3 kHz) with an A-M Systems
multichannel band-pass amplifier, digitized (10kHz), and stored and analyzed with
Spike2 software (Cambridge Electronic Design).
Data were collected in two separate experiments. In the first, electrodes were implanted into the olfactory bulb granule cell layer, the anterior piriform cortex, the basolateral amygdala and the dorsal hippocampus. Although not all sites were recorded simultaneously in each rat, at least 3 sites were recorded in each rat, with recordings from at least 4 rats obtained for every pair of sites (n = 4-6). In a subset of these rats, an additional electrode was positioned in the primary visual cortex as a monitor of neocortical slow-wave activity (Murakami et al. 2005). A monopolar stimulating electrode was placed in the lateral olfactory tract (0.1 ms, 500 µA pulses). Recordings in the piriform cortex were made from Layer III. In the second experiment (n = 5 rats), a bipolar electrode with vertical tip separation of 1 mm was placed in the anterior piriform cortex under physiological control, such that the ventral tip was in Layer I and the dorsal tip was in Layer III. Separate electrodes were placed in the olfactory bulb and the dorsal hippocampus in all rats. Recording sites were verified histologically at the end of the experiments.

Data analyses were performed on at least 50 sec of spontaneous activity in both fast-wave and slow-wave states. SWA was distinguished from fast-wave activity (FWA) based on the amplitude of delta frequency oscillations (Wilson 2010) and respiratory rate (see below). Fast-Fourier transform (FFT) spectral analyses were performed in Spike2 with 2.4 Hz resolution. Coherence analyses were performed across the entire 50 sec as a single block with the “cohere” script in Spike2 with 4.8 Hz resolution. The 50 sec duration was chosen to allow determination of stable coherence values representative of a given state, rather than detection of transient, rapidly changing conditions. Coherence within the delta (< 4Hz), theta (5-15 Hz) and beta (15-35 Hz) frequency ranges were determined for statistical analyses. Statistical comparisons between state and pathways were performed with repeated measures ANOVA and post-hoc tests in StatView (SAS Institute Inc., Cary, NC).

Granger causality analysis

Granger causality analysis was conducted on the LFP data from each animal in the second experiment. Granger causality analysis is a technique for determining whether one time series is effective in predicting another. Unlike regression analysis which
reflects correlations only, Granger causality analysis allows for interpretation on the
causality of two time series. The mathematical basis for Granger causality analysis was
well explained in previous studies (Ding et al. 2006; Granger 1969). The basic principle
is to test whether knowing the past of X can help predict Y better than knowing the past
of Y alone, if so, a statistical interpretation can be made that X “Granger causes” Y. The
Granger causality of two variables can be implemented based on two linear regression
functions. A restricted model for prediction of Y by its own past can be expressed as
below:

\[ Y(t) = \alpha_1 Y(t-m) + \epsilon_1(t) \]  

(1)

Where m is the time lag considered for estimation. And the unrestricted model for
prediction of Y by the past of X can be expressed as below:

\[ Y(t) = \alpha_2 Y(t-m) + \beta X(t-m) + \epsilon_2(t) \]  

(2)

An F test can be conducted to test whether the residual of the unrestricted model (2) is
significantly reduced than that of the restricted model (1):

\[ F = \frac{\frac{RSS_r - RSS_{ur}}{m}}{\frac{RSS_{ur}}{T-2m-1}} \]  

(3)

Where \( RSS_r \) is the sum of squares for the restricted model and \( RSS_{ur} \) is the sum of squares
for the unrestricted model. \( T \) is the total number of observations. If the F statistic is
significant, an interpretation can be made that X “Granger causes” Y. The mathematical
basis for multivariate estimation is much more complicated. The current study utilized
the implementation via multiple vector autoregressive (MVAR) modeling with the
assistance of the Granger Causal Connectivity Analysis Matlab Toolbox (Seth 2010) and
the BSMART toolbox (Cui et al. 2008). Detrending and demeaning was conducted as
preprocessing steps, to subtract the best-fitting line and to remove the temporal mean
separately. Covariance stationarity was tested on the time series matrix, as an effort to
restore the covariance stationarity, we used successive time window of 1000 time points
(1 sec) for low pass filtering, and calculated the Granger causality values within the
successive windows; stability of the Granger causality values was evaluated and each pair
of the Granger causality values maintained constant between the successive windows
over the 50 sec analysis block. The lag for estimation (corresponding to the “m” in function (1)) was 20 data points (20ms in our data). The order for regression was 10 based on our pilot study and a previous study (Brovelli et al. 2004). Granger causality density values together with accompanying significance parameters from the above analysis were used to generate the illustrative figures about the directionality (Figure 5), in a similar way as in a previous study (Seth 2005). Granger causality density values within the delta (< 4Hz), theta (5-15 Hz) and beta (15-35 Hz) frequency ranges were determined for further statistical analyses.

Since the Gaussian nature of the distribution of the Granger causality density values is not known, non-parametric statistics were applied for cross-condition comparison. For each frequency band, two-tailed 2-related sample Wilcoxon tests were applied to test the differences between two directions in each pair of recording sites. For each frequency band, two tailed 2-independent sample Mann-Whitney tests were applied to test the differences between the two states in each direction between each pair of recording sites.

RESULTS

Spontaneous local field potentials

Spontaneous activity during urethane anesthesia transitions between FWA and SWA as evidenced in the local field potentials (Murakami et al. 2005; Wilson 2010). In the piriform cortex, SWA is characterized by large delta frequency oscillations and relatively reduced beta frequency activity compared to FWA (Fig. 1). Transitions between states can occur rapidly and each state can remain stable for anywhere from 5 min to over 1 hour. The state transitions observed in piriform cortex occurred simultaneously with similar transitions in the neocortex (Fig. 1). It should be noted that respiration rate was significantly depressed during slow-wave activity (SWA) compared to fast-wave activity (FWA; FWA mean respiration rate = 2.4 ± 0.2 Hz; SWA = 1.7 ± 0.1 Hz, paired t-test, p < 0.02). Piriform cortex delta band power significantly, negatively correlated with respiration rate across animals (r = -0.75).

Electrical stimulation of the lateral olfactory tract evokes a surface negative potential, which reverses at the cell body layer (II) to a positive wave recorded in the
deeper layer III (Freeman 1959; Haberly and Shepherd 1973; Ketchum and Haberly 1993). In contrast, the slow oscillations characteristic of SWA include a large surface positive wave in the piriform cortex, which reverses at layer II to a large negative waveform in layer III. This differential positioning of the negative potentials suggests that in contrast to the excitation of synapses in layer I by afferent input from the olfactory bulb, slow-wave activity is dominated by intracortical and/or descending fiber synapses located in layer III (Behan and Haberly 1999).

It should be noted that lateral olfactory tract evoked potentials could also be recorded in the dorsal hippocampus, and these potentials reversed in polarity as the electrode passed through the different cell layers (Wilson and Steward 1978), suggesting that local field potentials recorded there were locally generated. Our monopolar recordings in lateral amygdala did not show similar polarity reversals due to the nuclear organization of this structure, and thus it is possible that these amygdala local field potentials may include contamination from locally surrounding tissue.

State-dependent changes in coherence

Coherence of spontaneous activity between the piriform cortex and olfactory bulb and forebrain regions was examined during both FWA and SWA in 50 sec blocks for each state. As shown in Figure 2, during FWA, both the olfactory bulb and piriform cortex were strongly entrained to respiration. During cortical slow-wave periods, however, the olfactory bulb did not display typical slow-wave activity, but rather maintained a strong entrainment to respiration. This resulted in a decrease in coherence of spontaneous activity between the olfactory bulb and piriform cortex during SWA compared to FWA, primarily in delta and theta band activity. In contrast, coherence between the piriform cortex and dorsal hippocampus was dramatically enhanced during SWA in the delta and theta frequency range compared to FWA. During FWA, the dorsal hippocampus would frequently enter a theta band oscillation, independent of the respiratory cycle driving piriform cortical activity.

As shown in Fig. 3, there was significant state-dependence in coherence between pathways in both the delta (pathway × state repeated measures ANOVA, main effect of pathway F(2,13) = 52.30, p < 0.001; main effect of state F(1,13) = 63.60, p < 0.001;
pathway X state interaction, $F(2,13) = 26.87$, $p < 0.001$), and theta (main effect of
pathway $F(2,13) = 47.75$, $p < 0.001$; main effect of state $F(1,13) = 24.62$, $p < 0.001$;
pathway X state interaction, $F(2,13) = 11.35$, $p < 0.001$) frequency bands, but no state-
dependence of beta frequency coherence (main effect of state, $F(1,13) = 1.73$, $p = 0.21$
N.S.).

During SWA, coherence between the piriform cortex (recoded in layer III) and
both the dorsal hippocampus and basolateral amygdala was significantly enhanced
compared to FWA in both the delta and theta frequency bands (post-hoc Fisher tests, $p <$
0.05). In contrast, the coherence between the piriform cortex and the olfactory bulb
showed the reverse pattern, with a nearly significant ($p = 0.06$) decrease during SWA
compared to FWA, especially in the theta band.

Laminar differences in coherence

In five separate rats, we recorded LFP’s in both layer I and layer III of the
piriform cortex, and compared functional connectivity between these individual lamina
with input (olfactory bulb) and output (dorsal hippocampus) regions, as well as directly
between layer I and III. As above, state-dependent effects on coherence were limited to
the delta and theta bands, with no effect in beta frequencies. State-dependent changes in
coherence (pathway and lamina x state, delta: $F(4,18) = 9.52$, $p < 0.001$; theta; $F(4,18) =$
7.03, $p < 0.01$) were observed between layer III field potentials and the olfactory bulb and
dorsal hippocampus, but not between layer I and those structures. That is, similar to that
reported above, coherence in the delta and theta frequencies was enhanced between
piriform cortex layer III and the dorsal hippocampus during SWA compared to FWA
(post-hoc Fisher tests, $p < 0.05$). Furthermore, coherence was significantly decreased in
both the delta and theta frequencies during SWA compared to FWA between piriform
cortex III and the olfactory bulb (post-hoc Fisher tests, $p < 0.05$). Importantly, coherence
between layer I and III was also decreased during SWA in the theta band (post-hoc Fisher
tests, $p < 0.05$), suggesting additional evidence for a state-dependent filter within piriform
cortex (Murakami et al. 2005; Wilson 2010).
The above results suggest that during SWA when functional connectivity (coherence) between the olfactory bulb and piriform cortex is decreased, functional connectivity between the piriform cortex and amygdala and hippocampal areas is enhanced. This enhanced synchrony/functional connectivity may allow greater information flow between the piriform cortex and these sites during SWA. In order to explore the directionality, if any, of this information flow, we performed Granger causality analyses on pathways into and out of the piriform cortex during the two different states. The laminar data from experiment 2 (Fig. 4), where all animals had the same collection of recording sites, were used for these analyses.

As shown in Figure 5, spontaneous activity showed a strong directionality, which in some cases was state-dependent. Only those pathways showing a consistent, reliable directionality and/or state-dependence are shown. Wilcoxon tests reveal that Granger causality in the direction of dorsal hippocampus toward piriform cortex layer III was significantly stronger than in the opposite direction across the delta and beta bands (p < 0.05). There was also greater Granger causality in the direction of the dorsal hippocampus to the olfactory bulb than in the reverse direction in the beta band (p < 0.05) accompanied by a marginally significant difference in the theta band (p = 0.093). Mann-Whitney tests revealed that in the theta band there was also a significant state difference in the Granger causality from the dorsal hippocampus towards the olfactory bulb, in the delta band there was a marginally significant state difference in Granger causality from the piriform cortex I towards the dorsal hippocampus (p = 0.076).

In delta band activity, there was stronger Granger causality of the dorsal hippocampus on piriform cortex (layer I and III) than in the reverse direction in both FWA and SWA. This was observed in 4 out of 5 individual rats. The hippocampal influence on piriform cortical activity increased during SWA. In spontaneous beta band FWA, again, Granger causality was strongest in the direction of hippocampus to piriform cortex, although this directionality was lost during SWA when there was no net difference between piriform cortex-to-hippocampus Granger causality versus hippocampal-to-piriform cortex Granger causality. A similar dominance for
hippocampal-to-olfactory bulb Granger causality was observed in that pathway, though most consistently within the theta frequency band.

Finally, a comparison between Figures 4 and 5 reveals that while coherence was highly state dependent in several pathways, the strength and direction of Granger causality across pathways was relatively more stable. For example, while coherence between the dorsal hippocampus and piriform cortex layer III was state-dependent, with significantly greater coherence during SWA in both delta and theta frequency bands, the relative strength of Granger causality in those bands was consistently in the direction of hippocampus to piriform cortex in both SWA and FWA, with only minimal state-dependence.

**DISCUSSION**

The present study was an examination of state-dependent functional connectivity during spontaneous activity between the piriform cortex and its upstream and downstream connections. The results demonstrate that during slow-wave sleep-like states, when the piriform cortex shows reduced sensitivity to odor input via the olfactory bulb, there is enhanced coherence with forebrain structures such as the amygdala and dorsal hippocampus. Using Granger causality analyses, it is apparent that the link between piriform cortical and hippocampal activity during SWA is in the direction from the hippocampus to the piriform cortex, rather than the other way around. The results suggest that slow-wave sleep-like states may provide an opportunity for the transfer and/or consolidation of information related to odor memories, specifically at a time when the piriform cortex is less sensitive to sensory input. This activity could contribute to plasticity critical for perceptual learning and/or associative memory.

As expected, the emergence of SWA in the urethane anesthetized rat piriform cortex occurs simultaneously with that of slow-wave in the rest of the forebrain. Associated with this state transition was a decrease in respiration rate. While activity in the piriform cortex became uncoupled from respiration during SWA (Fig. 1), the olfactory bulb maintained its respiratory entrainment. The uncoupling of the piriform cortex from respiration during SWA is also apparent in the decrease in coherence observed between the olfactory bulb (which maintains its respiratory rhythm) and
piriform cortex (Fig.'s 3 and 4) in low frequency bands, as well as the previously reported decrease in odor-evoked activity in piriform cortical units (Murakami et al. 2005; Wilson 2010). During SWA, piriform cortical neuron membrane potential becomes biphasic with an up- and down-state (Murakami et al. 2005) similar to that observed in thalamocortical systems (Steriade et al. 2001), and the cells become less responsive to odor or electrical stimulation of cortical sensory afferents (Murakami et al. 2005; Wilson 2010). Similarly, in both rats (Seelke and Blumberg 2004) and humans (Carskadon and Herz 2004) odors are less arousing during slow-wave sleep than during waking or fast-wave sleep states, though olfactory responses can be observed in the hippocampus during slow-wave sleep (Rasch et al. 2007). Given the maintained responsiveness of the olfactory bulb to sensory input during SWA while the piriform cortex becomes relatively uncoupled from sensory input, one may argue that the piriform cortex is serving a gating role similar to the thalamus in thalamocortical sensory systems (Shepherd 2005), though based on other aspects of information processing, such a role has been ascribed to the olfactory bulb (Kay and Sherman 2007). This region of the olfactory system may not fit easily into a thalamocortical analogy.

During this relative off-line SWA period, coherence between the piriform cortex and both the amygdala and dorsal hippocampus significantly increased, particularly in the delta and theta frequency bands. The enhanced coherence between the piriform cortex and amygdala during SWA could help facilitate the association of odor information with hedonic valence acquired during waking. For example, activity in the piriform cortex reflects not only odor quality information, but also varies with learned odor hedonics (Moriceau et al. 2006; Sacco and Sacchetti 2010). This association may be influence by co-activity in the piriform cortex and amygdala during post-training sleep. Granger causality analyses suggests the increase in coherence between the piriform cortex and dorsal hippocampus primarily reflects activity flow from the hippocampus to the olfactory system. In contrast, during active odor sampling in a behavioral task, directional information flow is primarily from the olfactory system to the hippocampus (Gourevitch et al. 2010). Thus, during periods when the piriform cortex is less responsive to sensory input, it is more strongly coherent with, and receptive to input from the hippocampus. Based on comparisons with thalamocortical sensory systems (Ji and Wilson 2007; Molle
et al. 2006; Sutherland and McNaughton 2000), this synchronous activation of distant neural circuits may contribute to synaptic plasticity involved in memory consolidation.

In the olfactory system, at least two forms of information may need to be remembered and consolidated following exposure to new odors. Experience-dependent plasticity in the olfactory system is not only involved in associative odor memory (i.e., this odor signals reward or foot-shock; (Brennan et al. 1990; Cohen et al. 2008; Mouly et al. 2001; Wilson et al. 2004)), but also is required for synthetic processing of odor objects which forms the basis of odor perception (Gottfried 2010; Kadohisa and Wilson 2006; Wilson 2003). Familiar odors can be more easily discriminated from similar odors than novel odors (Fletcher and Wilson 2002; Li et al. 2008; Mandairon et al. 2006; Rabin 1988), and this change in perceptual acuity is, in part, based on changes in association fiber synaptic strength within piriform cortex (Linster et al. 2009; Wilson 2001). Work ongoing in unanesthetized animals is exploring the role of sleep in these two forms of odor memory (Barnes et al. 2010).

Finally, the present results suggest that different frequency ranges may be differentially involved in the processes described here (Kay et al. 2009; Poo and Isaacson 2009). For example, gamma frequency oscillations in the olfactory bulb are believed to be mediated by local circuit interactions between granule cell GABAergic interneurons and output neurons, mitral and tufted cells (Halabisky and Strowbridge 2003; Rall and Shepherd 1968). Gamma oscillations can emerge either during expectation of odor sampling (Kay and Freeman 1998; Ravel et al. 2003) or during odor sampling (Beshel et al. 2007; Freeman and Viana Di Prisco 1986), and may contribute to fine-tuning spatiotemporal patterns of olfactory bulb output (Nusser et al. 2001). Gamma oscillations in the piriform cortex may also be influenced by pyramidal neuron-interneuron interactions (Eeckman and Freeman 1990; Luna and Schoppa 2008; Neville and Haberly 2003) and contribute to transfer of information from the olfactory bulb (Litaudon et al. 2008). Beta frequency oscillations appear to emerge from interplay between the piriform cortex and olfactory bulb (Hermer-Vazquez et al. 2007; Martin et al. 2006; Neville and Haberly 2003), and are strongly influenced by learning, behavioral state, expectation or attention, and task demands (Chabaud et al. 2000; Chapuis et al. 2009; Kay and Beshel 2010; Kay and Freeman 1998; Lowry and Kay 2007; Martin et al. 2007; Martin et al.
Activity in the delta and theta frequency ranges is known to be critical for several aspects of normal olfactory function, playing roles in stimulus sampling (breathing, sniffing) (Fontanini and Bower 2006; Kay 2005; Macrides and Chorover 1972; Schroeder et al. 2010) as well as induction of synaptic plasticity within piriform cortex (Jung et al. 1990). The present results suggest another role for these low frequency oscillations may be related to functional connectivity within olfacto-limbic circuits during slow-wave sleep and off-line odor processing.
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FIGURE CAPTIONS

Figure 1. Slow-wave activity in the piriform cortex. (A) During fast-wave activity cortical activity is driven by respiratory activity. Activity becomes uncoupled from respiration during SWA. The slow-wave are negative in Layer III and reverse to positive waves in Layer I. This is the opposite of lateral olfactory tract afferent evoked activity. (B) Slow-wave activity is evidenced by strong delta frequency (1-4 Hz) activity in the piriform cortex compared to FWA and a decrease in beta frequency activity. Note difference in scales of the FFT power plots. (C) Cycling between slow-wave and fast wave activity occurs at the same time in piriform cortex and neocortical regions such as the visual cortex (V1). Electrode locations shown. The onset of slow-wave activity is associated with a decrease in respiration rate and reduced variability in instantaneous frequency of respiration (Inst. Freq.). Simultaneous respiratory plethysmograph traces are shown with each trace (resp).

Figure 2. State dependent changes in coherence between the piriform cortex (Layer III) and afferent and efferent connections. On the left are raw traces of spontaneous activity recorded in the piriform cortex simultaneously with either the olfactory bulb or dorsal hippocampus. On the left are coherence measures taken over 50 sec of spontaneous activity in each state. (A) Activity in the main olfactory bulb (OB) is more closely coherent with the anterior piriform cortex (PCX) during FWA compared to SWA, particularly at lower frequencies. Note that the olfactory bulb does not demonstrate classic SWA. In contrast, activity in the piriform cortex and both the (B) basolateral amygdala (BLA) and (C) dorsal hippocampus (HPC) becomes more coherent during SWA compared to FWA. Note the presence of theta activity in the hippocampus during FWA while the piriform cortex is in phase with respiration. Recordings in A, B and C are from different animals. (D) Electrode location in piriform cortex Layer III. (E) Electrode location in dorsal hippocampus (dentate hilus).

Figure 3. Waveform coherence between the anterior piriform cortex and the olfactory bulb and forebrain structures is state dependent. Piriform cortex coherence in the delta and theta frequency bands is significantly enhanced during SWA compared to FWA. In
contrast, coherence between the piriform cortex and olfactory bulb is marginally reduced in the delta band during SWA. Asterisks signify significant difference between states, \( p < 0.05 \). “a” signifies nearly significantly different, \( p = 0.06 \). Abbreviations: PCX – piriform cortex; OB – olfactory bulb; BLA – basolateral nucleus of the amygdala; dHPC – dorsal hippocampus.

**Figure 4.** Laminar differences in state-dependence of waveform coherence. Changes in coherence were most pronounced for piriform cortex layer III recordings than layer I, with decrease coherence with the olfactory bulb and enhanced coherence with the dorsal hippocampus during SWA. Coherence between layer I and III in the theta band was also significantly reduced during SWA compared to FWA.

**Figure 5.** Granger causality measures of directionality during state changes. The pathways shown displayed either differential directionality or state-dependent changes. Other pathways tested did not differ in either direction or state-dependence. The strong delta and theta coherence between piriform cortex and hippocampus during SWA appears to be primarily driven by hippocampal feedback to piriform cortex (layers I and III), rather than piriform cortical input to hippocampus. Similarly, the fast-wave beta coherence between piriform cortex and hippocampus is primarily in the direction of hippocampus to piriform cortex.
A

FWA

OB
PCX
resp

SWA

OB
PCX
resp

B

BLA
PCX
resp

C

dHPC
PCX
resp

200µV
1 sec

D

E