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State-dependent functional connectivity of rat olfactory system assessed by fMRI

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Abstract

Functional connectivity between the piriform cortex and limbic and neocortical areas was assessed using functional magnetic resonance imaging (fMRI) of urethane anesthetized rats that spontaneously cycled between slow-wave and fast-wave states. Slow-wave and fast-wave states were determined indirectly through monitoring of respiration rate, which was confirmed to covary with state as determined by electrophysiological recordings. Previous electrophysiological data have suggested that the piriform cortex shifts between responsiveness to afferent odor input during fast-wave states and enhanced functional connectivity with limbic areas during slow-wave state. The present results demonstrate that fMRI-based resting state functional connectivity between the piriform cortex and both limbic and neocortical areas is enhanced during slow-wave state compared to fast-wave state using respiration as an indirect measure of state in urethane anesthetized rats. This state-dependent shift in functional connectivity may be important for sleep-dependent odor memory consolidation.

Keywords

piriform cortex; slow-wave sleep; functional magnetic resonance imaging; resting state; hippocampus; amygdala; memory consolidation

INTRODUCTION

Normal brain function requires extensive interaction between anatomically distinct brain regions. This functional connectivity mediates information flow essential for sensory processing, cognition, memory and behavior. Functional connectivity between regions is evident both during active information processing and behavior, as well as during

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“spontaneous” or resting states [4, 17, 30]. Functional connectivity between regions can be observed either by correlated single-unit activity [25], or more commonly through correlation and/or coherence between local field potentials recorded at the scalp in EEG or with depth electrodes [14]. In functional magnetic resonance imaging (fMRI) data, resting state functional connectivity emerges as a very low frequency (<0.1 Hz) correlation in activity [4, 17, 30], in part due to the temporal constraints of fMRI data collection.

Although modest to strong long-term stability has been shown for such “resting state” networks [27, 34], functional connectivity can vary based on ongoing task demands, past experience, and internal state [6, 8, 12, 15, 18]. Furthermore, functional connectivity varies over the sleep-wake cycle in limbic and neocortical circuits [12, 15, 28]. It has been hypothesized that tight coupling of activity between hippocampal and neocortical or limbic circuits during sleep may facilitate memory consolidation [22, 29]. For example, hippocampal replay of recent experiences during slow-wave sleep coincides with neocortical spindles which may facilitate transfer of information from hippocampal to neocortical circuits and/or enhanced binding of information in disparate cortical regions [28, 31].

Recent evidence suggests similar phenomena may occur in the olfactory system. The piriform cortex serves as an experience-dependent, auto-associative, pattern recognition device within the olfactory system [2, 10], promoting synthesis of odor objects from the spatio-temporal patterns of odorant features encoded in the olfactory bulb [13]. The piriform cortex is also reciprocally connected with other regions encoding information about odor hedonics, contextual associations and multimodal inputs, such as the basolateral amygdala, entorhinal cortex and orbitofrontal cortex. [21, 23]. Odor responses in the rodent piriform cortex are suppressed during slow-wave sleep compared to either awake or rapid-eye movement sleep [1], despite relatively maintained olfactory bulb activity [16]. Similar odor hypo-responsiveness can be observed during slow-wave states in urethane-anesthetized rats, which spontaneously cycle between fast-wave and slow-wave states [7, 20]. During this relatively ‘off-line’ state, piriform cortical single-unit activity is modified by recent fast-wave state odor experience [32], and functional connectivity among the piriform cortex and amygdala and dorsal hippocampus is enhanced [33]. These findings are consistent with a potential role for slow-wave sleep in the consolidation and transfer of odor memories within the olfactory cortex and limbic regions.

The present study further explored state-dependent changes in functional connectivity between the piriform cortex and other regions using fMRI as urethane anesthetized rats spontaneously cycled between fast-wave and slow-wave states. We took advantage of the known state-dependent change in respiration rate [33] to identify state in the MRI without the need for simultaneous electrophysiological recordings. The results confirm and extend our recent electrophysiological data [33] and show a strong enhancement in resting state connectivity between the piriform cortex and both limbic and neocortical areas during slow-wave compared to fast-wave states.

MATERIAL and METHODS

Subjects

Male Long-Evans hooded rats (200-350g) were used as subjects. Animals were housed individually in polypropylene cages on a 12 h light/dark cycle, with food and water available *ad libitum*. 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. All testing was performed during the light portion of the day-night cycle.

Electrophysiological data

The representative electrophysiological and respiration data used for verification of changes in respiration rate with brain state were from 3 rats used in a previously published study [33]. Briefly, animals were prepared the same as for MRI, with urethane anesthesia (1.5 g/kg) and maintained on heating pad for the duration of recordings. A tungsten microelectrode was implanted into the anterior piriform cortex for recording of spontaneous local field potentials (LFP), which were amplified (200×), band-pass filtered (0.5-300Hz) and digitized (10 kHz) for analysis by Spike2 software (Cambridge Electronic Design). Respiration was recorded simultaneously with a piezoelectric plethysmograph attached to the animals chest.

Slow-wave state was defined by the presence of large delta frequency (0.5-4 Hz) cortical oscillations and relatively low beta frequency (15-35 Hz) activity, while fast-wave state was defined as low delta activity and higher beta frequency activity [5, 20, 32]. Thus, slow-wave state could be reliably marked by the root-mean square (r.m.s.) of local field potentials band-pass filtered to delta frequency. Analyses were made of LFP mean delta r.m.s. and simultaneous respiratory rate (breaths/min; BPM) within each animal over time.

Animal Preparation for MRI

Animals were anesthetized with urethane (1.5 g/kg). Temperature was monitored through a rectal probe. An SA instruments Animal monitoring unit (model 1025, Stony Brook, NY) was used for physiological monitoring. This unit monitors animal temperature, ECG and respiration. Body temperature was computer controlled through a forced warm air unit interfaced to the SA instrument unit. The animals were secured in the RF coil with a bite bar and stereotaxic holder.

MRI Acquisition

The image acquisition consists of an interleaved snapshot Echo Planar Imaging (EPI) module. This approach splits the conventional EPI sequence into a series of excitation-acquisition blocks applied in immediate succession within a single repetition period. The full details of this acquisition strategy have been described previously [9]. Because there are virtually no delays between the acquisition blocks, there is almost no loss of temporal resolution in comparison with conventional EPI. The susceptibility distortions are minimized owing to shorter sampling intervals after each excitation, in much the same way as parallel acquisitions methods.

Briefly, there are four key elements: 1) Variable flip angles $\alpha_s = \sin^{-1}(1/\sqrt{n-s})$, $s = 0, 1, 2, \dots, n-1$ is employed to equalize the transverse magnetization among all n segments. 2) Polarity of the read gradient is reversed between the segments to preserve the k -space structure of a traditional EPI in the dataset combined from all interleaved segments. 3) Onset of acquisition in a segment s is delayed by $(s/n) T_{RL}$, where T_{RL} = readout length, to ensure smooth T^*_2 decay over the k -space data set, free of a step-wise modulation and the associated ghosting in the reconstructed image. 4) There are no other delays between the acquisition blocks to avoid any loss of temporal resolution in comparison with the conventional EPI.

Image processing was conducted in native space using the 1000 Connectomes project scripts [3](available at http://fcon_1000.projects.nitrc.org/). Briefly images were corrected for motion, despiked, skull-stripped, smoothed (2 mm FWHM) and band-pass filtered at 0.009 – 0.1 Hz. In addition, linear and quadratic trends were removed from the data.

Functional connectivity was determined for regions of interest (ROIs) placed in the piriform cortex, amygdala, dorsal hippocampus, and cerebral cortex on the rats' high resolution (fast

spin echo) image by one of the authors (DAW). The time series for each of these ROIs was extracted and regressed against the time series for all other voxels in the EPI image set. From this, we obtained functional connectivity measures for each animal and in each condition. Cross correlations between time series for different ROI pairs were determined within each animal and compared across all animals and both states using repeated measures analysis of variance (ANOVA).

RESULTS

Respiration as an assay of state

Previous reports have demonstrated that under urethane anesthesia, rodents cycle between fast-wave states and slow-wave states as determined by cortical LFP recordings [20, 33]. Furthermore, respiration rate is depressed during slow-wave state compared to fast-wave state [33]. This suggested that respiration rate could be used as an assay of brain state under urethane anesthesia. As shown in Fig. 1, although the specific respiratory rate and slow-wave delta power measures varied between animals, the state-dependence was reliable across all animals. Paired t-tests between slow-wave activity and fast-wave activity showed a significant increase in mean delta frequency r.m.s. ($t(17) = 10.25$, $p < 0.001$) during slow-wave activity. Respiration rate was significantly decreased during slow-wave state ($t(17) = 11.71$, $p < 0.001$). This steady state change in respiration rate allowed us to monitor slow-wave and fast-wave brain states indirectly while animals were in the MRI by monitoring BPM over time and observing transitions between periods of stable rate. Determining a state change required a large (> 20 BPM) transition between two stable (> 5 min) periods, and thus excluded respiratory changes due to sniffing or other transient causes. For the animals used in the MRI studies described below, the mean difference in respiration rate between putative fast-wave and slow-wave states was 42 ± 8 BPM (range 24 - 79 BPM; Fig. 2).

Functional imaging

All data were obtained on a 7.0 Tesla Agilent (Santa Clara, CA) 40 cm bore system. The gradient coil insert had an internal diameter of 12 cm with a maximum gradient strength of 600 mT/m and minimum rise time of 200 μ s. A Rapid (Rimpar, Germany) volume transmit (72 mm ID) and a 4 channel receive-only surface coil was used for one animal and a quadrature transmit/receive RF coil (Ekam Imaging, Shrewsbury, MA) with internal diameter of 38 mm was used for the other animals. Both coils had the same signal to noise ratio. All resting state data were acquired with the following parameters: field of view = 35 mm, slice thickness = 1mm, echo time = 13 ms, repetition time = 2 s, number of volumes = 120, 3 segments with variable flip angles = 35°, 45° and 90°.

Five rats showed clear cycling of respiration rate between two stable states and their resting state functional connectivities (ROI time series cross-correlation) were assessed in both putative brain states. As shown in Fig. 3, functional connectivity between time series in our ROI's was significantly enhanced during slow-wave activity compared to during fast-wave activity (repeated measures ANOVA, Pathway $\times$ State, main effect of state: $F(1,20) = 8.56$, $p < 0.01$). There was no significant main effect of Pathway ($F(4,20) = 1.11$, N.S.) and no significant Pathway $\times$ State interaction ($F(4,20) = 0.85$, N.S.). All pathways examined involving the piriform cortex or the dorsal hippocampus showed significant (post-hoc Fisher tests, $p < 0.05$) increases during slow-wave activity, with the exception of the piriform cortex-amygdala pathway.

DISCUSSION

The present results demonstrate that fMRI-based resting state functional connectivity between the piriform cortex and both limbic and neocortical areas is enhanced during slow-wave state compared to fast-wave state using respiration as an indirect measure of state in urethane anesthetized rats. These results extend previous work on state-dependent changes in piriform cortical functional connectivity [33] by showing greater SWA connectivity not only with the limbic system, but also neocortex. Further, they demonstrate a novel, non-invasive method of analyzing state-dependent changes in activity and connectivity through the combined use of respiration monitoring and fMRI. However, while the local and regional structure of neural activity under urethane anesthesia closely parallels that of the unanesthetized brain, urethane modulates a variety of neurotransmitter systems [11]. Thus, although shifts between fast-wave and slow-wave state are strikingly similar to shifts between waking and slow-wave sleep in the olfactory pathway [1, 20], care must be taken in extending these findings to interpretation of events during natural sleep states.

Nonetheless, the observed enhancement in piriform cortical connectivity with other central regions may contribute to odor memory consolidation during periods of reduced interference from outside odor stimuli [1, 20, 32]. In essence, during the odor hypo-responsive slow-wave sleep state, the piriform cortex shifts from monitoring olfactory bulb input to becoming functionally linked to emotion, memory and other central circuits. In fact, it has recently been reported that rats spend more time in slow-wave sleep immediately following odor-fear conditioning than pseudo-conditioned rats, and that the 24 hr expression of fear memory strongly correlates with this increase in post-conditioning slow-wave sleep [1].

The state-dependent changes in piriform cortical connectivity were most strongly expressed with dorsal hippocampus and neocortex; the piriform – amygdala connectivity was relatively stable across fast-wave and slow-wave states. This latter finding is in contrast to previous electrophysiological recordings showing an enhancement in piriform cortex – basolateral amygdala connectivity during slow-wave state as assessed with local field potentials [33]. This difference may reflect the greater spatial localization obtained with local field potential recordings, as recent work has demonstrated that the resting state functional connectivity of the human amygdala is highly dependent on which sub-region of the amygdala is examined [24]. Individual amygdala nuclei could not be isolated with the spatial resolution in the current study, and thus state-dependent changes in piriform-amygdala connectivity may have been masked by merging multiple nuclei together.

Using electrophysiological recordings, piriform cortex functional connectivity with other regions during slow-wave state has been shown to be most strongly expressed in the delta (0.1-5 Hz) and theta (5-15 Hz) frequency ranges [33]. In the olfactory system, delta frequency oscillations are often driven by, and in phase, with respiration, while theta frequency activity can coincide with active sniffing that occurs during arousal and exploration. The present results suggest that in addition to delta and theta frequency oscillations, very low frequency (<0.1 Hz) resting state oscillations may also contribute to functional network cohesion. Resting state oscillations at different frequencies may convey different forms of information and/or may differentially modulate circuit excitability in preparation for attention or action [5, 26]. Furthermore, the very strong functional connectivity between dorsal hippocampus and neocortex observed here during slow-wave state coincides with the known correlation between single-unit activity in these two structures during slow-wave sleep [19, 28, 31]. This coupling between sharp-wave activity within hippocampus and cortical slow-wave activity has been hypothesized to be critical for memory consolidation [31].

CONCLUSIONS

Functional connectivity between the piriform cortex and both limbic and neocortical areas is enhanced during slow-wave states compared to fast-wave state, as assessed by fMRI. This enhanced connectivity of primary olfactory cortex with areas involved with emotion and memory comes during a slow-wave state associated decrease in cortical response to odors, and is hypothesized to contribute to odor memory consolidation. These findings also demonstrate the feasibility of performing analyses of state-dependent changes in brain activity using fMRI in anesthetized animals with respiration as an indirect measure of brain state.

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Figure 1.

Respiration rate varies with shifts between slow-wave and fast-wave brain states under urethane anesthesia. **(A)** Piriform cortex local field potential (LFP) and respiration recordings from a urethane anesthetized rat. The top most trace show root mean square (r.m.s.) power within the delta frequency band. During slow-wave activity (SWA) LFP's are dominated by large slow-waves and strong delta frequency power. During this state, respiration frequency (breaths per minute; BPM) is relatively low and invariant. During fast-wave activity (FWA), LFP's are characterized by high-frequency, low amplitude activity with very little delta frequency power. During this state, respiration rates increase and become more variable. **(B)** Individual data from three different rats showing the strong correspondence between respiration rate and brain state. In each rat, three measurements of BPM and LFP delta power (30 sec sampling period) were taken during SWA, followed by six measures during FWA, followed by three more measures taken during a subsequent SWA period to confirm stability of the state-dependent measurements. Although the specific BPM and delta power measures varied between animals, the state-dependence was reliable across all animals.

Figure 2.

Stable respiratory rates of individual urethane-anesthetized rats recorded while in the MRI magnet. Each line represents one animal which showed repeated shifts in respiration rate between two stable states. Based on the electrophysiological data, these respiratory rate states were used to infer fast-wave activity (FWA) and slow-wave activity (SWA).

Figure 3.

(**TOP**) These image data were taken with the volume transmit coil and 4 channel surface receive only coil. The image is distortion-free because of the segmented EPI acquisition. The activity displayed in pseudocolor is correlated with piriform cortical ROI in one animal in the two states. For illustrative purposes, voxelwise analyses were carried out on the preprocessed data. For FWA, images were thresholded at $Z > 1.6$, $p \sim 0.05$, which is below conventional significance levels using voxelwise analyses. For SWA, Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $P = 0.05$. Colored pixels represent significant positive correlation ($p < 0.05$) with piriform cortex ROI. During slow-wave activity correlated activity was strongly enhanced. (**BOTTOM**) Functional connectivity (time series correlation) between different ROI's in the two respiration-defined states. Functional connectivity was significantly enhanced during slow-wave activity (SWA) in most pathways compared to during fast-wave activity (FWA). Asterisks signify significant difference between FWA and SWA, $p < 0.05$. Abbreviations: PCX – piriform cortex; AMYG – amygdala; dHIPP – dorsal hippocampus; CX – neocortex.