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Specific Increase of Human Entorhinal Population Synaptic and Neuronal Activity During Retrieval

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Abstract

Population transmembrane currents and neuronal firing in different layers of the human entorhinal cortex (ER) were recorded during semantic and episodic memory processes using a linear array of 24 laminar microelectrodes. Both measures, as well as local broadband spectral power, increased during retrieval of newly-learned characteristics, especially in superficial layers. No differences were observed in the activity evoked by remembering people as compared to places. Semantic retrieval evoked similar activity. In contrast, intentional encoding of declarative memory evoked relatively little activity. A double-dissociation of these responses with simultaneously recorded lateral inferotemporal recordings suggests that entorhinal cortex may be specifically engaged during retrieval, across multiple memory types and materials.

Introduction

Lesions of the medial temporal lobe (MTL) produce a profound and specific amnesia (Squire et al., 2004), and conversely, MTL electrical hyper-activation can evoke intense memories (Bartolomei et al., 2004; Halgren et al., 1978b), implying that the MTL contributes to memory retrieval. However, temporary MTL disruption produces a temporary inability to lay down new traces, implying that it is also necessary for encoding (Halgren, 1984). Direct recordings of MTL neurons in humans have found increased firing during both input and output phases of memory tasks (Cameron et al., 2001; Halgren et al., 1978a).

Rat hippocampal (HC) neurons fire in particular spatial locations, leading to theories that the MTL is particularly involved in spatial navigation (O'Keefe and Nadel, 1978). Consistent data were found in recent hemodynamic and unit studies in humans (Ekstrom et al., 2003; Maguire et al., 1998). Other data, however, suggest that the involvement of the HC in spatial processing

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is secondary to a more general role whenever novel configurations of elements are processed (Eichenbaum, 2000).

While many studies find that amnesics with bilateral hippocampal damage are unable to learn new semantic facts even when divorced from their context (Squire et al., 2004), studies of patients whose HC had been lesioned in early childhood found relatively preserved semantic knowledge, suggesting that the HC may only be necessary for episodic memory (Vargha-Khadem et al., 1997). In summary, although the critical role of the MTL for human episodic memory is one of the key findings of cognitive neuroscience, crucial aspects of this role remain in doubt: is the MTL involved in both encoding and retrieval? in spatial processing or in configurational learning? in semantic as well as episodic memory?

In order to address these issues, we recorded multiunit activity (MUA) and current source density (CSD) at 150 μ intervals across different cortical layers from the left ER of a patient suffering from medically refractory epilepsy. Time- and frequency-domain ER responses were compared to those simultaneously recorded in the inferotemporal cortex (IT) and nearby MTL locations. ER responses were largest during episodic retrieval, but were not selective for spatial information.

Patient and Methods

Patient

A 37-y old right handed male suffering from medically intractable temporal lobe epilepsy since early childhood was investigated with bilateral stereotactic invasive EEG depth recordings within the clinical presurgical evaluation for the localization of the seizure onset zone. The subject had normal neuropsychological and psychiatric function and left hemispheric language dominance. Invasive EEG recordings found the seizure onset zone in the right amygdala. A right sided selective amygdalohippocampectomy was performed and the patient has now been seizure free for over 3 years (Engel Ia). Histopathology revealed right medial temporal sclerosis. The position of the electrodes, and the duration of the invasive recordings were based entirely on clinical considerations. Before taking part in the study, the participant gave fully informed consent according to the guidelines of the Declaration of Helsinki, monitored and approved by the Institutional Review Boards of Beth Israel/Deaconess Medical Center and Massachusetts General Hospital, Boston.

Task

The paradigm was visually presented on a 19" computer monitor as white capital letters in Geneva font on a black background in the central $\sim 5^\circ$ of visual angle. The patient performed 60 trials of each of 6 conditions: EPISODIC ENCODING, EPISODIC RETRIEVAL, and SEMANTIC RETRIEVAL, crossed with PERSON and LOCATION.

Each trial regardless of condition was comprised of four frames presenting successively a cue, a name, a category and a characteristic. EPISODIC ENCODING trials began with a CUE to 'LEARN,' followed by a NAME of a previously unknown person or location, then a CATEGORY of attribute (e.g., 'HOBBY' for a person, 'LANGUAGE' for location), and finally the CHARACTERISTIC itself (e.g., 'PAINTING' or 'SPANISH'). Each NAME-CATEGORY-CHARACTERISTIC association was presented twice during learning. EPISODIC RETRIEVAL trials began with a CUE to 'ANSWER' followed by a NAME-CATEGORY-CHARACTERISTIC, and the patient indicated if they were correct. SEMANTIC RETRIEVAL trials had an identical format, but probed public knowledge (e.g. 'RONALD REAGAN-JOB-PRESIDENT' or 'AUSTRIA-LANGUAGE-GERMAN'). Half of the retrieval trials were correct, i.e. the presented characteristic matched that which had been presented previously in association with the same name and category. Thus both encoding and

retrieval trials included both novel and repeated characteristics, roughly balancing for repetition effects.

Probes and recording details:

Details of electrode construction, recordings and analysis techniques can be found elsewhere (Ulbert et al., 2001). Briefly, clinical electrodes were modified to be smaller in diameter (350 μm) in a 5 mm segment at their tips, containing 24 Platinum-Iridium contacts (each 40 μm diameter, 110 μm interelectrode distance). The tip of electrode was in the left ER (see Figure 1). Differential recordings were obtained from 23 pairs of successive contacts. Population trans-synaptic current flows were estimated using Current Source Density (CSD) analysis (Nicholson and Freeman, 1975; Ulbert et al., 2001). CSD approximates the depth distribution of the current sources in laminated structures such as the cerebral neocortex (Mitzdorf, 1985). Excitatory post synaptic currents (EPSCs) produce current sinks at the active synapses, with passive sources as current returns (Nicholson and Freeman, 1975). Conversely, at the membrane potentials typical of waking and active human neocortex, inhibitory post synaptic currents (IPSCs) should produce current sources at the active synapses, that would be accompanied by passive sinks as current returns. Simultaneously recorded multi unit activity (MUA) as a continuous estimate of the population neuronal firing can help distinguish if a sink reflects an active excitation or passive current return (Ulbert et al., 2001). In contrast to local field potentials, which do not distinguish between excitatory and inhibitory events, CSD current sinks with increased MUA are interpreted as representing local population excitatory postsynaptic currents (EPSCs). Due to the small size of the recording contacts (40 μm), the close spacing of the contacts (150 μm center-to-center), and the particular analytic methods, CSD and MUA decline steeply with distance, estimating activity of neurons in a volume roughly corresponding to that of a cortical column (Grover and Buchwald, 1970; Wang et al., 2005).

The temporal evolution of task modulation was examined by calculating on every trial the spectral power for each latency, frequency and cortical depth. For these measures, the single trial signal for each channel was convolved with complex Morlet's wavelets (Kronland-Martinet et al., 1987). Each Morlet's wavelet used here has a Gaussian shape both in the time domain (SD σ_t) and in the frequency domain (SD σ_f) around its central frequency f_0 : $w(t, f_0) = A \exp(-t^2/2\sigma_t^2) \exp(2i\pi f_0 t)$, with $\sigma_f = 1/(2\pi\sigma_t)$. Relatively constant temporal and spectral resolution across target frequencies was obtained by adjusting the wavelet widths, according to the target frequency (Wang et al., 2005). Wavelet widths were kept at 1 for frequencies 1, 2, and 3 Hz, resulting in σ_t of 159, 80, 53ms and σ_f of 1, 2, and 3Hz. Wavelet widths increased from 1 to 15 as the frequency went from 4 to 60Hz, resulting in a constant σ_t of 40 ms and a constant σ_f of 4 Hz. Tests with simulated data confirmed that the methods used here are accurate as low as 1 Hz (Wang et al., 2005). Phase-locking was calculated between microelectrode recordings in different layers of entorhinal cortex, and macroelectrode recordings in inferotemporal, entorhinal and temporal polar cortices (Lachaux et al., 1999). The resulting time-frequency and phase-locking plots were averaged across trials and normalized with respect to the pre-stimulus baseline for each frequency and channel (Halgren et al., 2002). To test the difference between conditions, two-tailed student t-tests were applied to baseline corrected spectral powers with the standard deviation from individual trials. All comparisons noted in the figures or text were significant at $p < 10^{-5}$.

Results

Behavioral Performance

The hit rate was slightly similar for the episodic condition (97%) and for the semantic condition (95%), and both hit rates differed significantly from chance ($p < 0.001$, two-sided binomial

test, $n = 320$). The reaction times (RT) were similar between episodic retrieval (1228ms) and semantic retrieval (1243ms), and for correct (1235ms) and incorrect (1236ms) trials.

Laminar recordings

EPSCs were evoked in both superficial and deep ER layers during episodic encoding and retrieval, as well as semantic retrieval (Figure 1). These responses were largest during episodic retrieval and significantly smaller during episodic encoding. CSD sinks and especially MUA increases were prolonged (~1s long). The responses mainly occurred following presentation of the characteristic when the subject was retrieving the recently-learned association rather than in earlier parts of the same trial when the context was being established. These excitatory currents were largest in the superficial ER layers. In the same layers, spectral analysis of the CSD from individual trials found that power in both lower frequency (delta/theta) and higher frequency (beta/gamma) bands again increased more strongly during retrieval than during encoding (Figure 1c). Thus, both time-domain and frequency-domain measures were elevated during retrieval as compared to encoding trials, during the part of the trial when active retrieval took place.

Macro-depth electrodes

In order to compare ER responses to those in different parts of the temporal lobe, we recorded local field potentials from macro-electrodes in white matter 5mm lateral to the ER laminar microelectrode array ('ER-lat'), in left lateral inferotemporal cortex (IT), and in rhinal cortex (RH) in the collateral fissure of the anteroventral temporal lobe (Figure 2). ER-lat macrorecordings replicated ER CSD recordings in demonstrating increased theta power during episodic retrieval as compared to encoding, but high frequency changes were absent suggesting that they are highly focal. In IT, local field potentials and low frequency spectral power increased sharply to all categories of visual stimuli, consistent with its known role in visual information processing. Similar to ER, the rhinal cortex (RH) showed the largest increase in delta, theta, alpha and beta frequencies during retrieval, and especially during episodic retrieval. The ER extends a variable amount into the collateral sulcus at this anterior level (Insausti and Amaral, 2004), so it is possible that this recording reflects ER activity. Increased event-related phase-locking was observed between both superficial and deep layers of ER, and IT (see Figure 2d), consistent with sustained event-related communication between ER and this neocortical area. This increased phase-locking was confined to lower frequencies (theta and delta), and was less regular between ER and the other macroelectrode contacts (see Supplemental figure 1). No difference was observed in either laminar or macro-recordings between items presenting or probing memory for places *versus* people (see Supplemental figure 2).

Discussion

Entorhinal cortex population EPSCs, neuronal firing, and spectral power across multiple bands were strongest during the retrieval of memories, especially of episodic memories, as compared to memory encoding. This difference was not seen in simultaneous recordings from inferotemporal cortex, supporting previous suggestions that different parts of the MTL may contribute primarily to different phases of memory (Brown and Aggleton, 2001). For example, in monkeys, while lateral inferotemporal (IT) firing strongly decreases when stimuli are repeated, this effect may not be present in ER, suggesting that IT may support priming whereas medial areas including ER are more involved in retrieval (Suzuki and Eichenbaum, 2000; Xiang and Brown, 1998). A similar dissociation has been noted in humans, when population firing and excitatory currents are suppressed by word repetition in IT and perirhinal sites, but not in ER (Halgren et al., 2006). Further support for a role of ER in conscious retrieval is the fact that

hyperactivation of this area may evoke vivid recollections (Bartolomei et al., 2004; Halgren et al., 1978b).

The elevated response during retrieval of different characteristics appeared equal regardless of whether those characteristics referred to people *versus* locations. This is consistent with views of the MTL as related to declarative memory in general rather than being restricted to spatial information (Eichenbaum, 2000). Similarly, although as with fMRI, the ER response was larger during episodic as compared to semantic retrieval (Steinvorth et al., 2005), it was present during both. This is consistent with the notion that the MTL may continue to be engaged by retrieval processes in general, even after the trace becomes either sufficiently consolidated (Squire et al., 2004) or interlinked with semantic knowledge (Nadel and Moscovitch, 1997).

Superficial ER layers are the main source of input from IT and other neocortical areas to HC, and deep ER layers relay HC activity back to neocortex (Insausti and Amaral, 2004). This HC-ER-neocortical interaction may be modulated and synchronized by theta and gamma oscillations (Chrobak and Buzsaki, 1998). Similarly, we found increased delta and theta phase-locking between ER and IT during our task. Human ER firing and theta and gamma coherence between rhinal and HC areas help predict subsequent memory, suggesting that the ER may be involved in encoding (Cameron et al., 2001; Fell et al., 2003). Our results are consistent with these previous studies in showing that the encoding and retrieval conditions evoked similar patterns of activity, and differed only in the intensity of activation.

Previous studies have found the onset latency of activity in IT and PR evoked by visual words to be at about 120ms, consistent with the IT recordings here (Halgren et al., 1994; Halgren et al., 2006). ER activity in these recordings began at about 180ms, and continued for over a second with the maximal response at about 500ms. Since IT and HC activity is also long-lasting, all three structures in this circuit are simultaneously active. The HC-EC and EC-IT relationships are bidirectional, and the conduction delays are about 2 orders of magnitude shorter than the period of co-activation, suggesting that this period is also one of cyclic interaction. This interaction is central to models that posit MTL participation in encoding during the creation of a neural network representing an event, and again in retrieval as the previous network is re-created (Alvarez and Squire, 1994; Halgren, 1984). In this view, the activation of superficial ER neocortex-recipient layers during retrieval is to be expected, because they are a key link in the cyclic interaction of MTL and neocortical structures as they progressively refine the re-created network.

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Supplementary Material

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Figure 1.

Estimates of population synaptic activity (CSD) and neuronal firing (MUA) in ER during memory. **a.** CSD responses from representative superficial and deep channels show a sustained sink beginning about 200ms after the name of the object or location is presented, and continuing for over 2 s, through the presentation of the Category and peaking about 500 ms after presentation of the individual characteristic to be learned or retrieved ($\uparrow$). This sink is larger during episodic retrieval (blue line) than during episodic encoding (green line). Significant intervals indicated by red bars in the baseline. **b.** MUA increases during the retrieval period during the retrieval tasks ($\downarrow$), suggesting that the sink in a represents an excitatory post-synaptic current. **c.** Time-depth maps of current sinks (red) and sources (blue). Current sinks in

superficial layers are greatest during the matching of a presented characteristic to that retrieved from recent episodic memory (*). **d.** Individual trial event-related spectral power (iERSP), plotted as z-scores relative to pre-stimulus baseline. Power in the delta/theta (↓) and beta/gamma (↑) bands significantly increases in the superficial channels for sustained periods during retrieval trials.

Figure 2.

Local field potentials simultaneously recorded in different left MTL areas during memory. **a.** MRI taken with the electrodes *in situ*, showing the microelectrode array in entorhinal cortex (ER) whose recordings are in figure 1, as well as macroelectrode contacts in the anterior collateral sulcus (RH, rhinal cortex), inferotemporal cortex (IT), and in the temporal white matter 5 mm lateral of the entorhinal cortex, (ER-lat). Please note that the sizes of the artifacts on the MRI images are larger than that of the probes themselves. **b.** Event-related potential gradients in IT show a sharp initial response peaking at ~170ms (↓), as well as later components peaking at ~540ms (↓). The responses to each stimulus in the sequence was similar, and did

not change between conditions, consistent with these responses representing visual processing. In contrast, ER-lat and RH contacts record mainly late and small responses (Δ). **c.** Spectral power maps showing activity in IT, ER-lat and RH during episodic encoding, episodic retrieval and semantic retrieval. Frequency spectra from 1 to 60 Hz are displayed. Spectral power in IT initially increases across delta, theta, alpha, and beta bands (*), followed by a sustained increase in delta activity (^), and does not change between conditions. ER-lat also shows a late delta increase (Δ), but it is greater during retrieval. RH shows a large broadband response, specifically to retrieval, and especially episodic retrieval ($\uparrow$). **d.** Phase locking between IT and ER-lat in the delta/theta band increases relative to baseline during the memory task ($\uparrow$). Shown here are all trials; increases were seen during both encoding and retrieval (see Supplementary Figure 1).