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Closed-Loop Interruption of Hippocampal Ripples through Fornix Stimulation in the Non-Human Primate

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ABSTRACT

Background: Hippocampal sharp-wave ripples (SWRs) arising from synchronous bursting in CA3 pyramidal cells and propagating to CA1 are thought to facilitate memory consolidation. Stimulation of the CA3 axon collaterals comprising the hippocampal commissure in rats interrupts sharp-wave ripples and leads to memory impairment. In primates, however, these commissural collaterals are limited. Other hippocampal fiber pathways, like the fornix, may be potential targets for modulating ongoing hippocampal activity, with the short latencies necessary to interrupt ripples.
Objective: The aim of this study is to determine the efficacy of closed-loop stimulation adjacent to the fornix for interrupting hippocampal ripples.
Method: Stimulating electrodes were implanted bilaterally alongside the fornix in the macaque, together with microelectrodes targeting the hippocampus for recording SWRs. We first verified that fornix stimulation reliably and selectively evoked a response in the hippocampus. We then implemented online detection and stimulation as hippocampal ripples occurred.
Results: The closed-loop interruption method was effective in interrupting ripples as well as the associated hippocampal multi-unit activity, demonstrating the feasibility of ripple interruption using fornix stimulation in primates.
Conclusion: Analogous to murine research, such an approach will likely be useful in understanding the role of SWRs in memory formation in macaques and other primates sharing these pathways, such as humans. More generally, closed-loop stimulation of the fornix may prove effective in interrogating hippocampal-dependent memory processes. Finally, this rapid, contingent-DBS approach may be a means for modifying pathological high-frequency events within the hippocampus, and potentially throughout the extended hippocampal circuit.

Introduction

Sharp wave ripples (SWRs) are hippocampal oscillations that are associated with widespread activation of neocortex, and as a consequence of this co-activation, SWRs are believed to underlie memory consolidation [1]. SWRs are produced by synchronous activity of CA3 pyramidal cells, which in turn excite CA1 pyramidal cells [2–4]. The ventral hippocampal commissure in rodents contains CA3 pyramidal cell collaterals, making it possible to interrupt ripples through online detection and stimulation of this pathway. Interruption using this method suppresses the synchronous spiking typical of SWRs and impairs performance on memory tasks, supporting a role for SWRs in memory consolidation and/or retrieval [5–8]. Sharp wave ripples are also seen in humans [9–11] and macaques [12,13]; but because ripples have not been experimentally manipulated in primates, their role in memory less clear. Unlike rodents, primates have a weak, sparsely connected ventral hippocampal commissure [14–16], making the murine approach to ripple interruption

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untenable in primate brains. The fimbria-fornix fiber tract, however, is robust and well-conserved [17–19] and its integrity is important for memory formation in primates [20–29]. Furthermore, preliminary and case studies using fornix stimulation in humans has been linked to memory improvements and changes in hippocampal structure and function [30–34]. The fornix contains projections from within the hippocampus proper and connected subicular complex, and from extra-hippocampal structures known to modulate ripples in rodents [35–39], suggesting the potential to interrupt ripples. The effect of fornix stimulation on hippocampal ripples, however, is unknown.

In this study, we sought to 1) detect ripples in real time in the primate brain and 2) measure hippocampal ripple-band and unit responses to fornix stimulation. We show that fornix stimulation evoked responses in hippocampus, that ripples were detected in real time, and that the closed-loop fornix stimulation interrupted ripples and suppressed ripple-associated hippocampal multi-unit activity.

Materials and methods

Subject and surgical implantation

All procedures were approved by the local ethics and animal care authorities. The 10 kg adult female macaque (*Macaca mulatta*) underwent electrode implantation surgeries conducted under sterile conditions and with the animal maintained under approximately 2% isoflurane anesthesia. The animal was implanted bilaterally with a 4-lead NuMed mini-DBS electrode alongside and anterior to the post-commissural fornix of each hemisphere, just caudal to the anterior commissure (Fig. 1A). The leads had contacts of 0.5 mm separated by 1.5 mm (model FTML4E, NuMed, Inc., Hopkinton, New York). Implantation of the stimulating electrodes was guided by preoperative MR images, aligned to fiducial markers, using the Brainsight system (Rogue Research Inc., Montreal, Quebec, Canada). Over the right hippocampus, we implanted an indwelling array of two electrode bundles, each containing 4 independently depth-adjustable platinum/tungsten multicore tetrodes (96 micron outer diameter; Thomas Recordings, Giessen, Germany; Neuralynx, Bozeman, Montana). Post-operatively, tetrodes were lowered into the CA1/2 (bundle 1) and CA3/DG region (bundle 2) of the right hippocampus (Fig. 1B), verified through MR/CT coregistration and functional characterization of brain structures during lowering, including the appearance of SWRs. SWRs were observed in limited ranges of depths across tetrodes, and were associated with unit activity. Once the electrodes had been lowered into the layer, ripple-band activity was stable across sessions. One electrode was placed outside of the hippocampal formation, to measure non-ripple activity such as muscle activity, and to determine the selectivity of responses evoked by the stimulating electrodes ('control' electrode). All sessions took place while the animal was sitting quietly in a darkened booth. SWRs from this animal prior to stimulating electrode implantation were reported previously [13].

Electrophysiological recordings

Local field potentials were referenced to the titanium tetrode-array recording chamber and sampled at 32 kHz using a unity-gain HS36 head stage (Neuralynx, Inc., Bozeman, Montana, USA). The headstage was connected directly to the electrode interface board on the animal head, and powered by the acquisition system. As seen in Fig. 2A, digitized channels were processed in two ways – first, through the Cheetah 32 system (Neuralynx, Inc., Bozeman, Montana, USA). Second, the signal was split and sent to the High Performance Processing unit ('HPP', Neuralynx, Inc., Bozeman, Montana, USA). This processing unit is equipped with a Field-Programmable Gate Array (FPGA – ARM Cortex-A9 processor, Xilinx Inc., Cambridge, UK) capable of carrying all digital processing required for ripple detection and triggering the stimulator in real time. Ripples are neural events with band-limited, high-frequency (80–150 Hz) power compared to background levels of power in that band, therefore ripples can be detected when the band power or envelope exceeds a threshold [9,12,13,41]. Selecting the electrode channel with the highest-amplitude ripple activity, the threshold level should be optimized to maximize the number of true positives by user inspection, while minimizing false detection rate on this channel. That is, low thresholds will produce more false positives while high threshold values may have lower detection rate. The optimal threshold value is well above the baseline activity because band-limited activity of the ripples is considerably higher than the instantaneous activity [13]. In this study, we set the threshold to 6-sd of the band activity, a value similar to rodent interruption studies [5–8]. The 6-sd threshold was estimated based on the average and variance of the ripple band from earlier recordings which were consistent over days in these experiments (9.5 ± 0.1 μV mean±SEM).

The ripple detection algorithm was implemented on the high performance processing unit in three steps. First, the signals were bandpass filtered using a custom-designed finite impulse response (FIR) filter with 512 taps (16 ms delay, which is about one cycle of ripple activity). Activities slower than 80 Hz or faster than 150 Hz are suppressed more than 20 dB (Fig. 2B). Next, amplitudes were compared to the threshold across the target channel and 'control' electrodes that were not observed to record true ripples. These control electrodes were used to reject artifacts such as electromyography (EMG) or other transients that might have otherwise crossed threshold on the target channel, thereby preventing the target's threshold-crossing from generating an output pulse to the stimulator described below.

Artifact rejection and false detection reduction

EMG artifacts and other transients can show broadband signals with considerable power at the ripple band. As such, they can be mislabeled as ripples if a single electrode channel is used for ripple detection. Thus, a simple method to identify the muscle and other common artifacts in real-time was to measure excessive ripple-band activity at other locations, such as the control electrode that had been placed outside the hippocampus. When activity of this control electrode exceeded threshold, crossings on the ripple-detecting electrodes were ignored as a false positive, and the stimulator was not triggered.

Electrical stimulation

Stimulating electrodes were connected to an STG4002 stimulator (Multichannel Systems, Reutlingen, DE). Bipolar stimulation was applied across pairs of contacts using a 100 μs biphasic pulse width of 2 mA. The proximal 2 contacts in both of the stimulating electrodes yielded the strongest responses (i.e. the dorsal most pair of leads, Fig. 1A), and were therefore used to measure hippocampal evoked responses. To measure the hippocampal response to fornix stimulation, the fornix was stimulated for 3 minutes at 1.75 Hz intervals varying by current and number of pulses. The parameter space was single, 4, or 8 pulses and 1, 2, or 3 mA stimulation current. Each stimulation session was epoched using the stimulation triggers such that the last pulses were aligned at $t = 0$. Then the hippocampal response to each stimulation protocol was measured by averaging the epochs obtained during the stimulation session.

For the ripple interruption experiments, we used bipolar stimulation across the adjacent, proximal 2 leads of each electrode, delivered bilaterally at 2 mA, in short bursts of 4 or 8 pulses at 2 ms