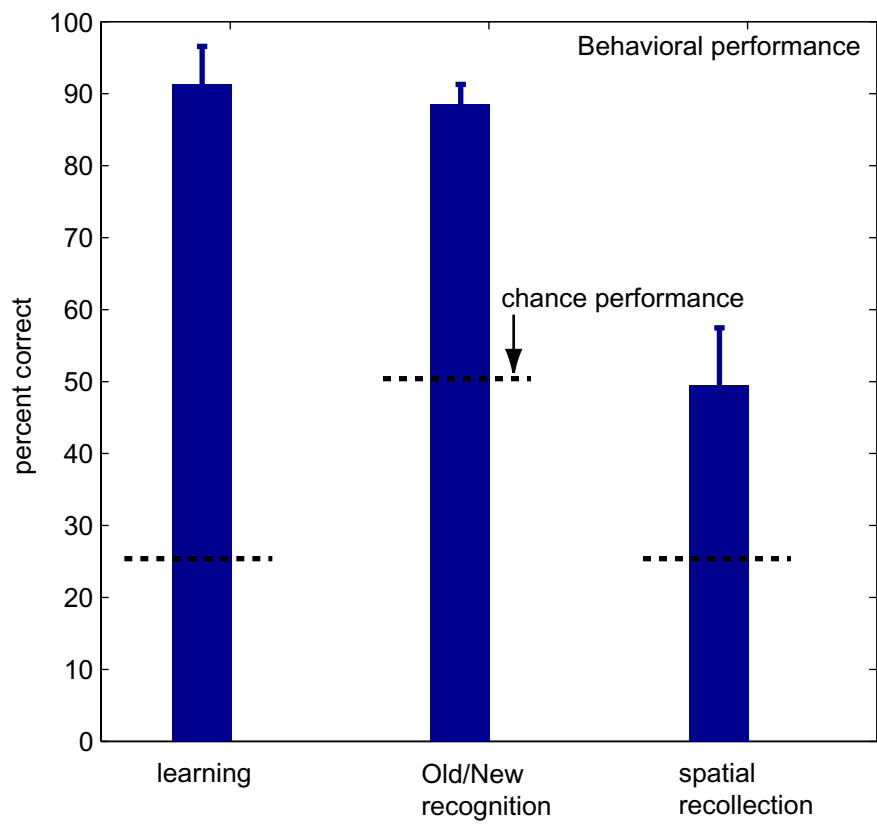


**Supplementary Data for "Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex"**

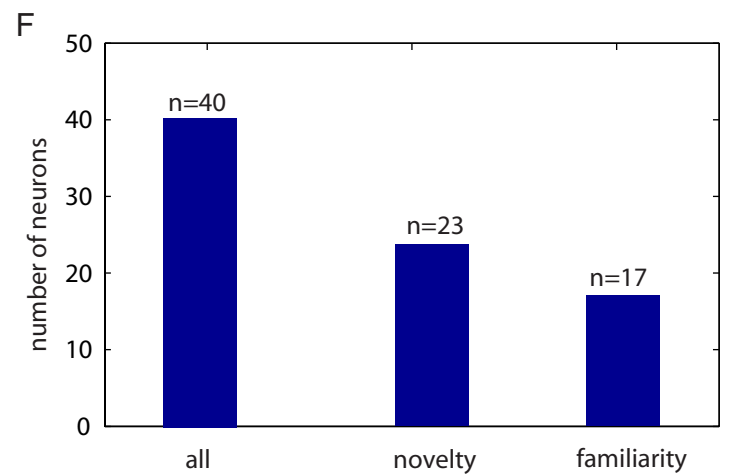
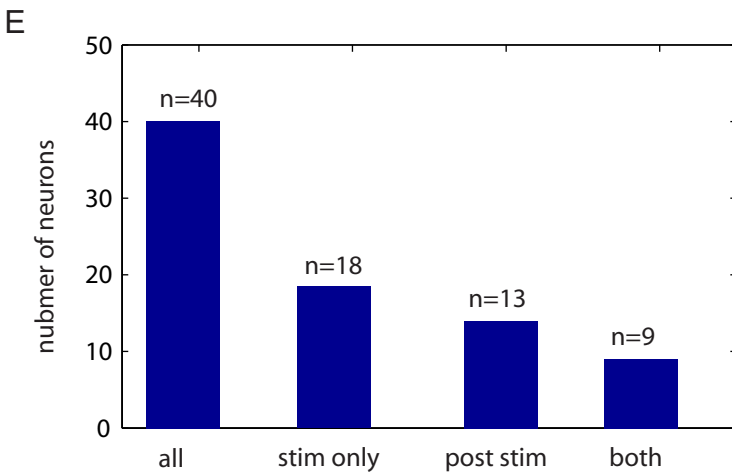
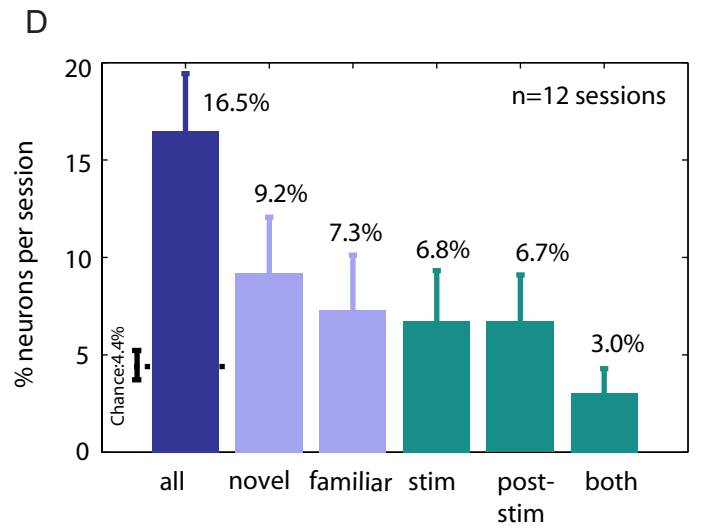
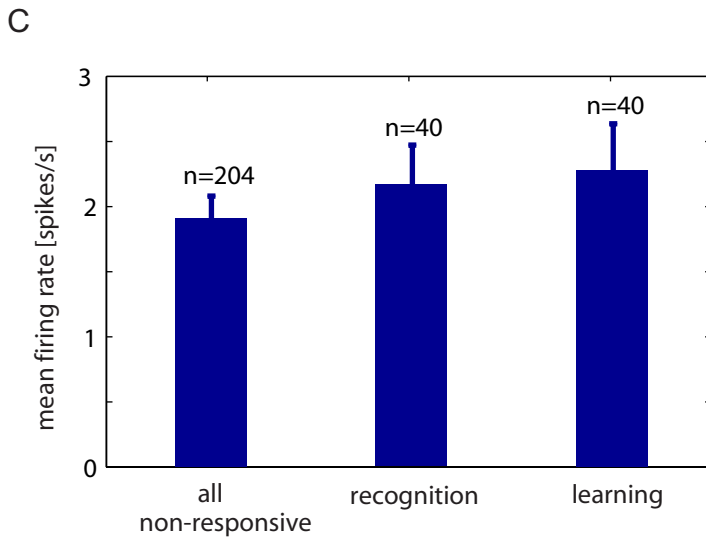
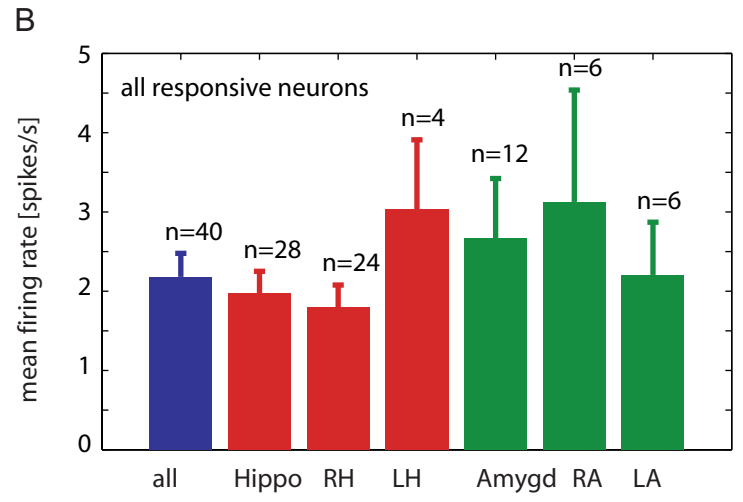
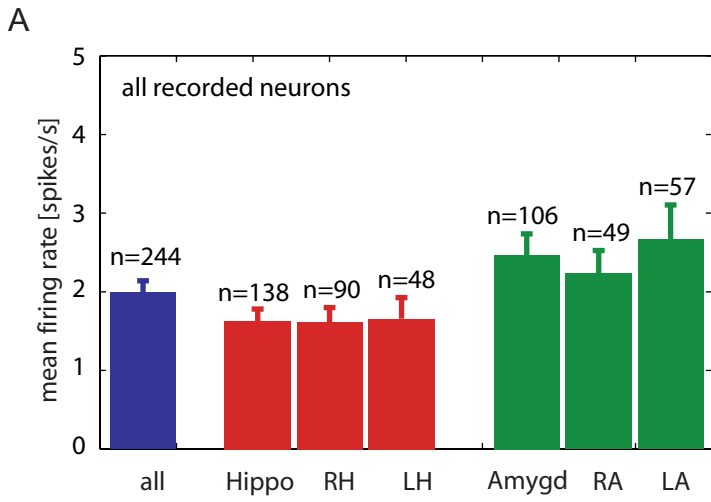
**Figure S1.** Behavioral performance of all subjects. Recognition performance (Old/New) was close to 90% (chance 50%) whereas spatial recollection, in which the subject reports the quadrant in which the images was presented for all images classified as "Old", was 49%. All performance levels are significantly different from chance ( $p < 0.05$ ).



Supp Fig 1

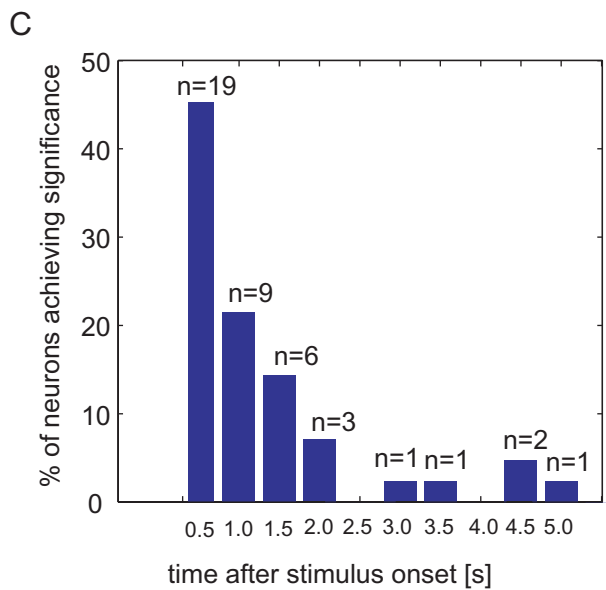
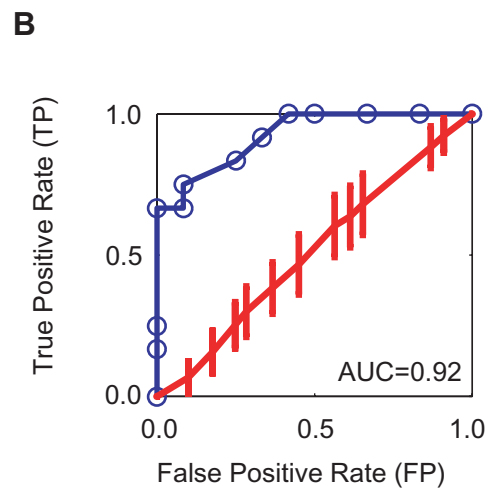
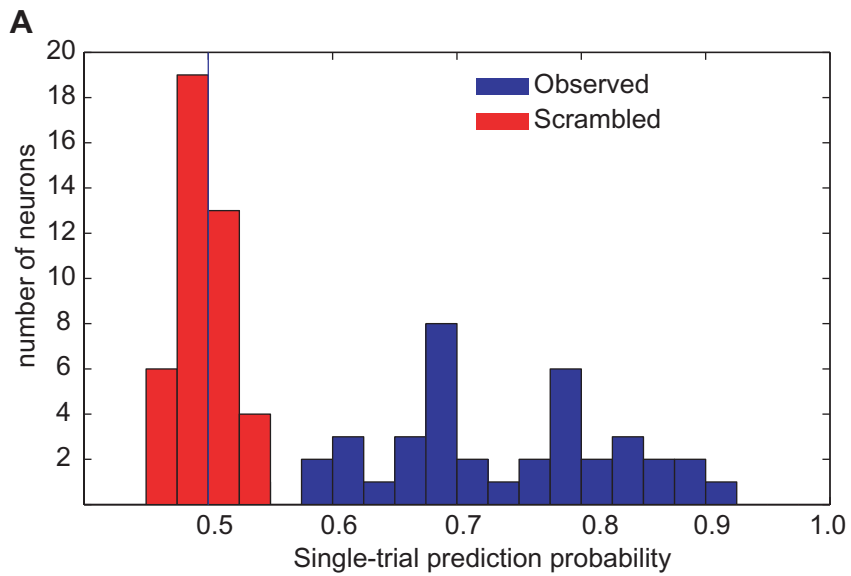
**Figure S2.** Population statistics for all neurons **(A)** as well as the subset of significantly responsive neurons **(B-F)**. **(A)** The mean firing rates of all neurons recorded (n=244) was  $1.96 \pm 0.14$  Hz. The mean firing rate was not significantly different among different brain areas (1-way ANOVA,  $p < 0.05$ ). **(B)** The mean firing rate of all responsive neurons (n=40) was  $2.17 \pm 0.30$  Hz, with no significant difference amongst different brain areas. **(C)** The mean firing rate for novelty and familiarity neurons was not statistically different from all other neurons recorded (1-way ANOVA,  $p < 0.05$ ) during either learning or recognition. **(D)** Considering all sessions, 16.5% of all recorded neurons indicated novelty or familiarity in every session (2 sessions each in 6 patients). There were slightly more novelty neurons (9.2%/per session) than familiarity neurons (7.3%/per session). **(E)** We found a total of 40 significant neurons, 18 of which signaled during the stimulus period, 13 during the post stimulus period and 9 during both; **(F)** There were 24 novelty and 18 familiarity neurons.

Abbreviations: RH, right hippocampus; RA, right amygdala, LH, left hippocampus; LA, left amygdala; hippo, hippocampus; amygd, amygdala. All error bars are  $\pm$ s.e and n always specifies number of neurons.



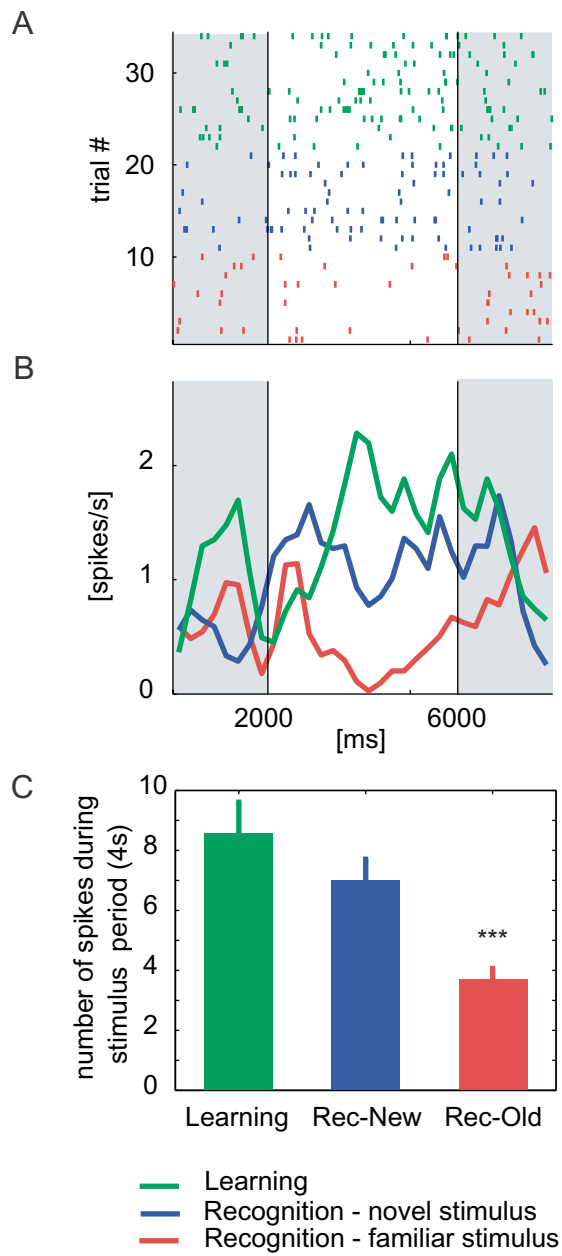
Supp Fig 2

**Figure S3.** (A) Histogram of the single-trial prediction probabilities for all 40 significant neurons. The mean probability was  $0.72 \pm 0.02$ . The prediction probability is equal to the area under the curve of the ROC of each neuron and specifies the ratio of recognition trials in which novelty or familiarity is successfully predicted on a trial-by-trial basis by observing a single neuron. Randomly shuffling (scrambled) the spike counts of new and old trials results in a mean of 0.5 (red in A, error bars are s.d.). The ROC for the same neuron as shown in figure 2 is shown in (B) (blue=real trials, red=randomly shuffled). (C) Latency of response for all neurons. Shown are, for each time following stimulus onset, the percentage of neurons which became significant for the first time in this time bin.



Supp Fig 3

**Figure S4.** Example of a novelty-sensitive neuron which increases firing to novel stimuli during both learning and recognition. (A) Raster for all spikes during learning (green), recognition old (red) and recognition new (blue). (B) Histogram summarizing the response. Note the decrease to familiarity. (C) Comparison of the number of spikes fired during the 4s stimulus period (white in B). The number of spikes fired for familiar items is significantly different from the number of spikes fired during learning and recognition of new items. ( $p < .001$  for both comparisons, 1-way ANOVA with posthoc multiple comparison.  $n=12$  (number of trials)).



Supp Fig 4



Table S1. Electrode position in stereotactic coordinates (Talairach).

<b>Patient</b>	<b>Amygdala (r/l)</b>	<b>Hippocampus (r/l)</b>
<b>P2</b>	-20,1,-19 26,-2,-20	-26,-9,-11 28,-11,-20
<b>P3</b>	-20,-3,-15 18,-4,-15	-23,-13,-12 33,-12,-16
<b>P4</b>	-19,4,-26 28,7,-26	-21,-9,-25 27,-7,-26
<b>P6</b>	-23,-2,-14 23,-6,-13	-25,-13,-12 29,-18,-12

Table S2. Location of resected tissue (temporal lobe lobectomy in each case)

<b>Patient</b>	<b>Side of temporal lobe lobectomy</b>
<b>P1</b>	left
<b>P2</b>	left
<b>P3</b>	right
<b>P4</b>	left
<b>P5</b>	left
<b>P6</b>	right

## **Supplemental experimental procedures**

### **Electrophysiology**

Recordings were conducted using a commercial (Neuralynx Inc, Arizona) acquisition system with specially designed, head-mounted pre-amplifiers. Signals were filtered and amplified by hardware amplifiers before acquisition. The frequency band acquired was either 1-9000Hz or 300-9000Hz, depending on the noise levels. Great care was taken to eliminate noise sources. This included using batteries to power the amplifiers, experimental computers, IV machines and heartbeat monitors. Recordings commenced the second day after surgery and continued for 2-4 days for about 1 hour per day. The experiments reported in this paper were done on two consecutive days for all 6 patients (12 sessions in total).

The amplifier gain settings, set individually for each channel, were typically in the range of 20000-35000 with an additional A/D gain of 4 (2 in some cases). The raw data was sampled at 25 kHz and written to disk for later filtering (300-3000Hz bandpass), spike detection and spike sorting. Spikes were detected using a local energy method (Bankman et al., 1993) and sorted by a template matching method (Rutishauser et al., 2006). Great care was taken to ensure that the single units used passed stringent statistical tests (projection test (Pouzat et al., 2002)). It is thus likely that we underestimate the number of single units present. Only neurons with mean firing rates  $\geq 0.25\text{Hz}$  were included in the analysis.

## **Electrodes**

In each macroelectrode, 8 microwires were inserted (Fried et al., 1999). One microwire was used as local ground and the other 7 were used for recordings. The impedance of a total of 56 microwires in 2 patients was, on average,  $135\pm 62\text{k}\Omega$  ( $\pm$ s.d.) with a range of 38-245k $\Omega$ .

Electrode position was determined by an experienced neurosurgeon (ANM) from structural MRIs taken 1 day after electrode implantation on a clinical 1.5 Tesla MRI system (Toshiba, Inc). We always recorded from 3 macroelectrodes simultaneously: left/right Hippocampus and either left or right Amygdala (total of 24 channels, 8 channels for each macroelectrode with 1 channel used as local ground).

## **Localization of electrodes**

We localized the position of each macroelectrode in a standardized stereotactic coordinate system (Talairach) in a subset of 4 patients for which high resolution structural MRIs were available (Supp Table 1). We transformed each structural 1.5T MRI scan to Talairach space by manually identifying the anterior-and posterior commissure as well as the anterior, posterior, superior and inferior points of the cortex. We used BrainVoyager (Brain Innovation B.V.) for this procedure. After co-registration we identified the Talairach coordinates by finding a consensus from the different structural scans. For each patient, we performed 4 different scans with 1x1mm resolution

in the following plane: coronal, sagittal and 2 axial with different pulse sequences (2TW and FLAIR).

### **Behavioral Task**

The experiment consisted of a learning block (Fig 1C) and a recognition block (Fig 1D). In each learning block 12 unique natural pictures were presented for 4 seconds each. Each of the 12 images was presented in one of 4 different positions (4 quadrants) on the screen. To facilitate learning and allow for subsequent spatial recollection, after each presentation the patient was asked to indicate where the image was presented (e.g. quadrant 1, 2, 3 or 4). After a ~30min delay (during which different tasks were performed: a virtual reality spatial memory and a reaction time task), the recognition block was administered. Twenty-four images were presented, in random order, for 4 seconds at the center of the screen. 12 of these images were previously presented during the learning block (“Old”) and 12 of the images were novel (“New”). After each image (2sec delay after offset), the patient was asked to indicate whether the image was Old or New (2 alternative forced choice). If the answer was Old, the patient was then asked to indicate where the image was presented (quadrant 1-4) during learning. For each patient, prior to beginning the above experiment, a short version of the task (with unique stimuli) was administered to familiarize subjects with the procedural aspects of the task.

The task was implemented using Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) in Matlab (Mathworks Inc) and ran on a notebook PC placed directly in front of the patient. Distance to the screen was approximately 50cm and the screen was approximately 30 by 23 degrees of visual angle. The pictures used were approximately 9 by 9 degrees. Specially marked keys ("New", "Old") on the keyboard were used to acquire subject responses. We chose to use natural pictures as stimuli rather than words or faces because it has been shown that pictures reliably result in bilateral fMRI activation of the MTL whereas words and faces result in primarily unilateral (left) activation (Kelley et al., 1998).

### **Data analysis**

We conducted all statistical analysis using bootstrap tests (see methods of main text). To be thorough, we repeated the same analysis using a two-tailed t-test ( $p < 0.05$ ) and found reasonable overlap with the pool of neurons determined to signal novelty or familiarity using the above bootstrap method. We found, however, that using the t-test more neurons were classified as novelty/familiarity detectors, some of which (by visual inspection) were likely false positives. Also, the chance performance determined by random shuffling was high (~ 10%). We thus decided to exclusively use the bootstrap method since it yielded the most consistent and

conservative results. Post-Stimulus Histograms (PSTH) were created by binning the number of spikes into 250ms bins. To convert the PSTH to an instantaneous firing rate, a Gaussian kernel with standard deviation = 300ms was used to smoothen the binned representation. Population averages (Fig 3C and 3D) were constructed by averaging the normalized firing rate of each neuron. Firing rates were normalized to the mean firing rate of the neuron during the particular part of the experiment (learning block or recognition block). We averaged the raw normalized PSTH of each neuron (above PSTH smoothening is not applied to normalized PSTH of each neuron nor to the population average).

### **Spatial recollection analysis**

To investigate whether the response observed during familiarity/novelty recognition required later successful spatial recollection we conducted additional data analyses. Based on several pieces of evidence we find that successful spatial recollection is not required for emergence of novelty/familiarity cells: i) In 4/12 sessions spatial recollection performance was at chance levels (mean  $21.7 \pm 7.9\%$ ) and yet we found that 14.8% of the recorded neurons in these sessions signaled novelty/familiarity during recognition and showed single-trial learning. This percentage is remarkably similar to the percentage of all neurons that signal novelty or familiarity (Fig S2). Thus despite the fact that these patients weren't able to correctly recollect the spatial location in any of the trials the same percentage of cells signaled novelty as in the

other sessions. ii) In the 8 sessions with above chance spatial recollection performance (mean  $63.91 \pm 7.02\%$ ), 28 neurons were found (17.2% of all recorded neurons). Repeating the analysis as described above, but only including trials with successful recollection, results in 26 of those 30 neurons remained significant. The number of selective neurons is thus decreased if only trials with successful spatial recollection are included and error trials are thus contributing valuable information. iii) In 9 sessions there were at least 4 spatial recollection error trials (correctly recognized as Old, but location wrong). Considering only these error trials (disregarding trials with correctly remembered locations), 20 out of originally 26 (77%) neurons remain significant. A high proportion of all originally identified neurons thus signal novelty/familiarity even in the absence of successful spatial recollection.

### **Single-neuron ROC analysis**

To determine how well the response of a single neuron during recognition predicts whether the patient is currently viewing a familiar or novel stimulus we conducted an ROC (receiver-operator characteristic) analysis (Britten et al., 1996; Green and Swets, 1966). This analysis assumes that an ideal observer, who only has access to the number of spikes fired by a single neuron during the presentation of the stimulus and the post-stimulus period (6s period), should be able to correctly classify individual neurons as signifying novelty vs. familiarity. Only trials where the subject correctly replied with "Old" or "New" were used for this analysis (this was 88.5% of all trials). We quantify the ROC for each neuron recorded by integrating the area

under the curve (AUC) of the ROC. This number equals the probability of correctly predicting, on a single-trial basis, whether the “subject” has viewed a novel or familiar stimulus. An AUC of 0.5 equals chance. We confirmed the validity of our analysis by randomly shuffling the labels "New" and "Old" while leaving the spike trains intact. Repeating this procedure 50 times for each neuron resulted in AUC values clustered around 0.5 (Fig 5A,B).

We conducted this ROC analysis without preclassifying neurons into novelty/familiarity detectors. This results in a cluster of neurons with a prediction probability significantly below 0.5 and one significantly above 0.5. Since Old/New is a binary state, this contributes equal information and we thus subtracted  $1-x$  for all ROC values  $x < 0.5$  to get an unimodal distribution, as shown in Fig 5A.

We repeated the analysis above for different time bins following stimulus onsets (step size 500ms), e.g. counting spikes in bins 2000-2500ms, 2000-3000ms, 2000-3500ms, etc. Using this analysis we defined for each neuron when it's ROC value became significantly above chance the first time (Fig 5C).

### **Epileptic v. non-epileptic tissue**

One concern regarding the neurons described in this paper is that they were recorded from epilepsy patients. To confirm that our findings are also valid for "healthy" tissue, we repeated our analysis but excluded all electrodes which were in tissue that was



later resected (Table S2). Of the total 244 recorded neurons, 138 were in tissue which was not resected. Of these 138 neurons, 22 signalled novelty or familiarity (15.9%).

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