Hippocampal CA1 and CA3 neural recording in the human brain: validation of depth electrode placement through high-resolution imaging and electrophysiology

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OBJECTIVE Intracranial human brain recordings typically utilize recording systems that do not distinguish individual neuron action potentials. In such cases, individual neurons are not identified by location within functional circuits. In this paper, verified localization of singly recorded hippocampal neurons within the CA3 and CA1 cell fields is demonstrated.

METHODS Macro-micro depth electrodes were implanted in 23 human patients undergoing invasive monitoring for identification of epileptic seizure foci. Individual neurons were isolated and identified via extracellular action potential waveforms recorded via macro-micro depth electrodes localized within the hippocampus. A morphometric survey was performed using 3T MRI scans of hippocampi from the 23 implanted patients, as well as 46 normal (i.e., nonepileptic) patients and 26 patients with a history of epilepsy but no history of depth electrode placement, which provided average dimensions of the hippocampus along typical implantation tracks. Localization within CA3 and CA1 cell fields was tentatively assigned on the basis of recording electrode site, stereotactic positioning of the depth electrode in comparison with the morphometric survey, and postsurgical MRI. Cells were selected as candidate CA3 and CA1 principal neurons on the basis of waveform and firing rate characteristics and confirmed within the CA3-to-CA1 neural projection pathways via measures of functional connectivity.

RESULTS Cross-correlation analysis confirmed that nearly 80% of putative CA3-to-CA1 cell pairs exhibited positive correlations compatible with feed-forward connection between the cells, while only 2.6% exhibited feedback (inverse) connectivity. Even though synchronous and long-latency correlations were excluded, feed-forward correlation between CA3-CA1 pairs was identified in 1071 (26%) of 4070 total pairs, which favorably compares to reports of 20%–25% feed-forward CA3-CA1 correlation noted in published animal studies.

CONCLUSIONS This study demonstrates the ability to record neurons in vivo from specified regions and subfields of the human brain. As brain-machine interface and neural prosthetic research continues to expand, it is necessary to be able to identify recording and stimulation sites within neural circuits of interest.

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KEYWORDS magnetic resonance; depth electrode recording; cross-correlation; functional connectivity; single neuron; memory; cognition

THE human hippocampus is essential for the processing of sensory information into retained memories.^{1,2} The transformation of sensory stimuli into memory in mammals occurs through a highly specialized, hierarchical network of interconnected neuronal ensembles of the CA1 and CA3 hippocampal subfields.^{3,4} Any

attempt to repair dysfunctional hippocampal circuitry in patients with memory impairment due to neurological disorders such as Alzheimer's disease requires network intervention to restore proper memory encoding.^{5,6}

Preclinical studies from this laboratory and others have shown that neuronal discharge relationships necessary for

ABBREVIATIONS DMS = Delayed-Match-to-Sample; NHP = nonhuman primate.

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TABLE 1. Patient recruitment and subject enrollment

Variable	No. of Patients	No. of Probes	No. of Neurons	No. of CA1 Neurons	No. of CA3 Neurons
Implant type					
Unilat anterior-only	7	7	201	94	107
Bilat anterior-only	7	14	281	127	154
Unilat anterior + posterior	5	10	81	42	39
Bilat anterior + unilat posterior	1	3	53	26	27
Bilat anterior + bilat posterior	3	12	128	66	62
Total	23	46	744	355	389

the performance of memory encoding can be predicted based on preceding encoding events that resulted in successful recall.^{6,7} Successful firing patterns can then be mimicked during working memory performance via delivery of electrical stimulation in a pattern that emulates an ensemble code for memory. Stimulated encoding has been shown to improve performance when hippocampal function is limited, or to replace memory encoding when hippocampal ensembles cannot generate a successful code to perform a task.^{4,6,8} Recording and stimulation of hippocampal neural ensembles has been demonstrated in preclinical rodent and primate models, although human subjects are currently being assessed.^{9,10} Successful implementation of such a hippocampal prosthesis in humans requires the following: 1) successful localization of electrodes within the specific hippocampal sublayers CA1 and CA3, 2) accurate recording of firing patterns from CA3 during memory encoding, and 3) evidence of successful stimulation of CA1 with the predicted neuronal firing patterns from prior successful encoded events.

Examination of patients with medically refractory epilepsy provides a unique opportunity to assess hippocampal neuronal firing during memory tasks. Intracranial monitoring for seizure localization requires stereotactic neurosurgical placement of depth electrodes into suspected regions of seizure origination, often involving the hippocampus and amygdala. Via intracranial electrodes, direct assessment of hippocampal ensemble activity can be performed with no additional risks to patients. Moreover, successful hippocampal electrode placement requires accurate identification of the hippocampal CA3 and CA1 sublayers and the exact location of the electrode, something not easily accomplished by imaging alone.^{11–13} Ulbert et al. provided one of the first descriptions of layer-specific electrophysiology within the human hippocampus through intraoperative recordings performed during resection of the medial temporal lobe and confirmed with immediate postoperative histology. Electrophysiological recordings were limited due to anesthesia, and this approach required additional time and risk associated with placing depth electrodes during planned temporal lobe resection.¹⁴

In this study we demonstrate a combined imaging and electrophysiological recording methodology that successfully confirms electrode placement within the CA1 and CA3 subfields of the human hippocampus without requiring postoperative histological confirmation. By demonstrating that firing patterns of CA3-to-CA1 neuronal

ensembles directly influence CA1 task responses, we are able to establish the necessary groundwork to initiate successful hippocampal stimulation for brain-computer interfacing and/or neural prosthetic research.

Methods

A multidisciplinary team examined 23 patients with medically refractory focal epilepsy requiring invasive monitoring prior to the planning of a potential resection. All patients underwent prior comprehensive preoperative testing including long-term noninvasive video-EEG analysis, preoperative MRI, and neuropsychological evaluation. Depth electrode placement was determined on the basis of presurgical data and clinical necessity for further evaluation. All patients had at least one hippocampal (anterior) electrode (Table 1).

Surgical procedures were performed at the Wake Forest Baptist Medical Center and approved by the IRB of Wake Forest School of Medicine. Postoperative monitoring and neurocognitive testing were performed at the Comprehensive Epilepsy Center at Wake Forest Baptist Medical Center. Twenty-three patients provided written informed consent prior to participation in this study, separate from consent for surgical procedures, standard seizure monitoring, and clinical management.

Electrode Implantation

FDA-approved intracranial depth probes capable of single-neuron and field-potential recording (Macro-Micro electrode, MM16C-SP05X-000, Ad-Tech Medical Instrumentation Corp.; Fig. 1D) were surgically placed using either a stereotactic head frame (CRW Precision Arc, Integra LifeSciences) or frameless stereotactic system (VarioGuide, Brainlab AG) at the discretion of the operating surgeon. Electrodes were placed through the head of the hippocampus perpendicular to its long axis in order to localize within CA3 and CA1 subfields (Fig. 1A). Calvarial entry points were created using either cranial burr holes or bone flaps as dictated by the clinical needs of each individual patient. A small puncture incision of the dura mater was performed and the electrode was placed at the pre-planned location, with at least one hippocampal electrode implanted in each patient. Intraoperative neuronal monitoring was performed in each patient to confirm adequate neural recording prior to securing each electrode to the scalp and closing the craniotomy and scalp incision.

FIG. 1. MRI-based assessment of hippocampal depth electrode positioning. **A:** 3T MRI of the neurosurgical “oblique” approach to the anterior hippocampus was performed to provide morphometric analysis of hippocampal cell layers for refinement of depth-recording probes. Calculations in the accompanying text and tables indicate the widths and distances of each region computed as an average across patients. **B:** Postoperative 3T MR image obtained in a patient with bilateral Ad-Tech macro-micro depth electrodes targeted at CA3 and CA1 cell layers in the human hippocampus. Electrode track and placement is evident as a shadow or artifact on MRI. The *dashed outlined area* is enlarged in panel C. **C:** Enlargement of the right hippocampal region shows the configuration of 1.6-mm EEG electrode sites (macro) as “beads” or swellings in the electrode artifact. Scale bars in B and C indicate 25- and 15- μ m scales in the respective images, as confirmed by tissue landmarks and electrode shadow. **D:** Schematic of the macro-micro electrode utilized in all patients in this study. Microwire (17- μ m) unit recording sites (micro 7–16) were located at the electrode tip and between the pairs of macro (1.6-mm) recording sites.

Postoperative localization of electrode placement was verified by MRI (Fig. 1B and C). Confirmation of electrode placement within the CA1 and CA3 subfields was determined by comparison with a morphometric survey of 3-Tesla MRI scans from 46 normal (i.e., nonepileptic) patients and 26 patients with a history of epilepsy but no prior depth electrode implantation, providing average dimensions of the hippocampus along typical implantation tracks. The typical duration of implantation was 10–20 days, as established by each patient’s care team to allow sufficient time to assess seizure localization.

Neurocognitive Testing and Recording

Depending on individual patient clinical needs, neurocognitive testing was typically performed on postimplantation days 3–7. Patients with electrode implantation in this study ($n = 23$) performed a custom-designed visual Delayed-Match-to-Sample (DMS) memory task modeled after the Cambridge Neuropsychological Test Automated

Battery (CANTAB, Cambridge Cognition Ltd.),¹⁵ adapted from prior implementation in nonhuman primate (NHP) studies.⁸ Patients were tested at bedside and were seated or reclining facing a touch-sensitive screen displaying the DMS trials. Each session consisted of 100 trials involving a “sample” phase in which a single image was presented, a randomized variable delay (1–75 seconds) with no images displayed, and a “match” phase in which the same sample image was displayed along with a randomized number of 1–6 distractor images. A correct response consisted of touching the same image in both the sample and match phases. Trials were separated by a 5-second intertrial interval.

Neurons were recorded on the microelectrode sites of the implanted electrodes using a Blackrock Cervello neural recording system (Blackrock NeuroMed). Extracellular action potential waveforms from neurons were isolated and identified for online and offline sorting of single-unit discharges.¹⁶ Continuous electrical digitized monitoring

TABLE 2. Hippocampal morphometry

Side	Skull Outer Table to Temporal Horn	Temporal Horn to Medial Wall	Subiculum/CA1 Region	Dentate Gyrus/CA3 Region	CA1–CA3 Inner Distance	CA1–CA3 Outer Distance	Mean CA1 Depth	Mean CA3 Depth
Normal/non epilepsy patients (n = 46)								
Rt HPC, mm	32.10 ± 2.92	1.78 ± 1.11	2.00 ± 0.57	1.75 ± 0.41	1.83 ± 0.39	5.08 ± 1.52	34.84 ± 2.78	38.11 ± 3.06
Lt HPC, mm	33.14 ± 2.88	2.02 ± 1.38	1.85 ± 0.55	1.60 ± 0.33	1.68 ± 0.35	4.92 ± 1.86	36.06 ± 2.88	39.26 ± 3.16
Epilepsy patients (not implanted, n = 26)								
Rt HPC, mm	32.51 ± 2.97	1.80 ± 0.36	1.33 ± 0.32	1.40 ± 0.34	1.36 ± 0.25	4.53 ± 0.62	34.98 ± 3.00	37.93 ± 3.06
Lt HPC, mm	32.84 ± 2.43	1.79 ± 0.38	1.24 ± 0.28	1.44 ± 0.33	1.34 ± 0.22	4.48 ± 0.55	35.26 ± 2.43	38.17 ± 2.41
Phase II patients (implanted, n = 23)								
Rt HPC, mm	32.92 ± 2.82	2.01 ± 0.62	1.76 ± 0.44	2.08 ± 0.52	1.92 ± 0.42	5.86 ± 1.10	35.82 ± 2.70	39.71 ± 2.75
Lt HPC, mm	33.08 ± 2.49	2.38 ± 0.53	1.63 ± 0.34	1.87 ± 0.40	1.75 ± 0.27	5.89 ± 0.86	36.28 ± 2.56	40.10 ± 2.78

HPC = hippocampal region.

(30,000 samples/sec) identified single-unit action potential waveforms (bandpass filtered to 500–5000 Hz, 30,000 samples/sec) and single-unit spike events (5000 samples/sec) during DMS task performance. These were merged with DMS task-event markers and retained for analysis.

Results

Neural Recordings

Neural recordings were obtained from 23 patients with electrodes implanted into the hippocampus. Figure 1B shows a coronal T1-weighted MR image captured at the region of the anterior hippocampus, demonstrating electrode placement bilaterally across the CA1 and CA3 cell fields. Figure 1B and C show optimal electrode placement, in which the electrode trajectory (linear shadow with beadlike swellings) is perpendicular to the long axis of the hippocampus, and the macroelectrode sites (Fig. 1D; see also “beads” on the electrode shadow in Fig. 1B and C) can be seen to overlie the CA1 and CA3 cell fields (Fig. 1C). Placement of Ad-Tech MM16C-SP05X-000 macro-micro depth electrodes were compared (where possible) to presurgical MR images to confirm the presence of morphologically distinct CA1 and CA3 cell fields at the electrode recording sites.

Hippocampal Morphometry

Electrode placement for all patients was compared to a hippocampal morphological study of the coronal 3T MR images of 46 normal (i.e., nonepileptic) patients and 26 patients with a history of epilepsy to confirm placement of the microelectrode recording sites (between the distal “beads” on the electrode shadow; Fig. 1C) within the average dimensions of human hippocampal CA1 and CA3 cell fields. The hippocampal morphometric study revealed that across normal patients, the CA1 region was encountered at an average depth of 36.06 ± 2.88 mm from the outer table of the skull on the left hemisphere, and 34.84 ± 2.78 mm on the right hemisphere. Notably, the CA1 and CA3

cell fields were separated by a minimum distance of 1.68 ± 0.35 mm on the left and 1.83 ± 0.4 mm on the right, with a maximum outer span from edge to edge of 4.92 ± 1.86 mm on the left and 5.08 ± 1.52 mm on the right. There was no significant difference between right and left hippocampal CA1 depths (pairwise t-test, $t_{[45]} = 1.86$, nonsignificant). In epilepsy patients without electrode implantation, the CA1 region was encountered at an average depth of 35.26 ± 2.43 mm from the outer table of the skull on the left hemisphere, and 34.98 ± 3.00 mm on the right hemisphere. There was no significant difference between left and right CA1 depths ($t_{[25]} = 0.59$, nonsignificant). The CA1 and CA3 cell fields were separated by a minimum distance of 1.36 mm on the left and 1.34 mm on the right, with a maximum outer span from edge to edge of 4.53 mm on the left and 4.48 mm on the right. There was also no significant difference between the depths of the left CA1 between normal and epilepsy patient populations ($t_{[24]} = 0.62$, nonsignificant), nor were there any significant differences between the implanted and nonimplanted epilepsy patient populations (Table 2). Thus, these distances were consistent with probe implantations with the most distal macro (EEG) recording site approximately 45 mm from the outer table of the skull and micro (single-unit) recording sites between 33 and 42 mm from the outer table of the skull.

Recordings of electrical signals from the microelectrode recording sites were used to isolate single-neuron activity from the human hippocampus. Each signal was recorded with a 60-Hz hardware notch filter to remove line noise, and then digitally bandpass filtered (500–5000 Hz) to isolate single-neuron action potential waveforms. Figure 2 illustrates the digitization and filtering of neural activity to yield isolated single-unit recordings from the 17-μm microelectrode sites on the macro-micro electrodes. Microelectrode neuron recordings were digitized at 30,000 samples/sec (Fig. 2A). Signals were alternating current coupled and bandpass filtered at 500–5000 Hz to isolate single-neuron action potentials (Fig. 2A, part b, ex-

FIG. 2. Digital recording and isolation of single-unit activity from the human hippocampus. **A:** Filtered (b) and unfiltered (c) neural activity from each 17- μ m microelectrode was digitized at 30,000 samples/sec using a Blackrock Cervello system. Bandpass digital filtering (500–5000 Hz) and notch filtering (60 Hz) were applied to assist in isolating single-neuron action potential waveforms. **B:** Expansion of the bandpass-filtered recording (red trace labeled “b” in panel A) at the same amplification revealed waveforms of hippocampal single-unit action potentials. Computer-isolated single units (1–3) were identified by consistent waveform shape and amplitude. **C:** Expansion of 1 msec of unfiltered recording (blue trace labeled “c” in panel A) at twice the amplitude illustrates insufficient identification of single-neuron waveform from non-bandpass-filtered recordings. **D:** Mean action potential waveforms (left) and autocorrelograms (right) for the unique neurons 1–3 identified in panel B. Note the presence of a 2-msec “gap” at 0 msec consistent with a single-neuron refractory period. A/C = alternating current; D/C = direct current.

panded in Fig. 2B) or direct current coupled and notch filtered at 60 Hz to preclude inclusion of artifactual “spikes” due to amplifier overdriving (Fig. 2A, part c, expanded in Fig. 2C). Individual neurons (i.e., “single units;” see 1, 2, and 3 in Fig. 2) were identified by consistent waveform shape and amplitude from filtered recordings (Fig. 2B). Figure 2D shows mean action potential waveforms and autocorrelograms for neurons 1–3 identified in Fig. 2B, thus positively identifying single neuronal recordings. A 2-msec “gap” at 0 seconds is visualized, consistent with the time span of single-neuron refractory periods.

Validation of Hippocampal Neural Recordings

Table 1 summarizes the data gathered from 744 neurons recorded from the 23 patients examined in this study.

Neurons were recorded from a total of 46 implanted electrodes as follows: 7 patients had a single electrode implanted (unilateral anterior hippocampus); 5 patients had 2 unilateral electrodes (anterior and posterior); 7 patients had 2 bilateral electrodes (anterior); 1 patient had 3 electrodes placed (bilateral anterior and 1 posterior); and 3 patients had 4 electrodes (bilateral, anterior, and posterior). Neurons were sorted by recording electrode location within the putative boundaries of CA1 and CA3 cell fields (Fig. 1, Table 2), and then by firing rate (Table 3). Neurons with steady-state resting firing rates > 20 Hz (i.e., clearly interneurons) were not recorded. An additional 27 CA1 and 41 CA3 neurons were putatively identified as interneurons due to steady-state firing rates between 10 and 20 Hz and were recorded, but not subjected to further analy-

TABLE 3. Functional connectivity and putative cell localization in 23 patients

Variable	Total	Mean $\pm$ SEM (per patient)	Min (per patient)	Max (per patient)
No. of putative CA1 neurons	381	16.5 $\pm$ 2.4	7	47
0.5–5.0 Hz	291	12.6 $\pm$ 2	2	43
5.0–10.0 Hz	63	2.7 $\pm$ 0.4	0	9
10.0–20.0 Hz	27	1.1 $\pm$ 0.2	0	6
No. of putative CA3 neurons	432	18.7 $\pm$ 2.8	7	53
0.5–5.0 Hz	325	14.1 $\pm$ 2.2	4	47
5.0–10.0 Hz	66	2.8 $\pm$ 0.3	0	8
10.0–20.0 Hz	41	1.7 $\pm$ 0.3	0	6
No. of CA1–CA3 pairs	4070	101.7 $\pm$ 8	30	252
No. of synchronous correlations (0-msec lag)	2044 (50%)	51.1 $\pm$ 5.6	4	181
No. of feed-forward correlations (2- to 5-msec lag)	814 (20%)	20.3 $\pm$ 1.4	6	45
No. of feed-forward correlations (>5-msec lag)	257 (6%)	6.4 $\pm$ 0.7	0	19
No. of feedback/negative correlations (negative lag)	107 (2%)	2.6 $\pm$ 0.5	0	22
No. of no connections (no correlation)	849 (21%)	21.2 $\pm$ 2.4	1	58

sis. The remaining neurons were further sorted into categories with firing rates between 0.5 and 5.0 Hz consistent with hippocampal pyramidal cells in lower vertebrates^{2,8,17} and rates between 5.0 and 10 Hz that have been shown to

occasionally include hippocampal neurons with behavioral correlates in NHPs.¹⁷ Table 3 shows that approximately 5 times as many neurons exhibited firing rates below 5.0 Hz than above 5.0 Hz, although both categories of neurons

FIG. 3. Mean peri-event firing across CA3 ($n = 135$) and CA1 ($n = 131$) neurons correlated to DMS sample and match responses. Solid arrows (horizontal) denote latency shift in pre-response peak firing. Open arrows (vertical) indicate suppression of peak firing on error trials. Mean firing was computed across neurons for 1555 correct and 355 error trials performed by 23 patients with intracranial electrodes targeting CA3 and CA1 cell fields.

FIG. 4. Heat maps depict mean ensemble firing, averaged with respect to DMS sample (SR) and match (MR) responses. Task-relevant firing of CA1 and CA3 neurons was localized by electrode position, imaging, and cross-correlation as in Fig. 3, with the addition of anterior (Ant.) and posterior (Post.) hippocampal localization. Neurons were sorted as a function of intensity of firing ± 2 seconds around SR and MR responses in the DMS task.

were retained for analysis. Neurons recorded from depth electrodes targeted at the human hippocampus were thus subjected to 1) firing rate, 2) behavioral correlation, and 3) functional connectivity validation and quantification to verify localization of CA1 and CA3 principal cells.

Behavioral Correlates

Peri-event histograms of putative hippocampal neurons were examined for single-neuron firing correlation to behavioral events in the DMS task. Figure 3 shows the mean firing of neurons recorded from hippocampal recording sites targeting the CA3 and CA1 cell fields. Sample and match responses exhibit significant firing peaks ($Z = [\text{peak within sample or match phase} - \text{mean outside of sample and match phases}] / \text{standard deviation outside of sample and match phases}$; significant peak = $Z > 3.09$). Significant amplitude differences or latency shifts in peaks between conditions are indicated by arrows in Fig. 3.

A detailed indication of behavioral correlation of putative hippocampal neurons is shown in Fig. 4. Heat maps indicate the mean firing rate averaged across DMS trials for 203 neurons recorded from anterior and posterior hippocampal recording sites. Individual neurons comprise each horizontal row sorted by region and latency of prominent firing peaks. Time relative to DMS trial events is indicated on the horizontal axis. The neuronal firing rate is indicated by the color scale, with blue indicating mean or background firing rates < 1.0 Hz and red indicating peak firing rates > 10 Hz. These examples demonstrate that putative anterior hippocampal neurons fire primarily in response to sample response, with some firing to the match response. Conversely, putative posterior hippocampal neurons ap-

pear to fire at elevated rates between the sample response and match response peaks. These behavioral correlates are consistent with hippocampal response types observed in both rodents^{18,19} and NHPs.¹⁷

Functional Connectivity

Proposed CA1 and CA3 neuron pairs were constructed between neurons recorded on the same electrodes, and then cross-correlograms were computed as spike-triggered histograms. As shown in Fig. 5 left, putative CA3 unit firing occurred at time = 0 msec, with mean CA1 unit firing plotted from -50 to $+50$ msec relative to the CA3 spike occurrence. Correlograms were analyzed via standard score: $Z = (\text{peak firing} - \text{baseline firing } [-50 \text{ to } -30 \text{ msec}]) / \text{standard deviation of firing } [-50 \text{ to } -30 \text{ msec}]$. Five characteristic correlation patterns were identified by peaks ($Z > 3.09$, $p < 0.001$) in which 1) firing peaks occurred at 0 msec (i.e., 0-msec lag); 2) peak firing was shifted in the positive direction (2- to 5-msec lag); 3) peak was shifted more positively (> 5 -msec lag); 4) no peak was detected (no correlation); and 5) peak firing was shifted negatively (inverse correlation). These correlation patterns were consistent with simultaneous driving of CA3 and CA1 (0 msec), feed-forward monosynaptic (2–5 msec) or multisynaptic (> 5 msec) connectivity, no correlation, or feedback connectivity (inverse correlation) incompatible with CA3-to-CA1 synaptic connectivity. Putative hippocampal principal cells with mean firing rates < 10 Hz as identified in Table 3 were examined for functional correlation as shown in the lower portion of the table.

Figure 5 right shows the distribution of correlation types

FIG. 5. Cross-correlation consistent with functional synaptic connectivity of putative CA3-CA1 pairs. **Left:** Cross-correlograms from putative CA3-CA1 cell pairs illustrate 5 differential patterns of functional connectivity listed in Table 3. Synchronous activity (0-msec differential) indicates common input, consistent with perforant path projections to both CA3 and CA1. Feed-forward short-lag (2–5 msec) correlation is consistent with correctly categorized CA1 and CA3 neurons connected monosynaptically by Schaffer collateral projections. Long-lag feed-forward correlation (> 5 msec) may reflect mono- or multisynaptic connectivity, due to the presence of interneurons. Inverse correlations indicate misidentification of CA3 neurons as CA1, and CA1 neurons as CA3. No correlation indicates lack of functional connectivity under the testing conditions irrespective of localization. **Right:** Bar graphs compare frequency distribution of correlation types listed in Table 3. Corr. = correlation; Fwd = forward; Inv = inverse; NoCorr = no correlation.

within the population of putative CA1 and CA3 neuron pairs recorded and analyzed from the human hippocampus. Only 2.6% of pairs exhibited a feedback (inverse) correlation. Misidentification of CA3 and CA1 neuron pairs would be expected to yield a higher percentage of inverse correlation. While only the 2- to 5-msec feed-forward correlations (20.3%) can be definitively identified as CA3-to-CA1 synaptic connectivity, the predominance of positive correlations (77.8%) and infrequent occurrence of inverse correlations suggest that the synchronous and multisynaptic correlate cell pairs are correctly identified with respect to CA3 and CA1 localization, and are further consistent with known common projections from entorhinal cortex to hippocampus, as well as indirect projections within the CA3 and CA1 cell layers.

Discussion

Continuous and randomized stimulation of the human hippocampus has been shown to be detrimental to recall in memory-associated tasks.^{20,21} In contrast, model-based patterned stimulation, previously assessed in rodents, NHPs, and humans, has been shown to significantly improve performance in a DMS memory task.^{6,8,16} To accurately assess the performance of a human neuroprosthetic that utilizes model-based stimulation for enhanced memory storage and recall,^{22,23} placement of stimulating electrodes within the CA3 and CA1 subfields of the hippocampus must be confirmed.

Hippocampal neurons have previously been identified with specific encoding of spatial, object, and task-related

features in rodents and NHPs through MRI verification in combination with assessment of neuronal firing patterns.^{9,17,18} In humans, prior efforts at stimulation of mesial temporal lobe structures were based on approximating depth electrode placement from operative radiographs.²⁴ These efforts^{20,21} indicated that random hippocampal stimulation resulted in memory impairment. Suthana et al.,¹² however, reported memory enhancement of spatial information from electrical stimulation of the entorhinal cortex via implanted intracranial depth electrodes. Once again, evidence of placement of the electrode within the hippocampus and entorhinal cortex was based on imaging (pre- and postoperative MRI). To accurately establish the pattern by which human hippocampal neural ensemble firing patterns encode information for memory processing for future utilization in neural prosthetics,¹⁶ definitive localization of depth electrode contacts within the CA3 and CA1 subfields must be established. It is therefore imperative to utilize more precise methods for this confirmation.

To accomplish this objective, we performed a morphometric anatomical survey in the coronal plane of 3T MR images in 46 normal patients and 26 patients with diagnosed epilepsy. The locations of the temporal horn, subiculum/CA1, and dentate gyrus/CA3 were determined. The distances obtained in this morphometric survey assisted in the targeting of depth electrode placement in epilepsy patients who were to undergo preplanned depth electrode placement for seizure monitoring. The mean distances were again used to assess depth electrode placement on postoperative MRI. Localization using standardized temporal stereotactic systems, such as that described by Miller et al.,²⁵ would be useful for future iterations or for standardization across surgical centers.

To confirm localization and accuracy of electrode placement for this study, we combined this MRI-based localization technique with detailed electrophysiological analysis of neural recordings. Using this technique, a total of 813 neurons were recorded and analyzed from 23 patients. A critical advantage of our approach is that correlation between CA3-CA1 pairs was identified in 1071 of a total 4070 pairs, similar to the 20%–25% feed-forward correlation noted in animal studies.^{26,27} Misidentification of CA3 and/or CA1 neurons, as evidenced by feedback (negative) correlation, was noted in 2.6% of pairs, while multisynaptic correlations were noted in 6.4% of pairs (again, consistent with animal studies²⁶). This critical step allowed us to determine functional connectivity of the identified neurons, which will be necessary for the further design of specific, targeted stimulation interfaces to enhance neuronal encoding.¹⁶ Interestingly, characteristic differences were noted in the neuronal firing of the anterior and posterior hippocampus during the DMS trials; future studies will determine broadly if differential function occurs with respect to this or other memory tasks.

Conclusions

In this study we have presented data supporting placement and localization of depth electrodes within the CA3 and CA1 cell fields of the human hippocampus. Verification of placement was established through a combination

of high-resolution MRI and validation of hippocampal neural recordings utilizing baseline firing rate analysis, behavioral correlation, and functional connectivity. This study provides confirmation of the ability to record neurons in vivo from identified structures and subfields of the human brain and represents an important step in validating and targeting coordinates for intracranial recordings in the human hippocampus.¹⁶ As brain-machine interface and neural prosthetic research continues to expand, it is essential to be able to record and stimulate critical sites within the neural circuits of interest.

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Disclosures

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Conception and design: Hampson, Wicks, Witcher, Popli, Whitlow, Deadwyler. Acquisition of data: Hampson, Wicks, Witcher, Couture, Laxton, Popli, Fetterhoff, Dakos, Roeder. Analysis and interpretation of data: Hampson, Wicks, Witcher, Whitlow, Fetterhoff, Dakos, Roeder. Drafting the article: Hampson, Wicks, Witcher, Whitlow. Critically revising the article: Hampson, Wicks, Witcher, Dakos, Roeder. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Hampson. Statistical analysis: Hampson, Wicks, Witcher. Administrative/technical/material support: Hampson, Couture, Laxton, Popli, Deadwyler. Study supervision: Hampson, Couture, Laxton, Whitlow, Deadwyler. Neurosurgeon for study patients: Couture, Laxton. Supervising physician for IRB: Couture. Clinical care oversight, oversight of Epilepsy Monitoring Unit, head neurologist for study patients: Popli.

Supplemental Information

Videos

Video Abstract. <https://vimeo.com/426970861>.

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