Coding of Episodic Memory in the Human Hippocampus

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Neurocomputational models have long posited that episodic memories in the human hippocampus are represented by sparse, stimulus-specific neural codes. A concomitant proposal is that when sparse-distributed neural assemblies become active, they suppress the activity of competing neurons (neural sharpening). We investigated episodic memory coding in the hippocampus and amygdala by measuring single-neuron responses from 20 epilepsy patients (12 female) undergoing intracranial monitoring while they completed a continuous recognition memory task. In left hippocampus, the distribution of single-neuron activity indicated that only a small fraction of neurons exhibited strong responding to a given repeated word and that each repeated word elicited strong responding in a different small fraction of neurons. This finding reflects sparse distributed coding. The remaining large fractions of neurons exhibited a concurrent reduction in firing rates relative to novel words. The observed pattern accords with longstanding predictions that have previously received scant support from single-cell recordings from human hippocampus.

Hippocampus | Episodic memory | Single units | Amygdala

Episodic memory affords the capacity to recollect past events that occurred at a particular time and place (1). In humans, episodic recollection allows for the re-experiencing of an event through a process of mental time travel (2). The ability to encode new episodic memories depends on the hippocampus, but it is not clear how episodic memories are coded by the activity of individual hippocampal neurons. We investigated the activity of isolated hippocampal neurons in epileptic patients undergoing intracranial monitoring while they encoded and retrieved episodic memories. Memory was tested using a continuous recognition procedure (3) in which words were presented in a continuous stream and were sometimes repeated. Throughout the task, patients were asked to classify each word as "new" upon its first presentation and as "old" if it was repeated. A correct "old" decision in response to a repeated word is an instance of successful episodic memory (i.e., memory for the prior occurrence of the word in the experimental context). Hippocampal lesions impair performance on continuous recognition tasks for words (4).

Neurocomputational models (5-8) have long posited that coding in the hippocampus is sparse and distributed. Thus, individual episodic memories are represented by the activity of small and typically non-overlapping sets of neurons. Under such a coding scheme, activity associated with the retrieval of a specific episodic memory would be hard to detect because only a small proportion of hippocampal neurons would exhibit increased firing rates. Perhaps for this reason, single-unit studies of recognition memory in humans and nonhuman primates have often failed to detect any activity related to episodic memory in the hippocampus (9-12). Moreover, when activity related to episodic memory has been detected, the identified neurons responded non-specifically, coding whether stimuli were novel or familiar (13-19), and leaving open the question of whether neurons can be found that sparsely code some recently studied items and not others.

A standard procedure for detecting stimulus-specific, single-unit activity involves repeatedly presenting a stimulus to determine if a neuron responds reliably only when that stimulus is presented. Notably, this approach has identified neurons that respond selectively to the presentation of a photo of a particular person or landmark (20-21). In studies of episodic memory, one would expect to find not only neurons that code stable semantic knowledge about the material being learned but also neurons that code aspects of the learning event itself. By definition, episodic memory involves retrieving an episode that occurred only once, i.e. a learning event such as remembering the earlier presentation of a word. Note that repeating a studied word not only prompts retrieval of its prior occurrence but also creates a new and distinct episodic memory, potentially coded by a different set of neurons. In that case, a neuron that responded the first time a word was repeated might not respond to its repetition. Moreover, if a given neuron did respond to every repetition of a word, the neuron might be responding to the word's context-free semantic meaning, not to the word's episodic occurrence in the experimental context. For these reasons, instead of searching for neurons that respond reliably to words repeated multiple times, we used an approach that is capable of detecting rare spiking events that theoretically signal episodic memory in response to words that were repeated only once.

Results

Behavioral Performance. Invalid trials (13.6% of all trials) were excluded from analysis. These trials had either no responses, early responses, or multiple keys pressed. From the remaining valid trials, hit rates (proportions of correctly classified repeated words) and false alarm rates (proportions of novel words mistakenly classified as repeated) were computed for each session. These hit and false alarm rates were used to compute a standard discriminability measure (d'). Performance measures for the 37

Significance

Neurocomputational models hold that episodic memories are represented by sparse, stimulus-specific neural codes. In tests of episodic memory, single unit recording studies of the human hippocampus have found neurons that operate as general novelty detectors or general familiarity detectors. Here, we investigated whether neurons can be found that sparsely code some recently studied items and not others. In left hippocampus, but not the amygdala, we found that small fractions of neurons exhibited strong responses to specific repeated words. The remaining large fractions of neurons exhibited a concurrent reduction in firing rates relative to novel words. Both findings are consistent with predictions made by neurocomputational models of how episodic memory is coded in the hippocampus.

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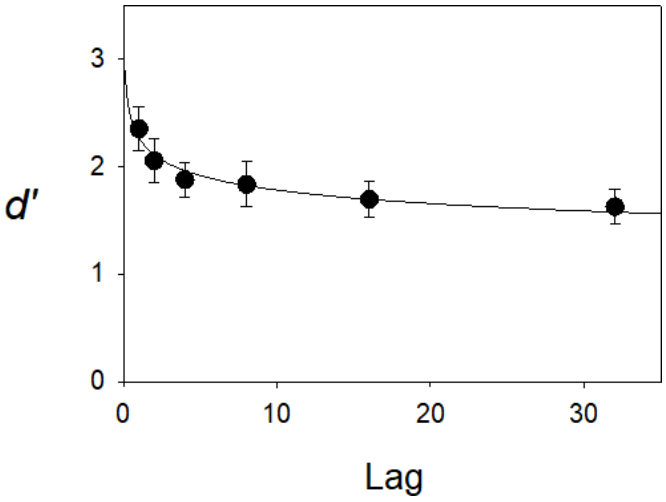

Fig. 1. . Behavioral forgetting function for the continuous recognition task. Discriminability (d') declined significantly as a function of the number of intervening items (lag) according to a repeated-measures ANOVA ($p < .001$). Each patient's d' score was first computed by averaging across recognition test sessions. Each point in the figure represents the average across all 20 patients. The smooth curve represents the least-squares fit of a power function, $d' = a*Lag^{-b}$, where a and b are free parameters. Error bars represent standard errors.

sessions were computed separately for each patient (averaged across sessions), and were then averaged across patients. The average false alarm rate was .21. Unlike the false alarm rate, hit rates could be computed separately by lag and showed a monotonic decline as lag increased (hit rates = .88, .80, .76, .75, .71 and .70 for the six lags, respectively). The corresponding *d'* scores exhibited the typical power law of forgetting (Fig. 1).

Analysis of Single Unit Activity in Hippocampus and Amygdala. Across all patients and all 37 sessions, we recorded 275 single units in the amygdala (161 left, 114 right) and 243 single units in the hippocampus (128 left, 115 right). The average background firing rates for these units were 2.20 and 1.60 spikes/s in left and right hippocampus, respectively, and 1.30 and 1.04 spikes/s in left and right amygdala, respectively.

In all four regions, some neurons exhibited spiking activity that significantly differed, on average, for repeated vs. novel items ("Significant Units" in Table 1), but only in the left amygdala were significant units detected with a frequency (27 out of 161) that exceeded chance expectations ($p < .0001$). This effect was largely attributable to increased firing rates to novel words. Of the 27 significant units in the left amygdala, 25 showed a novelty-detection pattern, whereas 2 showed the opposite pattern. Due to chance alone, under the null hypothesis, one would expect to find approximately $.05 * 161 \approx 8$ significant units in left amygdala, with equivalent counts of "novelty detectors" and "familiarity detectors." Thus, observing 2 familiarity detectors likely reflects chance alone, but this is unlikely to be the case for the 25 novelty detectors. Among the novelty detectors, the average normalized firing rate to novel items was 0.54 σ units above baseline, whereas the average normalized firing rate to repeated items was only 0.14 σ units above baseline.

Analysis of Spike Count Distributions from the Hippocampus. In the hippocampus, units distinguishing repeated vs. novel items were not detected at a significant frequency. Yet, if a given neuron in the hippocampus strongly responds on only a handful of repeated trials (e.g., < 5%), as in a sparse distributed coding scheme, a significant difference in the average firing rate for novel vs. repeated items is unlikely to be detected. To detect such activity in the hippocampus, if it exists, one should instead examine the full distributions of normalized spike counts (pooled across single units recorded from all patients) from trials involving novel items and, separately, from trials involving repeated items. In right hippocampus, no significant differences were observed in either the means (Fig. 2A) or the standard deviations (Fig. 2B) of the full distributions for repeated and novel items. In left hippocampus, the means of these two distributions also did not differ significantly (Fig. 2A), but a reliable difference was observed for their standard deviations (Fig. 2B).

Two distributions that have similar means and different standard deviations can differ in more than one way (Fig. 3). To investigate the source of the standard deviation difference between the distributions in left hippocampus, we constructed empirical quantile-quantile (QQ) plots (22). An empirical Q–Q plot is a graphical method of analysis that essentially displays one rank-ordered dataset (i.e., the sorted normalized spike counts for the repeated items) against another independently rank-ordered dataset (i.e., the sorted normalized spike counts for the novel items). We recently used this approach in a study of episodic memory (23), but because only 34 single units were recorded, the analysis was based primarily on multi-units, and convincing evidence of sparse distributed coding at the level of single units was not demonstrated. The present analysis is based on a much larger sample of 243 single units, and no multi-units were included.

The QQ plot from left hippocampus (Fig. 4A) is consistent with a bimodal distribution for repeated items (as illustrated in Fig. 3B). The pattern is similar for trials in which patients made a correct response (hit or correct rejection) and trials in which they made an error (miss or false alarm, Fig. S1). As predicted by the sparse distributed coding account, the points fall mostly along the diagonal line and then exhibit a sharp upward deflection at the upper-right end of the plot. When broken down by lag, the pattern did not vary in any systematic way as lag increased (Fig. S2). In right hippocampus, the QQ plot (Fig. 4B) shows no apparent departure from the diagonal line, which is consistent with the finding of similar means and standard deviations for the repeated- and novel-item distributions in right hippocampus (Fig. 2A and 2B, Right H). The data in Fig. 4A reflect an episodic memory signal in that the upward deflection at the upper-right end of the QQ plot indicates that some neurons responded strongly to a few repeated words even though the same neurons did not respond to those words when they were novel.

The significant difference in standard deviations for trials involving novel items vs. repeated items in left hippocampus (Fig. 2B, Left H) is consistent with visual evidence of bimodality in the QQ plot from left hippocampus (Fig. 4A). If a small upper distribution for repeated items is responsible for both the increased standard deviation and the visual signature of bimodality in the QQ plot (as illustrated in Fig. 3B), then removing a small percentage of scores from the upper tails of both the novel-item and repeated-item distributions should eliminate the difference in the standard deviations as well the visual evidence of bimodality in the QQ plot. In agreement with that prediction, when the highest 2.5% of spike counts were removed from both distributions, the QQ plot for the left hippocampus became essentially linear (Fig. 4C), and the difference in standard deviation between novel and repeated item distributions was eliminated (Fig. 5B, Left H). Note that the pattern observed after removing the highest 2.5% of the scores remains evident when larger proportions of each distribution are removed (Fig. 6). These results indicate that, when 100% of the data is analyzed, the standard deviation difference in left hippocampus (Fig. 2B, Left H) arose because of strong neural responses that occurred on a small percentage of repeated-word trials (the same trials responsible for the non-linear QQ plot in Fig. 4A). Our procedure was a verbal memory task, which likely explains why the effects were evident in only left hippocampus.

Table 1.

Region	Side	Recorded Units	Significant Units	Fraction	adj *p*
Amygdala	L	161	27	0.17	< .001
	R	114	4	0.04	0.827
Hippocampus	L	128	11	0.09	0.114
	R	115	7	0.06	0.469

Number of recorded units and number of significant units (i.e., units for which, using an unadjusted t-test, mean spikes for repeated items differed significantly from mean spikes to novel items) from left (L) and right (R) amygdala and hippocampus. Fraction = significant units / recorded units. The *p*-value (adj *p*) is the probability of observing that fraction by chance alone, after correcting for multiple testing using the Benjamini-Hochberg procedure. The same procedure was used to compute adjusted *p*-values in the subsequent analyses.

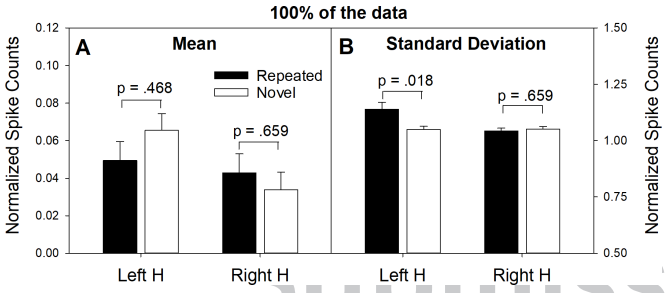

Fig. 2. . Mean and standard deviation statistics associated with 100% of normalized spike counts (collapsed over patients and sessions) for repeated and novel items. Mean (**A**, left y-axis) and standard deviation (**B**, right y-axis) of normalized spike counts associated with the full distributions (100% of the data) for repeated items (n = 12,854 spikes) and novel items (n = 13,822 spikes) in left and right hippocampus (H) collapsed over lag. The normalized spike counts are expressed in standard deviation (sd) units. In left hippocampus, repeated words elicited a mean increase in firing that was .05 sd units above baseline (similar to novel words). However, the standard deviation of the normalized spike counts was larger for repeated words than novel words (1.14 vs. 1.05). The *p*-values represent the probability of obtaining the observed difference (for repeated vs. novel items) by chance, under the null hypothesis of no difference (adjusted for multiple comparisons). The standard deviation effect tracked item status (repeated vs. novel), not the behavioral decision. More specifically, the standard deviation scores for hits and misses (repeated items) were 1.14 and 1.16, respectively, and the corresponding values for correct rejections and false alarms (novel items) were 1.06 and 1.00, respectively.

Removing the upper 2.5% of repeated- and novel-item scores from the aggregate distributions for the left hippocampus not only eliminated the standard deviation difference between the repeated- and novel-item distributions but also revealed another effect. Specifically, in left hippocampus, the mean normalized spike count of the remaining 97.5% of repeated-item scores was now significantly reduced, relative to the mean of the remaining 97.5% of novel-item scores (Fig. 5A, Left H). This finding suggests that strongly activated neurons (representing an episodic memory trace) inhibited competing neurons, an effect that has been termed neural sharpening (8). Upon close inspection, this effect in left hippocampus is visually apparent in the QQ plot (Fig. 4C), in that points on the left end of the plot consistently fall slightly below the diagonal line.

We next examined which patients and which repeated words contributed to the highest 2.5% of normalized spike counts. Of the 20 patients tested, single unit activity was detected in left hippocampus in 13 of them. Of those 13 patients, 11 yielded normalized spike counts in response to at least 4 unique words (mean = 22.3 words) that fell in the top 2.5% of normalized spike counts for repeated items. Thus, the increased standard deviation associated with repeated items in left hippocampus (Fig. 2A) was not caused by a single patient or a single repeated word but was instead a more general phenomenon.

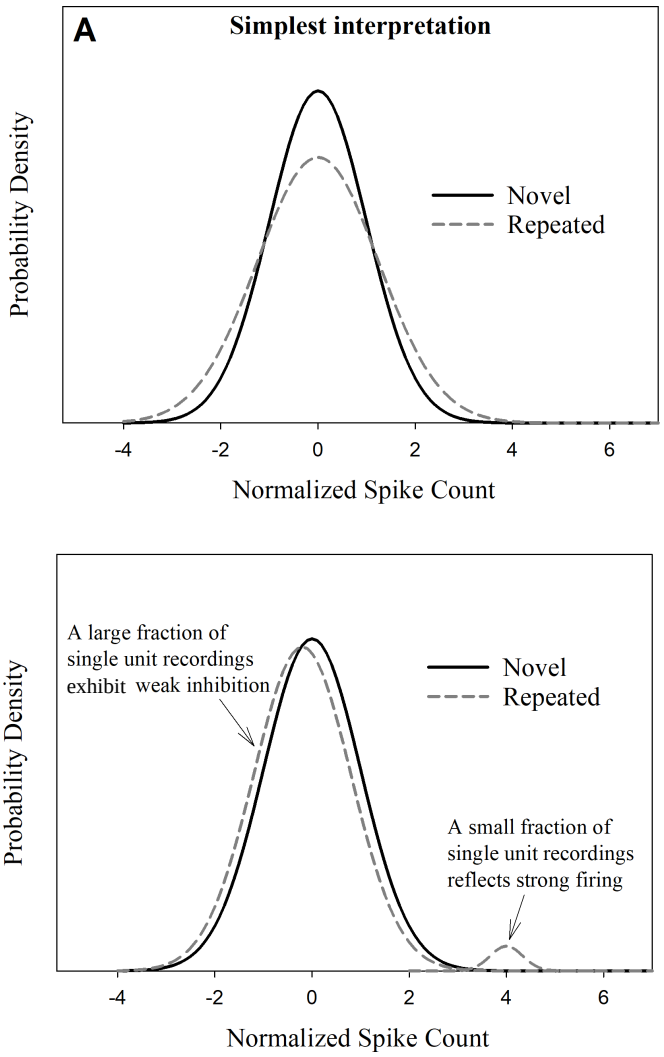

Fig. 3. . Hypothetical novel- and repeated-item aggregate distributions of normalized spike counts with the same means but different standard deviations. **A.** Distributions with the same shape but different standard deviations. **B.** Distributions with *different* shapes and different standard deviations. As predicted by a sparse distributed coding account, a small percentage of recordings made to repeated items (~2.5%) would yield strong responses and the remainder (~97.5%) would yield weakly inhibited responses. The strong responses would increase the standard deviation of the repeated-item distribution. The data conform to this pattern.

Analysis of Spike Count Distributions from the Amygdala. As noted earlier, a general novelty signal in the left amygdala was strong enough to be detected at the level of individual single units (Table 1). How does that effect manifest itself in an analysis of

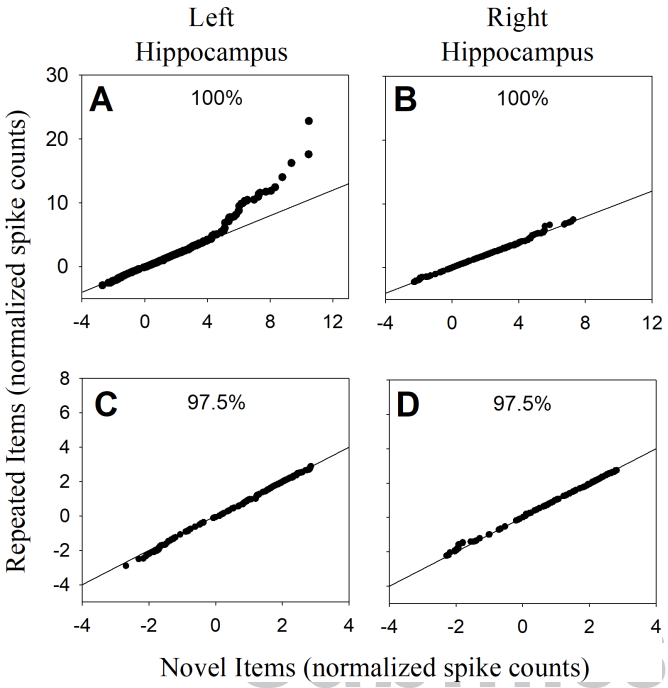

Fig. 4. . QQ plots for left and right hippocampus for 100% of the data (A and B, respectively) and after excluding 2.5% of the data with the highest spike counts from both the repeated-item and novel-item distributions (C and D, respectively). Each point on a QQ plot represents the normalized average spike count recorded on a single test trial. The plot displays those values aggregated across trials and patients. In left hippocampus, the 100% plot displays 12,854 and 13,822 normalized spike counts for repeated and novel items, respectively. In right hippocampus, the corresponding values are 11,089 and 11,955 normalized spike counts.

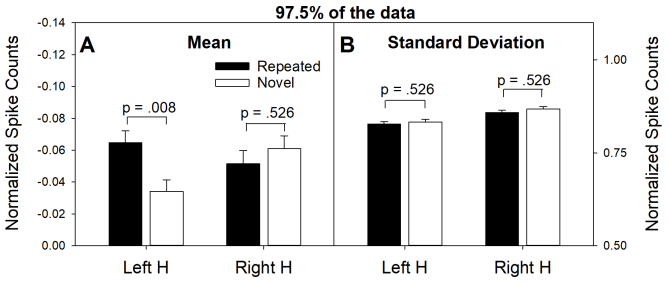

Fig. 5. . Mean and standard deviation statistics associated with 97.5% of normalized spike counts (collapsed over patients and sessions) for repeated and novel items. Mean (**A**, left y-axis) and standard deviation (**B**, right y-axis) of normalized spike counts after excluding the highest 2.5% of the scores for each distribution (retaining 97.5% of the data) in left and right hippocampus. Note in left hippocampus that the mean firing rates were now significantly different for repeated vs. novel words, and the standard deviations were similar. Because the y-axis in **A** covers a range of negative values, the mean for novel items is greater (i.e., closer to 0) than the mean for repeated items. The p-values for these statistical tests were also adjusted for multiple comparisons

the full novel-item vs. repeated-item distributions of single unit recordings? In the left amygdala (but not in the right amygdala), the overall mean and standard deviation of the full distributions were both significantly greater for novel compared to repeated items (Table S1). This overall novelty signal in left amygdala is consistent with the fact that 25 single units were identified in left amygdala that were general novelty detectors. The QQ plots for recordings made from left and right amygdala (Figures S3A and S3B, respectively) show no evidence of bimodality. When

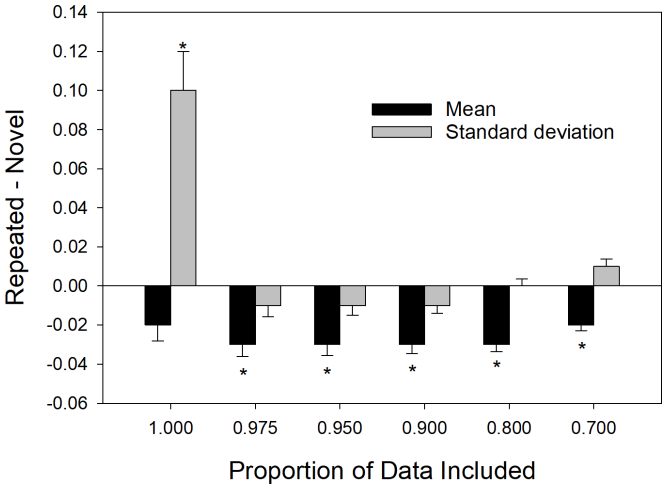

Fig. 6. . Statistics for the full distribution of scores represented as the difference in normalized firing rates for repeated vs. novel items in left hippocampus. The figure shows means and standard deviations as a function of the proportion of the scores from each distribution that was included in the analysis. The mean and standard deviation difference scores for proportions of 1.00 and .975 correspond to the data for left hippocampus shown in Fig 2A and 5A respectively. The asterisks indicate that the difference was significant at $p < .05$ (not corrected for multiple comparisons).

2.5% of the highest scores were eliminated, the statistical pattern of results was unaffected, unlike in left hippocampus. More specifically, whether 100% of the data are considered (Table S1A) or 97.5% of the data are considered (Table S1B), a significant difference is evident for both the mean and standard deviation in left amygdala. These differences remain significant even when 20% of the highest values are removed from the analysis. Thus, the pattern for both single unit activity (Table 1), and the means and standard deviations of spike activity aggregated across single units (Table S1), differs for left amygdala compared to left hippocampus.

Discussion

In studies of semantic memory, single neurons in the medial temporal lobe have been identified that respond reliably to repeated presentations of a known place or landmark, such as the Eiffel Tower (20, 24, 25). Similarly, in studies of episodic memory (16-18), single neurons in the medial temporal lobe have been identified that respond reliably to repeated presentations of items drawn from a general stimulus class (e.g., novel items). However, repeated stimulus presentations cannot be used to identify stimulus-specific, episodic representations. A neuron that codes episodic memory for a previous, context-specific presentation of a particular stimulus should respond selectively to the first repetition of that stimulus in a recognition test (at which time retrieval of the original experience may occur), but it will not necessarily respond to any subsequent presentations of that same stimulus. When repeated a second time, the stimulus may occasion retrieval of the first repetition (coded by different neurons), not the original experience. We therefore investigated stimulus-specific episodic coding in the human hippocampus, using an analysis performed on once-presented test items.

We found evidence for two complementary episodic memory signals in the human hippocampus, both of which have long been predicted by neurocomputational models (5-8). First, we identified a sparse-distributed memory signal, characterized by strong neural firing in response to repeated items (relative to novel items) for a small fraction of recordings (< 2.5%). Typically, a neuron exhibited a strong response to only 2 or 3 repeated items but not to any of the other repeated items. Moreover,

small fractions of neurons responded to different repeated items. Second, we identified a general suppression of firing rates in response to repeated items for the remaining large fraction of recordings (~97.5%). This neural-sharpening pattern was observed in the hippocampus (where sparse distributed coding of episodic memory is theorized to occur). A similar phenomenon, termed response suppression, has also been described in perirhinal/inferotemporal cortex in monkeys performing a recognition memory task (26, also see discussion in 27). Suppression of firing rates was not observed in the amygdala. Instead, in the left amygdala, we identified individual neurons that function as general novelty detectors (16-18).

The complementary effects observed in the hippocampus may reflect Hebbian learning coupled with inter-neuron competition, now a cornerstone of neurocomputational models (8). Empirically, a pattern consisting of a small group of cells with high firing rates coupled with the global suppression of a large group of cells with much lower firing rates has been reported in area CA1 as rats formed memories of a novel maze (28). The pattern we observed in the human hippocampus may reflect similar effects with respect to the encoding and retrieval of episodic memories. That is, small neural assemblies, when active, spread inhibition across many other neurons. This interpretation accords with other findings showing that interneurons impose surprisingly widespread inhibition throughout cell layers (29, 30).

In our study, fewer than 2.5% of single units in the hippocampus were strongly activated when an item was repeated on the continuous recognition test. This 2.5% figure reflects a combination of lifetime sparseness (percentage of stimuli that a given neuron responds to) and population sparseness (percentage of neurons that respond to a given stimulus). These two measures of sparseness are typically assumed to be similar to each other (31), and they have been found to be highly correlated in mouse V1 (32). Assuming the same is true of our data, we estimate that both lifetime sparseness and population sparseness in the human hippocampus are less than 2.5%.

Recent evidence suggests that the absolute number of neurons used to represent an experience is relatively stable between nonhuman primates and rats (33). Because nonhuman primates have a larger hippocampus than rats, the implication is that population sparsity (proportion of active cells) would be smaller in the nonhuman primate compared to the rat. The nonhuman primate population sparsity estimate in that study was approximately 4% in CA1, CA3 and DG (for rodents the estimate was approximately 30%). Because humans have a larger hippocampus than nonhuman primates, our estimate of less than 2.5% in humans is consistent with the idea that the absolute number of neurons used to represent an episodic experience is evolutionarily preserved in humans, nonhuman primates and rats.

Neurocomputational models predict sparse coding of episodic memory in the hippocampus but not in the amygdala. In accordance with that prediction, we found evidence of sparse coding only in the hippocampus. In the amygdala, a generalized novelty detection signal was observed. In contrast to the pattern we observed here, other recent studies of recognition memory in epilepsy patients reported evidence for both general novelty detectors and general familiarity detectors, in both the hippocampus and the amygdala (16-18, 34, 35). Similarly, another recent study of one-trial associative learning in epilepsy patients reported changes in stimulus-specific single unit activity as a function of learning in both the hippocampus and the amygdala (36). The fact that similar memory signals were observed in both structures in these studies is somewhat surprising given that recognition memory is a hippocampus-dependent task (but not an amygdala-dependent task) and that neurocomputational models predict that memory-related neural activity associated with episodic memory will be detected in the hippocampus (but not in the amygdala). It is unclear why memory-related activity of single units in the hippocampus and amygdala are sometimes similar and sometimes different.

Although we previously found evidence for a sparse-distributed memory code in the human hippocampus using an old/new recognition procedure (23), we did not detect any evidence of either neural sharpening in the hippocampus or novelty detection in the amygdala, as in the present study. However, that study involved many fewer single-units than we analyzed here, so there may have been insufficient power to detect the effect. Alternatively, the disparate pattern may reflect task differences. In continuous recognition memory, novel items carry greater significance, relative to study-test recognition, as the participant must simultaneously classify novel items as "new" and also encode them for later recognition. By this interpretation, the novelty signals we observed in left amygdala may reflect the high task relevance of novel items on the continuous recognition task (36).

Methods

Participants. The participants were 21 patients with drug-resistant epilepsy requiring the implantation of depth electrodes (Ad-Tech Medical, Racine, WI) for clinical evaluation and consideration of possible surgical resection of their seizure foci. The mean age of the patients was 40 (range 20 - 61 years), 12 were female, 20 were right-handed, and all had temporal lobe epilepsy. All patients provided informed consent to participate in the research, using a protocol approved by the Institutional Review Board of St. Joseph's Hospital and Medical Center (Phoenix, AZ). The final analysis included data from only 20 patients because the recognition memory performance of one patient was close to chance (see below).

Materials and procedure. The patients were tested using a continuous recognition task with words as stimuli. The words were 120 one-syllable, 120 two-syllable, and 120 three-syllable words, all taken from the MRC Psycholinguistic database (37). Each word was presented in either the Bradley or Impulse fonts (the font manipulation had no effect on any dependent measure, so we collapsed across fonts for all analyses.) One set of stimulus materials consisted of 40 each of the one-, two-, and three-syllable sets in both fonts. Another 15 one-syllable words in each font were used as fillers and never repeated. There were 3 separate sets of stimulus materials that could be presented, and these were used for patients who volunteered for multiple sessions.

Each experimental session consisted of a sequence of 255 trials, including 15 filler trials. (Filler words were presented only once in order to make the overall probability of repetition equal 50%.) In each trial, a word was shown for 1.5 seconds, followed by a question mark. Up to 2 seconds were allowed for a key press, indicating either that the word was repeated (previously seen in this experimental session) or novel. Repeated words were presented after 0, 1, 3, 7, 15, or 31 intervening words. In total, we administered 45 recognition tests to 21 patients. Five patients took more than 3 tests and saw a stimulus set repeated one or two times, but repetition of the stimuli had no significant effect on performance (i.e., recognition accuracy was unaffected by having previously seen a particular stimulus set.) Across patients, eight recognition tests resulted in poor recognition scores ($d' < 0.5$) and were excluded from further neural analysis, leaving 37 sessions to be analyzed from 20 patients.

Microwire Implantation. Electrode implantation was performed stereotactically (Medtronic StealthStation) using a preoperative structural MRI. This procedure localizes the tips of the microwires to within 2 mm (38). Bundles of nine 38-μm-diameter platinum-iridium microwires (California Fine Wire, Grover Beach, CA) were introduced through a lumen within the clinical intraparenchymal electrode during surgery. The implantation sites were chosen according to clinical criteria, which limits the potential recording sites. For the 20 patients studied here, however, the sites included the hippocampus and amygdala, bilaterally. In the hippocampus, the wires were targeted to be in the mid-body of the hippocampus, just behind the head of the hippocampus, opposite the apex of the cerebral peduncle. In the amygdala, the wires were targeted to be in the center of that structure.

Filtering and event detection. Extracellular potentials were recorded from the tips of the microwires using techniques previously described (39) and digitized at 29,412 Hz with 16-bit resolution. Possible action potential events (APs) were detected using digital filtering and thresholding (39). Because more than one neuron may be recorded near any given electrode, APs were sorted into several clusters of similar waveform shape using the open-source clustering program KlustaKwik (Klustakwik.sf.net). After sorting, each cluster was graded as being noise, multi-unit activity (MUA), or single-unit activity (SUA) based on criteria such as the waveform shape (Fig. S4), size of the waveform relative to noise, evidence of a refractory interval, and lack of powerline interference, using the criteria described previously (39).

In our experience, this technique produces results comparable to prior reports in other laboratories (19) in terms of recorded waveform shapes, interspike intervals, and firing rates. While it is important to note that these

and other reports of human single-unit recordings (40, 41) do not achieve the quality of unit separation achievable in animal recordings (42), they nonetheless represent neural activity at a much finer spatial and temporal scale than is achievable using other methods such as fMRI. Measurement at a fine spatial and temporal scale (not necessarily the measurement of single units per se) is necessary to test the predictions of neurocomputational models that assume a sparse distributed episodic memory coding scheme.

We recorded from a total of 1546 clusters of events representing neural activity in the medial temporal lobe (amygdala and hippocampus), 518 of which satisfied the criteria for SUA and 1028 of which were categorized as MUA. In this report, we focused on SUA (161 neurons in left amygdala, 114 neurons in right amygdala, 128 neurons in left hippocampus, and 115 neurons in right hippocampus). Post-stimulus spike counts for each unit were recorded 200 to 1000 ms after the onset of the test stimulus, and pre-stimulus (baseline) spike counts were recorded 1000 to 200 ms before the onset of the test stimulus. The test period during which post-stimulus spike counts were recorded was chosen because a previous study (20) found that selective responses of hippocampal neurons began approximately 300 ms after stimulus onset and because nearly all behavioral responses occurred after 1 s.

Data Analysis. For every recorded neuron, we computed normalized spike counts for each trial (*i*), where a "trial" refers to the presentation of a novel or repeated word. For each neuron (*j*), its baseline mean and standard deviation of spike counts (μ_j and σ_j, respectively) were computed across all trials. Normalized post-stimulus spike counts for a given trial (N_{ij}) in which s_{ij} raw spike counts were recorded on trial *i* for neuron *j* is given by $N_{ij} = (s_{ij} - \mu_j) / \sigma_j$. Trials in which a behavioral response occurred during the 1.5-s stimulus presentation (and, therefore, before the signal to respond was presented) were denoted as "early" responses and were excluded from the analysis.

The data were analyzed separately for each of four brain regions (left hippocampus, right hippocampus, left amygdala and right amygdala). First, we performed a conventional analysis on the normalized spike counts (using ANOVA) to identify individual neurons in the hippocampus and/or amygdala that were responsive to the general class of novel or repeated items, with word repetition status (novel vs. repeated) as the independent variable. Second, an aggregate analysis was performed on the full distributions of normalized single unit activity in a given region (collapsed over patients and sessions) for novel and repeated items. The question was whether the mean and/or standard deviation of the repeated-item distribution differed significantly from the corresponding parameters of the novel-item distribution (e.g., in left hippocampus). The statistical reliability of any difference in either the mean or the standard deviation of the two distributions was tested using a bootstrap procedure. For each test (e.g., comparing the standard deviations for novel and repeated items in left hippocampus), 10,000 bootstrap trials were performed in which (1) the data from all repeated words ($n_{Repeated}$) and all novel words (n_{Novel}) were combined, (2) $n_{Repeated}$ bootstrap "targets" and n_{Novel} bootstrap "foils" were randomly sampled with replacement from that combined data set, and (3) the difference between the statistic of interest (e.g., standard deviation) of those two bootstrap samples was computed. The resulting *p*-value was the proportion of bootstrap trials in which the absolute value of the difference was greater than the observed difference. A similar bootstrap analysis yielded the estimated standard errors shown in Fig. 2, Fig. 5 and Fig. 6.

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