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Analysis of coherent activity between retrosplenial cortex, hippocampus, thalamus, and anterior cingulate cortex during retrieval of recent and remote context fear memory

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ABSTRACT

Memory for contextual fear conditioning relies upon the retrosplenial cortex (RSC) regardless of how long ago conditioning occurred, whereas areas connected to the RSC, such as the dorsal hippocampus (DH) and anterior cingulate cortex (ACC) appear to play time-limited roles. To better understand whether these brain regions functionally interact during memory processing and how the passage of time affects these interactions, we simultaneously recorded local field potentials (LFPs) from these three regions as well as anterior dorsal thalamus (ADT), which provides one of the strongest inputs to RSC, and measured coherence of oscillatory activity within the theta (4–12 Hz) and gamma (30–80 Hz) frequency bands. We identified changes of theta coherence related to encoding, retrieval, and extinction of context fear, whereas changes in gamma coherence were restricted to fear extinction. Specifically, exposure to a novel context and retrieval of recently acquired fear conditioning memory were associated with increased theta coherence between RSC and all three other structures. In contrast, RSC–DH and RSC–ADT theta coherence were decreased in mice that successfully retrieved, relative to mice that failed to retrieve, remote memory. Greater RSC–ADT theta and gamma coherence were observed during recent, compared to remote, extinction of freezing responses. Thus, the degree of coherence between RSC and connected brain areas may predict and contribute to context memory retrieval and retrieval-related phenomena such as fear extinction. Importantly, although theta coherence in this circuit increases during memory encoding and retrieval of recent memory, failure to decrease RSC–DH theta coherence might be linked to retrieval deficit in the long term, and possibly contribute to aberrant memory processing characteristic of neuropsychiatric disorders.

1. Introduction

Cognitive function is associated with the concurrent activation of a distributed network of brain regions, with the synchronization of activity between these regions often directed by one or more distinct “hubs.” Disruption of activity within such networks is associated with pathological cognitive and emotional states (Grimm et al., 2009; Hamilton et al., 2011; Yu, Shen, Zeng, Ma, & Hu, 2013), underlying the need to understand how network activity in the brain is generated and how it relates to behavior. In rodents, studies of coherent activity between brain regions has typically focused on learning, but much less is known about how network activity underlies memory retrieval or how such networks change as memories age.

One measure of network activity in the brain is the coherence of oscillatory activity across brain regions. Oscillations in the theta (4–12 Hz) and gamma (30–80 Hz) frequency ranges have been especially implicated in mnemonic functions in both rodents (Colgin, 2015; Fitzgerald et al., 2015; Kay, 2005; Vertes, 2005) and humans (Barr et al., 2009; Klimesch et al., 2001; Lega, Jacobs, & Kahana, 2012; Rutishauser, Ross, Mamelak, & Schuman, 2010) due to their role in synchronizing and integrating activity across distributed networks of brain regions (Kirk & Mackay, 2003). Of the many regions in which these oscillations have been observed, retrosplenial cortex (RSC) is especially interesting because it (1) provides an essential conduit for the propagation of hypothalamic-generated oscillatory activity to other brain regions (Destrade & Ott, 1982) while also generating local theta oscillations during learning (Talk, Kang, & Gabriel, 2004), (2) is

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the cortical region in humans most consistently activated by emotionally salient stimuli (Maddock, 1999), and (3) unlike most other brain areas, plays a time-independent role in the retrieval of contextual (Corcoran et al., 2011) and spatial (Haijima & Ichitani, 2008) memories. Thus, RSC is uniquely situated to act as a hub of activity underlying the retrieval of both recently and remotely acquired memories. It is unknown, however, whether coherent activity between RSC and other regions correlates with memory retrieval, or how the patterns of such coherence change as memories age.

To address this issue, we trained mice in a contextual fear conditioning experiment and recorded simultaneous LFP activity in RSC and three regions with which it is robustly interconnected: DH, which has been implicated in the retrieval of recently acquired memories (Anagnostaras, Maren, & Fanselow, 1999); ACC, which has been implicated in the retrieval of remotely acquired memories (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004); and ADT, which provides one of the largest subcortical inputs to RSC (Berger, Milner, Swanson, & Thompson, 1980; van Groen & Wyss, 2003). The use of simultaneous multi-site LFP recordings allowed us to examine coherent activity between pairs of these structures prior to fear conditioning, during memory retrieval, and during fear extinction. Coherence in the theta band between RSC and the other three structures increased during memory encoding and recent memory retrieval, whereas decreased RSC–DH and RSC–ADT theta coherence were associated with successful retrieval of remote memory. Increased RSC–ADT theta coherence was observed in mice that successfully extinguished recently acquired fear memory. In contrast, RSC–ADT gamma coherence prior to fear conditioning predicted successful extinction. RSC–ACC did not show significant changes in coherent activity with memory age.

2. Methods

2.1. Subjects

A total of 38 nine-week-old male C57BL6/N mice obtained from a commercial supplier (Harlan, Indianapolis, IN) were used in this study. Mice were individually housed in a facility on a 12/12 h light/dark cycle (lights on at 7 a.m.), and allowed free access to food and water. All procedures were approved by Northwestern University's Animal Care and Use Committee in compliance with National Institutes of Health standards.

2.2. Surgery

Mice were anesthetized with Avertin (1.2%) and implanted with insulated silver wires (100 μm diameter) aimed at RSC (1.8 mm posterior, 0.4 mm lateral, 0.75 mm ventral to bregma), DH (1.5 mm posterior, 1.0 mm lateral, 1.75 mm ventral), ADT (0.8 mm posterior, 0.75 mm lateral, 2.75 mm ventral), and ACC (1.3 mm anterior, 0.4 mm lateral, 1.75 mm ventral). All electrodes were placed in the left hemisphere. A gold screw lowered into the skull near the right parietal/occipital bone suture served as a reference and ground electrode. Two stainless steel jeweler's screws were inserted in the skull to anchor the headcap. All wires were soldered to a 6-pin connector to which the recording devices were later attached, and the assembly was fixed to the skull with acrylic. Mice were allowed at least 72 h to recover from surgery prior to behavioral procedures.

2.3. Fear conditioning

Fear conditioning took place in a 35 × 20 × 20 cm Plexiglas chamber with a stainless steel rod floor (4 mm diameter, 0.9 cm center-to-center) in a sound-attenuating cabinet with black inner walls (TSE Inc., Bad Homburg, Germany). Mice were placed in the chamber and presented with a mild footshock (2 s, 0.7 mA, constant current) after 3 min. The chamber was cleaned after each mouse with 70% ethanol. Subsequent 3 min tests for fear to the conditioning context began 1 d (Recent group) or 35 d (Remote group) post-conditioning.

2.4. LFP recordings

Continuous recordings were made at a sampling rate of 600 Hz using wireless 4-channel recording devices (NeuroLogger, TSE Systems), which were attached to the mice prior to each session. Preamplification, analog-to-digital conversion (unity gain buffer, AC input range ±750 μV, 1000× gain, ADC resolution 8 bits), and data storage all occurred on the NeuroLogger. After each session, the NeuroLogger was removed and data were downloaded to a PC. Prior to fear conditioning, mice in the Recent group were connected to the NeuroLoggers for a recording session in their home cages to habituate them to the recording devices (home cage recording), followed 24 h later by a 3 min recording session in the conditioning chamber during which no shock was delivered (novel context recording). Fear conditioning occurred the following day, and retrieval/extinction tests began 24 h post-conditioning. For the Remote group, fear conditioning occurred prior to electrode implantation, home cage recordings occurred on post-conditioning day 34, and retrieval/extinction tests began on post-conditioning day 35 (Fig. 1A). LFPs were recorded during all 8 extinction sessions for all mice (data are only presented for the first and last extinction sessions). Mice were not connected to the recording devices during fear conditioning.

2.5. Data collection and analysis

Freezing during tests for context fear was scored every 5 s by a trained observer, and expressed as the percentage of the total number of observations that the mice remained motionless. During all sessions, locomotor activity in the form of infrared beam crosses was collected automatically. After fear conditioning, successful memory retrieval was defined as greater than 30% freezing during the first post-conditioning test session. LFP recordings were downloaded to a PC in compressed hexidecimal format and converted to a Matlab-compatible format for analysis. Spectral analyses were performed using open-source Chronux (http://Chronux.org) algorithms as described previously (Kay & Freeman, 1998; Rojas-Líbano, Frederick, Egaña, & Kay, 2014). Coherence spectra were computed for the theta and gamma frequency bands across each 3 min recording session using 35 half-overlapping 10 s windows with 4 tapers, (resulting in a frequency resolution of 1.4 Hz), and then transformed using the Fisher z-transform. There was no filtering. For both theta and gamma, the peak frequency within each band was taken as the center frequency, and coherence at this peak was used as the dependent measure. This z-coherence is normalized by power, allowing for direct comparison across subjects and sessions. Peak coherence in the theta and gamma bands were calculated for each mouse in each session and used for statistical analysis. To estimate the magnitude of coherence that could be expected by chance, coherence values were again calculated for each site-pair, except that data for the two structures in each site-pair were taken from separate recording sessions (e.g., RSC home cage vs. DH novel context, RSC recent retrieval vs. ADT home cage, etc.). All structure by session combinations were analyzed for each mouse at each time point for both the theta and gamma frequency bands. Significance of peak coherence values was determined by one-sample *t* tests against these shuffled coherence values. Group differences were determined using ANOVA or