J Neurophysiol 103: 97–107, 2010.
First published October 28, 2009; doi:10.1152/jn.91323.2008.

Responses of Human Medial Temporal Lobe Neurons Are Modulated by Stimulus Repetition

Carlos Pedreira,[1] Florian Mormann,[2,3] Alexander Kraskov,[2,4] Moran Cerf,[2,3] Itzhak Fried,[3,5] Christof Koch,[2] and Rodrigo Quian Quiroga[1,2,3]

[1]*Department of Engineering, University of Leicester, Leiscester, United Kingdom;* [2]*Computation and Neural Systems, California Institute of Technology, Pasadena, California;* [3]*Department of Neurosurgery, David Geffen School of Medicine, and Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, California;* [4]*University College London, Institute of Neurology, Queen Square, London, United Kingdom; and* [5]*Functional Neurosurgery Unit, Tel-Aviv Medical Center and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

Submitted 15 December 2008; accepted in final form 20 October 2009

Pedreira C, Mormann F, Kraskov A, Cerf M, Fried I, Koch C, Quiroga RQ. Responses of human medial temporal lobe neurons are modulated by stimulus repetition. *J Neurophysiol* 103: 97–107, 2010. First published October 28, 2009; doi:10.1152/jn.91323.2008. Recent studies have reported the presence of single neurons with strong responses to visual inputs in the human medial temporal lobe. Here we show how repeated stimulus presentation—photos of celebrities and familiar individuals, landmark buildings, animals, and objects—modulates the firing rate of these cells: a consistent decrease in the neural activity was registered as images were repeatedly shown during experimental sessions. The effect of repeated stimulus presentation was not the same for all medial temporal lobe areas. These findings are consistent with the view that medial temporal lobe neurons link visual percepts to declarative memory.

INTRODUCTION

The recognition of visual objects is processed along the ventral visual pathway, extending from primary visual areas (V1) to the inferotemporal cortex (IT) (Logothetis and Sheinberg 1996; Tanaka 1996). IT cortex has direct projections to the medial temporal lobe (MTL) (Lavenex and Amaral 2000; Saleem and Tanaka 1996; Suzuki 1996), where single cell studies in monkeys reported visual responses, among others, by stimulus-selective neurons that were related to the learning and rehearsal of association between visual cues (Naya et al. 2001; Sakai and Miyashita 1991; Wirth et al. 2003). In humans, it has been shown that neurons in the MTL respond strongly to visual inputs (Fried et al. 1997; Gelbard-Sagiv et al. 2008; Kreiman et al. 2000a,b, 2002; Quian Quiroga et al. 2005, 2008b, 2009). However, based on findings with patient H.M.—and similar patients with lesions or resections of the hippocampus and other parts of the MTL—it is widely accepted that the MTL is not necessary for visual recognition (but see Buckley and Gaffan 2006). Rather, the hippocampus is involved in declarative memory storage (Corkin 2002; Eichenbaum 2000; Rosenbaum et al. 2005; Scoville and Milner 1957; Squire et al. 2004). This raises the question of why MTL neurons respond strongly to images if this area is not part of the visual perception system. Based on *1*) the well-established findings about the role of the MTL in memory storage, *2*) the relatively long latency of MTL responses at ~300 ms or longer (Mormann et al. 2008; Quian Quiroga et al. 2005) compared to ~120 ms in monkey IT (Hung et al. 2005), and *3*) the fact that MTL neurons encode abstract information and not particular visual details (Quian Quiroga et al. 2005), it has been suggested that MTL neurons link visual perception to memory formation (Quian Quiroga et al. 2005, 2008a).

Brain imaging studies of stimulus repetition in humans showed a decrease in activity for different areas, including the MTL, which has been related to processes involving perception, attention, learning, and memory (for reviews, see Grill-Spector et al. 2006; Henson and Rugg 2003; Ranganath and Rainer 2003). In addition, experimental stimulus repetition paradigms induced response suppression patterns in monkey IT neurons (Li et al. 1993; Liu et al. 2009; Miller et al. 1991; Sawamura et al. 2006). Given these previous findings, here we set up to study whether a similar pattern of decreased responses with stimulus repetition was also present in the visual responses in the human MTL. We hypothesize—considering the abovementioned studies about MTL function—that such a finding with neurons in this area may be because of its role in declarative memory.

METHODS

Subjects and recordings

Subjects were 26 patients with pharmacologically intractable epilepsy (15 men; 22 right handed; 17–54 yr old). Extensive noninvasive monitoring did not yield concordant findings corresponding to a single resectable epileptogenic focus. Therefore patients were implanted with chronic depth electrodes for typically 7–10 days to determine the focus of the seizures for possible surgical resection (Fried et al. 1997). All studies conformed to the guidelines of the Medical Institutional Review Board at UCLA. The electrode locations were based exclusively on clinical criteria and were verified by MRI or CT co-registered to preoperative MRI. Here we report data from sites in the hippocampus, amygdala, entorhinal cortex, and parahippocampal cortex. Each electrode probe had a total of nine microwires at its end, eight active recordings channels, and one reference (Fried et al. 1997). The differential signal from the microwires was amplified using a 64-channel Neuralynx system, filtered between 1 and 9,000 Hz, and sampled at 28 kHz. Each recording session lasted ~30 min.

The data reported here were recorded during 44 experimental sessions. Subjects laid in bed facing a laptop computer on which pictures of animals, objects, landmark buildings and known and unknown faces were shown. After image offset, subjects had to respond whether or not the picture contained a human face, by pressing the Y and N keys, respectively. This simple task, on which performance was virtually flawless, required them to attend to the pictures (Quian Quiroga et al. 2005).

Address for reprint requests and other correspondence: R. Quian Quiroga, Dept. of Engineering, Univ. of Leicester, Leicester, UK LE1 7RH (E-mail: rqqg1@le.ac.uk).

0022-3077/10 $8.00 Copyright © 2010 The American Physiological Society

Downloaded from jn.physiology.org on January 12, 2010

Images covered ~1.5° of the visual angle and were presented for 1 s at the center of the screen, six times each in pseudo-random order. The mean number of images shown to the patient was 114.2 (range, 83–192). In a slightly different version of this paradigm, for 13 sessions, the presentation time was 500 ms, and the key responses were omitted. These sessions were considered together with the 1-s presentation sessions because there were no clear differences in the response patterns.

Of the 44 experimental sessions, 26 corresponded to the first experiment done with each of the 26 patients, so that the first trial for each picture was the first time the patient saw the image at the UCLA ward. The remaining 18 sessions corresponded to second sessions collected from 18 of the 26 patients, carried out on a following day. All the pictures considered from the second sessions were already presented in the first session. Because of the variability of spike shapes, it was, in general, not possible to follow the activity of single neurons across different experiments.

Data analysis

From the continuously recorded data, spikes were detected and sorted using the Wave_clus software package (Quian Quiroga et al. 2004). As in previous studies (Quian Quiroga et al. 2005, 2007), a response was considered significant if it was larger than the mean plus 5 SD of the baseline period (1,000 to 300 ms before stimulus onset) for all stimuli and had at least two spikes in the time interval between 300 and 1,000 ms after the stimulus onset. For those pictures eliciting significant responses, we computed the total number of spikes between 300 to 2,000 ms after stimulus onset for each trial. To account for the fact that different neurons have different firing rates, the responses were normalized by dividing by the maximum number of spikes across trials. The normalized number of spikes across trials was statistically compared using a one-way ANOVA (*test 1*), where the independent variable was the trial number and the repeated measures were the normalized responses. This analysis was performed for the whole population of responses in the MTL and for each of the four subregions separately.

For a further characterization of the response patterns, for each of the significant responses, we calculated a linear regression of the number of spikes with trial number. Differences in the slope values of these linear fits were compared for the different MTL areas and between the first and second experimental sessions using a two-way ANOVA (*test 2*). The independent variables were MTL area and session number, and the repeated measures were the slopes of the responses. Post hoc, we evaluated differences of the slope values to the "zero slope" response pattern (i.e., a response with the same number of spikes for every stimulus presentation) for each area separately using a paired *t*-test (*test 3*).

To evaluate the time profile of the responses, the instantaneous firing rate was computed by convolving the normalized spike trains with a Gaussian kernel (sampling period = 0.5 ms, σ = 100 ms). From the average instantaneous firing rate (across all responses) for each trial, we defined *1*) the peak amplitude; *2*) its latency; *3*) the onset of the response, as the point where the instantaneous firing rate crossed 4 SD above baseline and stayed above for ≥100 ms; and *4*) the duration, as the time interval between response onset and offset (Fig. S1).[1] Offset was defined similarly to onset but crossing the 4 SD line downward and staying below it for ≥100 ms. The effect of stimulus repetition on each of these parameters was assessed using one-way ANOVA with independent variable trial number (*test 4*). The repeated measures were the values of the corresponding parameters for each response.

RESULTS

In 26 first experimental sessions for each patient, we recorded from 1,210 MTL units (515 single units and 695 multiunits), with an average of 46.6 units per session. Of these 1,210 units, 262 (22%; 132 single units and 130 multiunits) had a statistically significant response to a total of 725 pictures (an average of 2.77 responses per unit). For the second experimental sessions, we recorded from a total of 745 units (328 single units and 417 multiunits), with an average of 41.3 units per session. Of these 745 units, 110 (15%; 57 single units and 53 multiunits) had a significant response to a total of 289 pictures (2.63 responses per unit). The decrease in the responsiveness of the recorded units between experimental sessions (22% for the 1st session against 15% for the 2nd one) was significant (χ^2, $P < 0.001$).

Single cell responses

Figure 1 presents four examples of significant responses recorded in four different patients. For each response, we display the raster plot (1st trial at the *top*), the number of spikes per trial, and the peristimulus time histograms. The neuron in Fig. 1*A* was located in the entorhinal cortex. Its average baseline activity was 2 Hz, and it fired with up to 20 Hz to the patient's own picture. The neuron in Fig. 1*B* was located in the amygdala and, from a mean baseline activity of ~7 Hz, it responded with up to 50 Hz to the picture of a monkey. The neuron in Fig. 1*C* was located in the hippocampus and, from a baseline of ~3 Hz, it responded with 30 Hz to the picture of a squirrel. Finally, the neuron in Fig. 1*D*

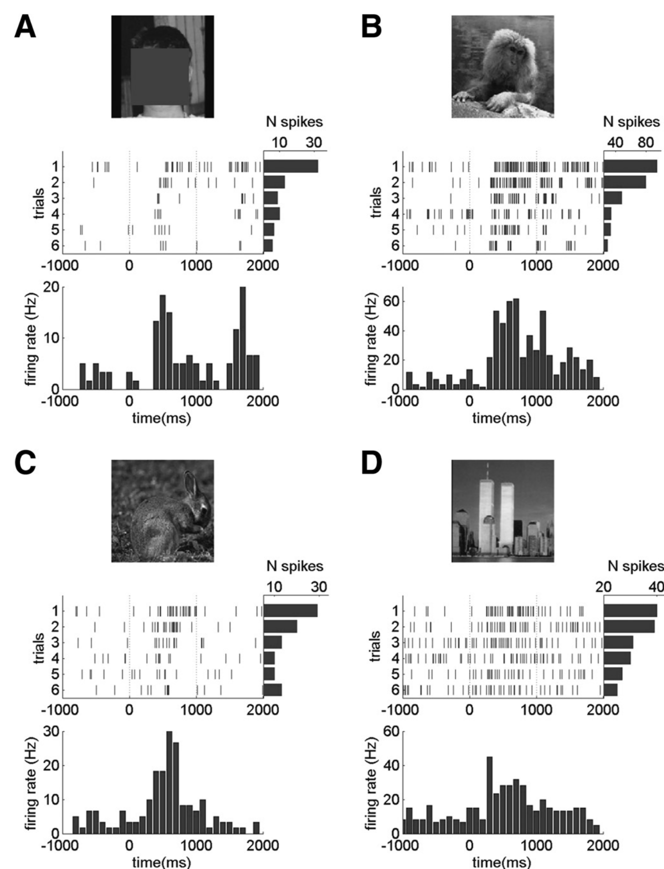

FIG. 1. Single cell responses. The 4 panels (*A–D*) correspond to the responses to the preferred stimulus for 4 different neurons. Responsive neurons were located in entorhinal cortex, amygdala, hippocampus, and parahippocampal cortex, respectively. For each response, we display the raster plot (1st trial at the *top*), the number of spikes within a 300- to 2,000-ms time window (*right*) and the peristimulus time histograms (*bottom*). A decrease in the number of spikes with trial number can be observed for each neuron. The onset (t = 0 ms) and offset (t = 1,000 ms) of the pictures are marked by dotted lines.

[1] The online version of this article contains supplemental data.

Downloaded from jn.physiology.org on January 12, 2010

was in the parahippocampal cortex and it responded to a picture of the World Trade Center with ~45 Hz from a baseline of 10 Hz. All these units increased their firing at least three times in response to their preferred pictures. However, this change was not equally distributed across the six trials. In fact, in the four examples, a clear decay in the number of spikes with trial number can be observed, as shown by the spike counts for each trial.

Population results

For each trial Fig. 2*A* shows the mean normalized number of spikes of the 725 responses recorded in the first sessions: 238 neurons in the amygdala, 311 in the hippocampus, 105 in the entorhinal cortex, and 71 in the parahippocampal cortex. As shown in the single cell examples of Fig. 1, there was a significant decay of the normalized number of spikes with trial number [$F(5,4084) = 19.34$, $P < 10^{-15}$, *test 1*, see METHODS]. Note that the normalized spike number did not reach a value of 1 because the maximum firing rate for different responses was not always at the same trial. Interestingly, this pattern of decay was not the same for all MTL areas, as shown in Fig. 2, *B–E*. Considering each area separately, this effect was statistically significant for the responses in amygdala [$F(5,1345) = 16.87$, $P < 10^{-15}$], hippocampus [$F(5,1726) = 6.03$, $P < 10^{-4}$], and entorhinal cortex [$F(5,588) = 2.52$, $P < 0.05$], whereas responses in the parahippocampal cortex [$F(5,407) = 0.98$, $P = 0.43$] had no significant dependency on trial number.

To further study differences between the four MTL regions, for each response we computed the slope of the best linear fit and statistically compared the results for different areas (*test 2*, see METHODS). As shown in Fig. 3, there was a significant difference between areas [$F(3,721) = 7.1$, $P < 10^{-3}$], which was mainly because of the smaller slope values in the re-

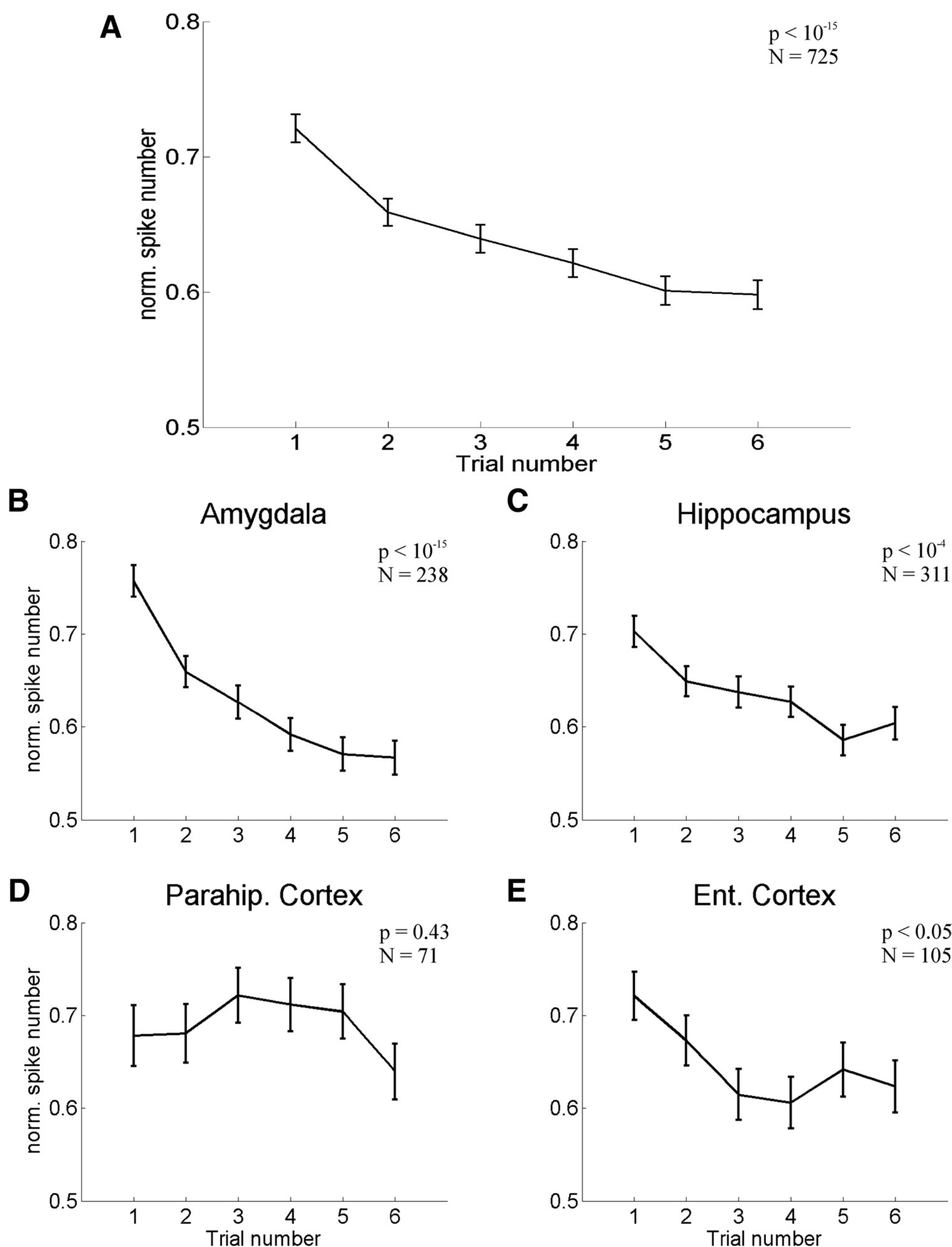

FIG. 2. Normalized mean number of spikes per trial. *A*: result for all 725 responses. There was a significant effect of the trial number on the mean firing rate. The mean number of spikes was reduced by 17% from the 1st trial to the last one of the session. *B–E*: responses divided by area. There was a significant decay with picture repetition for responses in the amygdala, hippocampus, and entorhinal cortex but not for the parahippocampal cortex. *N*, number of responses. Bars denote SE.

Downloaded from jn.physiology.org on January 12, 2010

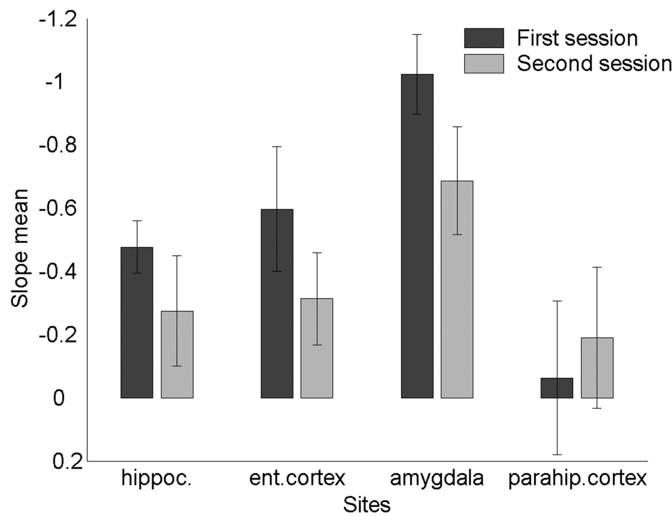

FIG. 3. Mean slopes of the decay in response magnitude with trial number. Slopes of the responses grouped by location. The 1st and 2nd sessions are represented in dark and clear gray, respectively. The average slope value in *session 2* was 50% lower for responses in hippocampus and entorhinal cortex and 30% for the amygdala responses. Parahippocampal responses had flat slopes, reflecting a similar firing for all trials in both sessions. Bars denote SE.

sponses from the parahippocampal cortex. In line with this observation, a separate *t*-test analysis for each MTL location (*test 3*) showed that the slope of the responses from entorhinal cortex, hippocampus, and amygdala were statistically different from zero ($t = -3.04$, df = 104, $P < 0.005$; $t = -5.77$, df = 310, $P < 10^{-7}$; and $t = -8.11$, df = 237, $P < 10^{-13}$; respectively), whereas the ones from parahippocampal cortex were not ($t = -0.26$, df = 70, $P = 0.8$).

Time profile of the responses

Figure 4*A* shows the instantaneous firing rates for each trial (see METHODS) averaged over the 725 responses. Responses for all trials are clearly larger than baseline activity, and the decrease of the number of spikes with trial number shown in Fig. 2 seems to be caused both by differences in the duration of the responses and differences in the peak amplitudes. Moreover, it is apparent that for all trials, the onset of the responses did not differ significantly. Figure 4, *B–E*, shows the firing rate of the responses divided by areas. A higher amplitude for the first trial can be seen only in the responses from amygdala. A delayed maximum response in the first trial can be seen in the hippocampus and the entorhinal cortex. To verify these observations, we assessed and statistically compared the onset, duration, peak amplitude, and latency of the responses, as defined in Fig. S1 (*test 4*).

Figure 5*A* presents the average peak latency for the entire population of neurons, for each of the six trials. There was a statistically significant decay of the peak latency with trial number [$F(5,3998) = 6.41$, $P < 10^{-5}$]. The same statistical analysis performed for each area separately, presented in Fig. 5, *B–E*, showed significant peak latency shifts with trial number for the responses in amygdala, hippocampus, and entorhinal cortex [$F(5,1422) = 7.82$, $P < 10^{-6}$; $F(5,1860) = 7$, $P < 10^{-5}$; and $F(5,624) = 3.36$, $P < 0.01$; respectively, as shown in Fig. 5, *B, C,* and *E*]. Figure 6*A* shows the analysis of the peak amplitude. As for the peak latency, there were significant differences with trial number [$F(5,3998) = 2.83$, $P < 0.05$)]. However, as can be seen in Fig. 6, *B–E*, in this case, a separate analysis for each area showed that this effect was significant only for the responses in the amygdala [$F(5,1442) = 2.92$, $P < 0.05$], whereas it was not significant for the responses in the hippocampus, entorhinal cortex, and parahippocampal cortex [$F(5,1860) = 0.98$, $P = 0.43$; $F(5,624) = 0.91$, $P = 0.47$; and $F(5,246) = 0.48$, $P = 0.79$; respectively]. Figures 7 and 8 show the effect of trial number on the duration and the onset latency of the responses. As observed in Fig. 4, Fig. 7*A* shows a significant decrease with trial number for the duration of the responses [$F(5,3531) = 3.09$, $P < 0.01$], but this effect was only significant for the responses in the amygdala [$F(5,1205) = 2.43$, $P < 0.05$; Fig. 7*B*]. Responses from hippocampus showed a similar behavior, but without reaching significance [$F(5,1505) = 1.62$, $P = 0.15$]. Responses in entorhinal cortex and parahippocampal cortex had a similar duration for all trials [$F(5,469) = 0.23$, $P = 0.95$ and $F(5,334) = 0.48$, $P = 0.79$, respectively]. Figure 8 shows that there were no significant differences for the response onset latencies both for the entire population of neurons [$F(5,3531) = 0.26$, $P = 0.9$; Fig. 8*A*] and for data divided by area [$F(5,1205) = 0.35$, $P = 0.88$; $F(5,1505) = 0.66$, $P = 0.65$; $F(5,469) = 0.25$, $P = 0.94$, and $F(5,334) = 0.13$, $P = 0.99$; for amygdala, hippocampus, parahippocampal cortex and entorhinal cortex, respectively; (Fig. 8, *B–E*)].

Results for the second experimental sessions

Next, we compared the results of the first sessions with those obtained in the following experiments, usually performed in a different day. These sessions were available for 18 of the 26 patients. All the pictures considered from these sessions were already presented in the first sessions.

As for the first sessions, we computed the slope of the best linear fit to the number of spikes per trial for each of the 289 responses obtained in the second sessions: from the amygdala (138), hippocampus (68), entorhinal cortex (45), and parahippocampal cortex (38). The slope values for the different locations are presented in Fig. 3 (the normalized responses divided by area, as reported in Fig. 2 for the first session, are available in the supplementary material; Fig. S2). The response pattern was similar to the one obtained for the first sessions, with responses in amygdala having the largest rate of decay with trial number, followed by the responses in entorhinal cortex, hippocampus, and finally the ones in the parahippocampal cortex. A *t*-test analysis of each MTL subregion showed that only responses in amygdala and entorhinal cortex had a slope significantly different from zero ($t = -4.01$, df = 137, $P < 0.001$ and $t = -2.16$, df = 44, $P < 0.05$, respectively; *test 3*). Note that, in general, the responses in the second sessions had lower slope values compared with the first sessions.

Statistical differences in the slope values between the different brain areas and between the first and the second sessions were evaluated with a two-way ANOVA (*test 2*). There were significant differences across the different MTL regions [$F(3,1006) = 8.28$, $P < 10^{-4}$]. The comparison between the first and the second sessions showed a general trend nearly reaching significance with smaller slope values for the second sessions [$F(1,1006) = 3.55$, $P = 0.06$]. Because responses in the parahippocampal cortex did not show an effect with stimulus repetition, we repeated the analysis excluding these responses and found that the difference of the slopes between the first and second sessions was statistically significant [$F(1,899) = 4.68$, $P < 0.05$].

Downloaded from jn.physiology.org on January 12, 2010

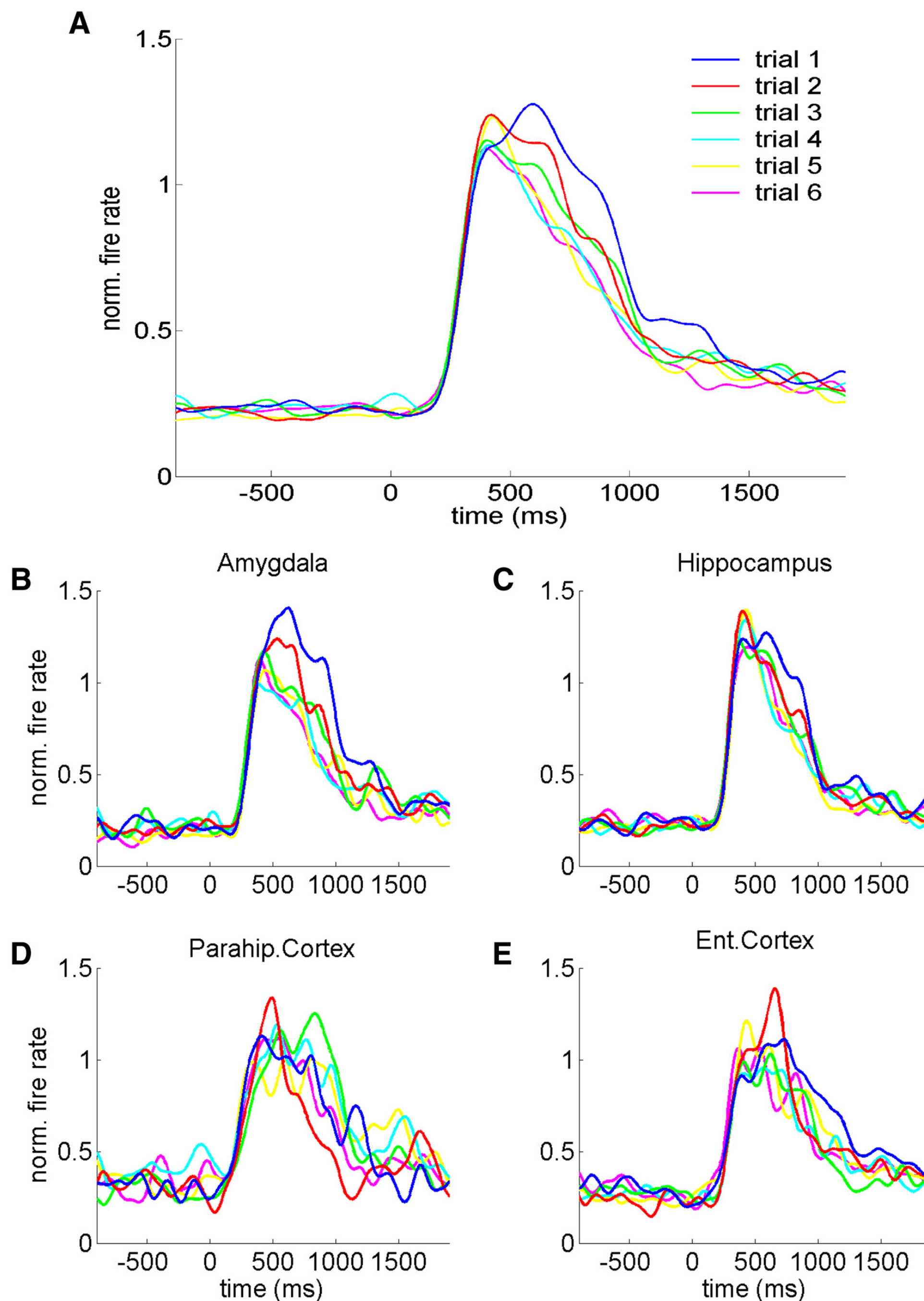

FIG. 4. Mean instantaneous firing rate for each trial. *A*: average over the whole set of responses (725). Note that all 6 trials had similar onset latencies. Responses for the 1st trials, especially for *trial 1*, in dark blue, had a larger duration, as well as a delayed and slightly higher peak value. *B–E*: data broken down by areas. In *B*, responses from amygdala (238 responses) showed higher and later peak values for the 1st presentations. In *C,* responses from hippocampus (311) showed a late peak value for the 1st trial. *D*: no differences in the responses from parahippocampal cortex (71) were observed for the different trials. *E*: responses from entorhinal cortex (105) showed a similar delay pattern as those in hippocampus.

DISCUSSION

In this study, we showed a decrease of the number of spikes fired by neurons in the human MTL in response to repeated picture presentations. This effect was not homogeneous across the different MTL areas. In particular, a decrease in the response peak amplitude with trial number was significant only for the amygdala responses. Moreover, there were significant decreases of the response peak latencies for the responses in the amygdala, hippocampus, and enthorinal cortex (but not for parahippocampal cortex). Given that the onset of responses was not different for the different trials (see Fig. 8), the decrease in peak latency can be attributed to a "time-sharpening"—i.e., responses were more localized in time for the later trials—in agreement with the pattern observed in the instantaneous firing rate curves shown in Fig. 4. The fact that in our study the time-sharpening of the responses was accompanied by a decrease in duration only for the amygdala neurons can be attributed to the less accurate estimation of the response durations, which accumulate inaccuracies in estimating both the onset and offset of the responses. In agreement with the previous observations, there was a decrease of the total number of spikes elicited in response to the stimulus for neurons in the amygdala, hippocampus, and entorhinal cortex.

Long-term response suppression effects have been reported by recent studies in monkey IT cortex during visual fixation and stimulus classification tasks (Anderson et al. 2008; Freedman et al. 2006). Interestingly, IT cortex has large projections to the MTL areas we record from, with direct projections to the

Downloaded from jn.physiology.org on January 12, 2010

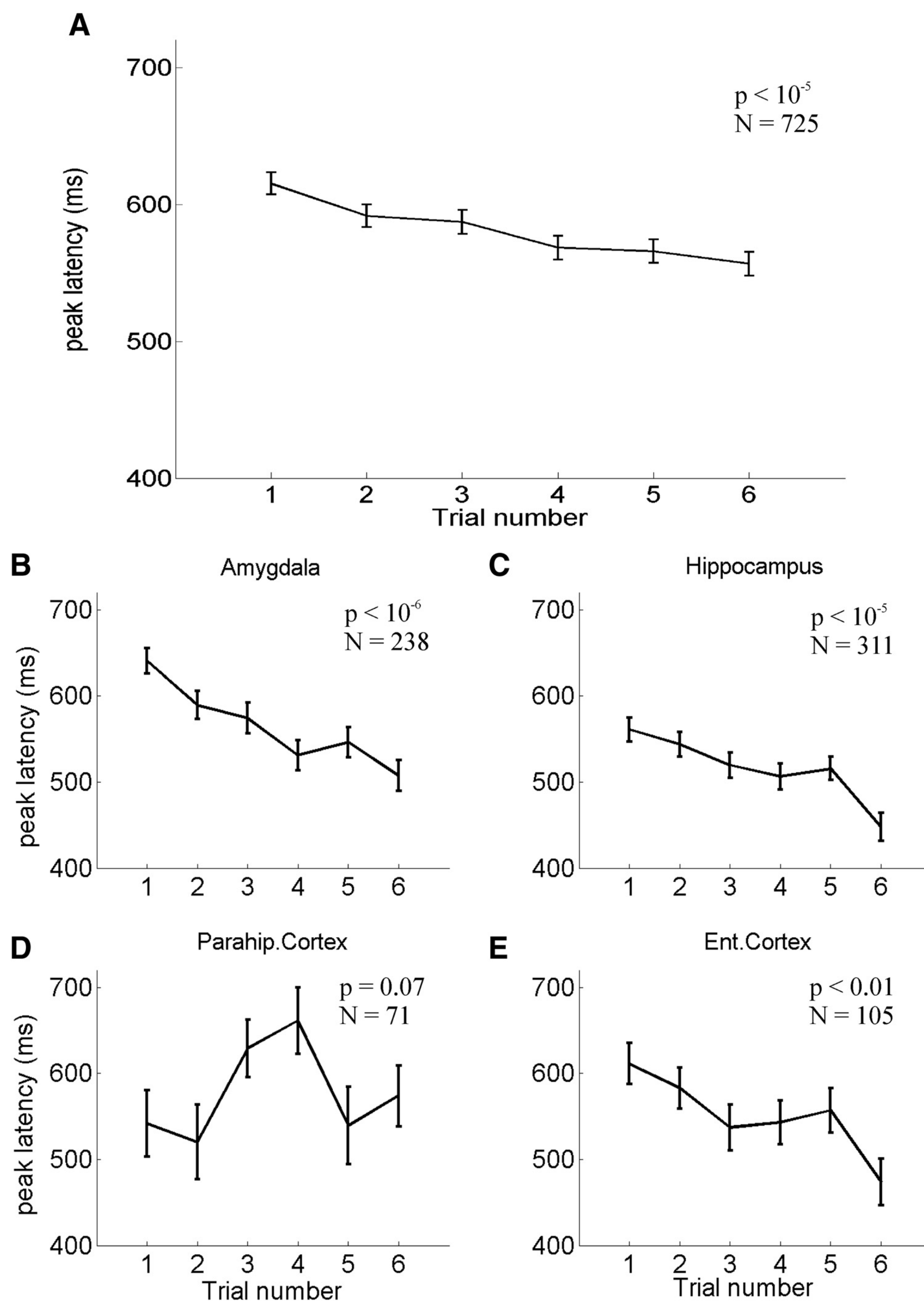

FIG. 5. Average peak latencies. *A*: average peak latencies for all the 725 responses. Latencies were significantly earlier for later trials. *B–E*: the same analysis separated by area. A significant latency decrease with trial number can be seen in the responses from amygdala (*B*), hippocampus (*C*), and entorhinal cortex (*E*) but not in the responses from parahippocampal cortex. *N*, number of responses. Bars denote SE.

amygdala and parahippocampal cortex, the latter one (together with the perirhinal cortex) giving the main input to the entorhinal cortex, which in turn is the main gateway to the hippocampus (Lavenex and Amaral 2000; Saleem and Tanaka 1996; Suzuki 1996). More related to our findings are reports of short-term response suppression in mere visual fixation tasks (Liu et al. 2009; Sawamura et al. 2006).

There has been extensive research on response suppression in humans using noninvasive techniques such as EEG and functional MRI (fMRI) (Breiter et al. 1996; for reviews, see Grill-Spector et al. 2006; Ranganath and Rainer 2003). However, it has to be noted that these studies can only give indirect evidence about the firing of single neurons, because they only measure the activity of large populations (Logothetis 2008; Logothetis et al. 2001). Closer to our findings, using single cell recordings in patients implanted with intracranial electrodes for clinical reasons, two recent studies have reported novelty and familiarity effects in human MTL neurons (Rutishauser et al. 2006; Viskontas et al. 2006). In these studies, previously unseen pictures of unknown places and faces were shown in a two-session protocol. In the first session, a set of these pictures was presented and subjects were instructed to remember them. In the second session, the previously presented pictures were mixed with some novel ones and subjects were asked to remember whether the picture had been shown previously or not. In the study of Viskontas et al., neurons from the hippocampal and parahippocampal regions showed a higher firing rate for novel pictures. Moreover, neurons from the hippocam-

Downloaded from jn.physiology.org on January 12, 2010

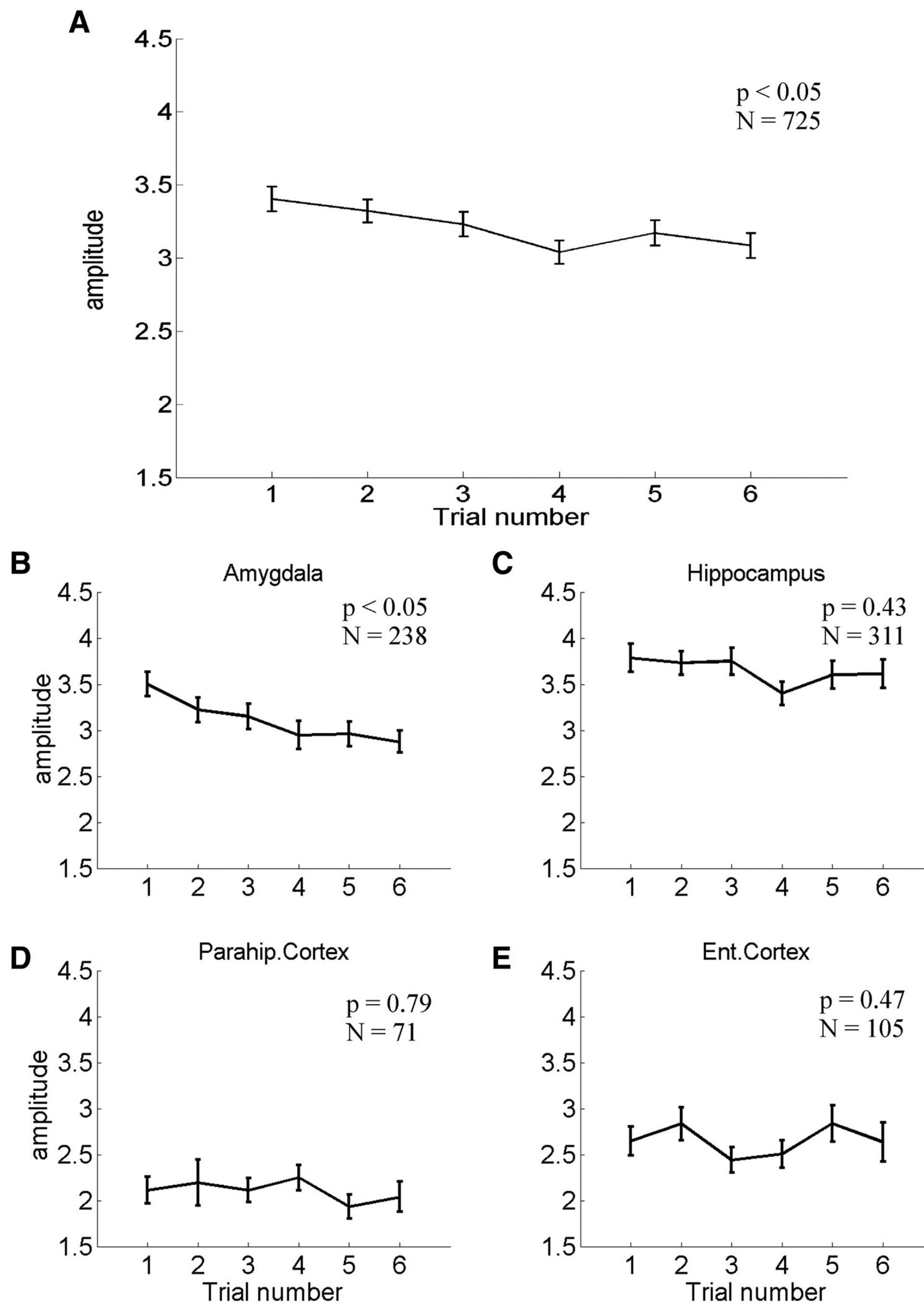

FIG. 6. Normalized average peak amplitude of the significant responses. *A*: average peak amplitude for all the 725 responses. The peak amplitude was significantly higher for the 1st trials. *B–E*: data broken down by areas. Only responses from amygdala showed a significant decrease trial number. *N,* number of responses. Bars denote SE.

pus presented a decrease of their firing below the baseline activity for subsequent presentations. Rutishauser et al. described two subsets of cells in hippocampus and amygdala: one group of cells increased their firing when the stimulus presented was new and another one increased their firing when it was shown few moments before (Rutishauser et al. 2006). Although related, there are two main differences between these two studies and the one presented here. First, the neurons described by Rutishauser et al. (2006) and by Viskontas et al. (2006) were not stimulus selective, because their behavior was independent of the particular (either novel or familiar) picture shown. This lack of selectivity compared to our study can be attributed to the fact that *1*) we used familiar stimuli, which are more likely to elicit responses (Viskontas et al. 2009); *2*) we used an optimal spike sorting algorithm that is particularly suited to detect sparsely firing neurons, which typically have very low baseline firing rates (Quian Quiroga et al. 2008b, 2009); and *3*) we recorded the continuous data and used optimal off-line analysis. Note that to avoid large data volumes many acquisition systems detect spikes on-line based on amplitude thresholds set by hand. These thresholds may be set to nonoptimal values, especially if the experimenter is dealing with a relatively large number of channels. In particular, this approach may miss or nonoptimally detect very selective neurons because these may be silent when the thresholds are set. A similar “dark matter” problem arises when using movable electrodes because silent neurons may not be identified as the electrode passes by, unless the right stimulus is shown (Olshausen and

Downloaded from jn.physiology.org on January 12, 2010

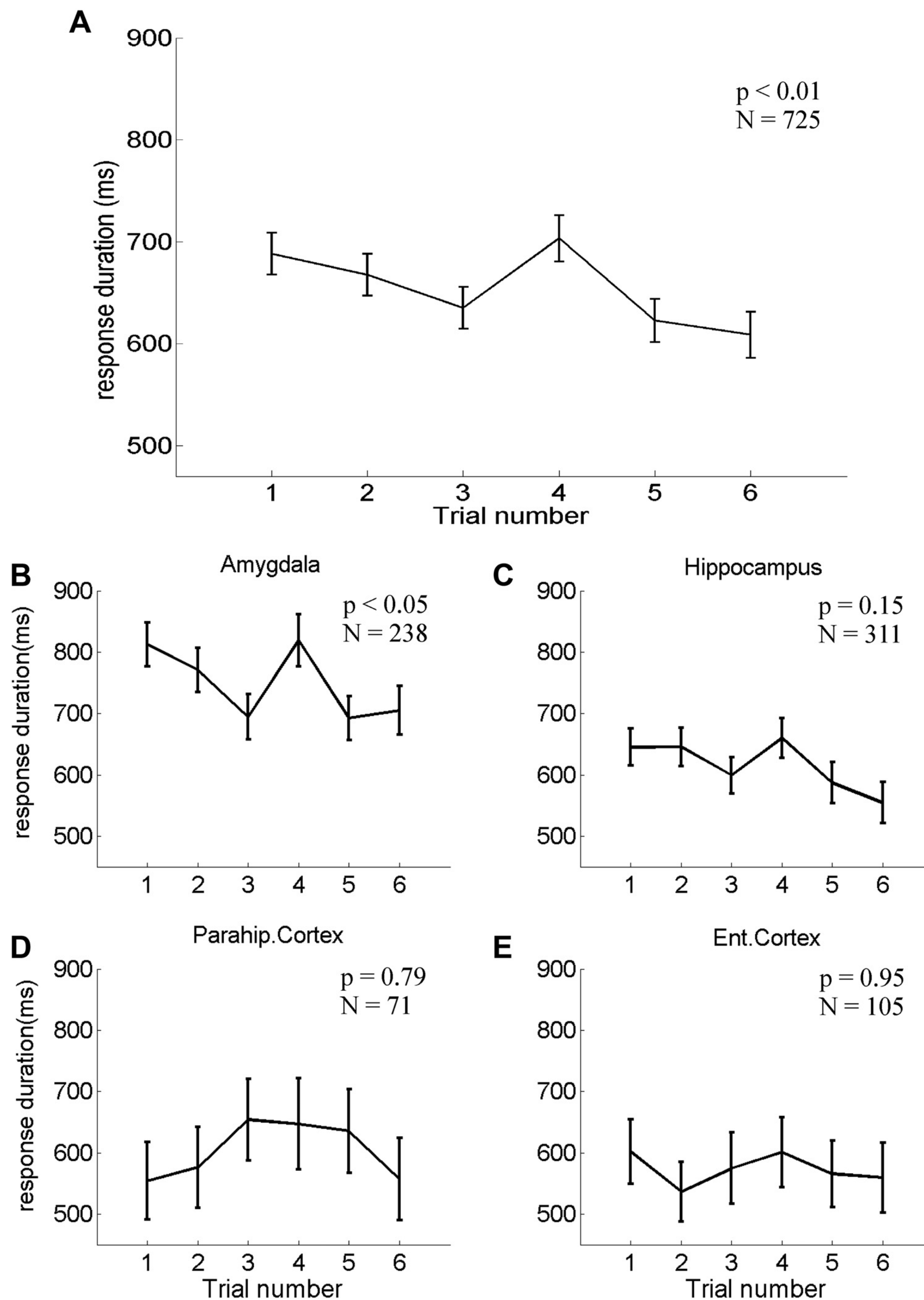

FIG. 7. Average duration of the significant responses. *A*: average response duration for all the 725 responses. The duration decreased significantly for later trials. *B–E*: data broken down by areas. *N*, number of responses. Bars denote SE.

Downloaded from jn.physiology.org on January 12, 2010

Field 2004; Quian Quiroga et al. 2008b; Shoham et al. 2006). Although we currently have no direct evidence to assess the contributions of each of these factors for comparing our responses to those described in Rutishauser et al. and Viskontas et al., it is likely that due to these differences our study describes a different set of cells with much higher selectivity. Understanding the role of these different types of neural responses is a subject for further investigation. The second main difference with the works from Viskotas and colleagues and Rutishauser and colleagues is that in their case the neuronal responses were elicited by an active memory task, whereas in our case the responses occurred in a nearly-passive viewing task. However, the fact that we did not have an explicit memory task does not rule out a memory effect because subjects can still remember seeing a particular picture at the UCLA ward even if not explicitly asked to do so. Interestingly, it has been recently shown that these percepts can trigger strong responses when later recalled (Gelbard-Sagiv et al. 2008).

With the very few notable exceptions mentioned above, direct studies of the response patterns of single neurons with stimulus repetition have only been done in animals (see Ranganath and Rainer 2003 for a review). In a series of studies using delayed matched-to-sample (DMS) and recognition memory tasks, Brown and colleagues reported neurons in the IT cortex and the MTL—more specifically the perirhinal and entorhinal cortices and the hippocampus—that responded differentially based on the familiarity, recency and novelty of the

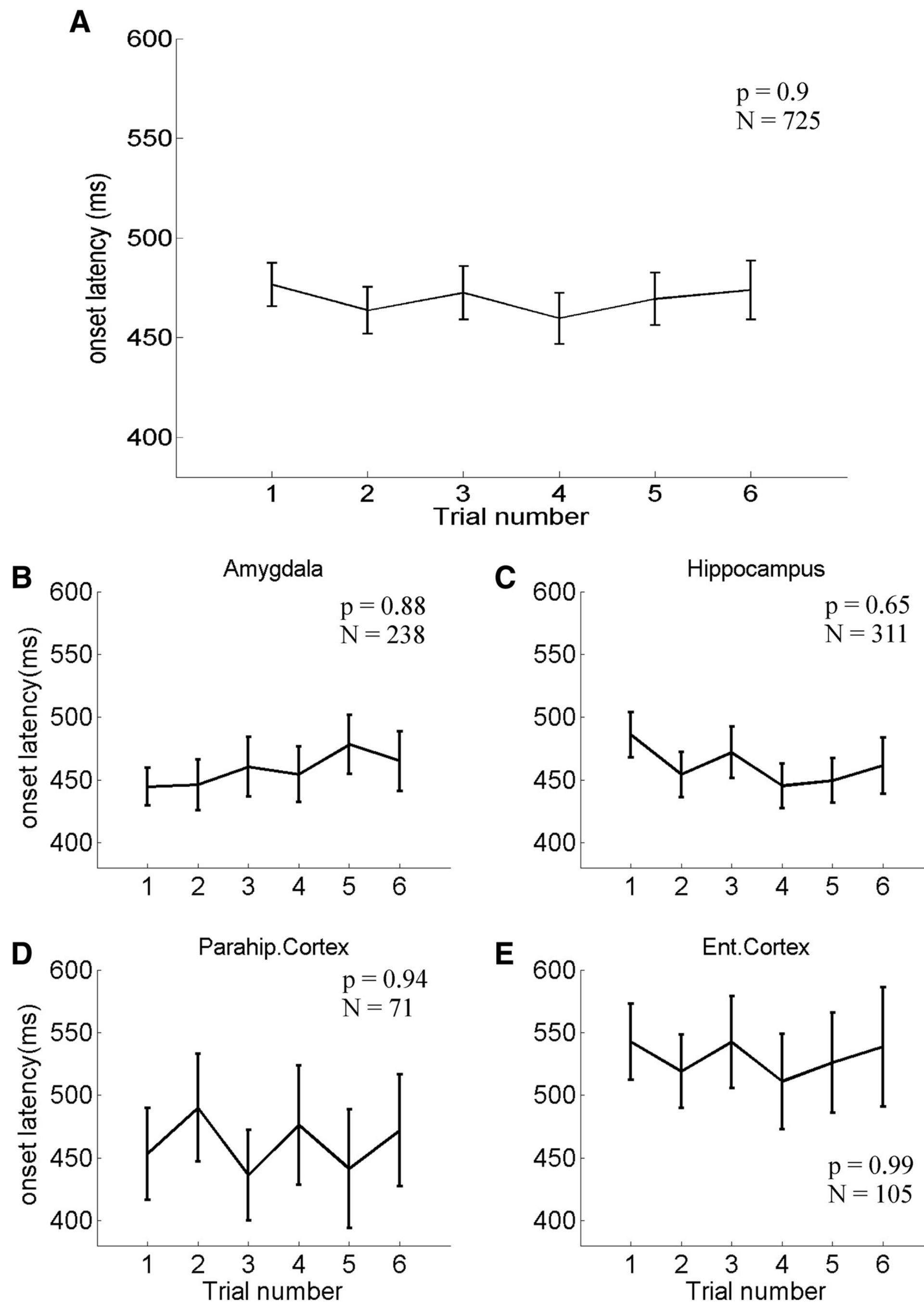

FIG. 8. Average onset latencies of the significant responses. *A*: averaged onset latencies for the 725 responses. *B–E*: data broken down by areas. There were no significant differences with trial number. *N*, number of responses. Bars denote SE.

stimulus (Fahy et al. 1993; Riches et al. 1991; Xiang and Brown 1998). Rolls et al. (1982) described neurons in the anterior border of the macaque thalamus that responded only to familiar stimuli in a recognition task, and in another study with a similar task, they found that ~2% of the recorded neurons in the hippocampus responded differently to novel and familiar stimuli (Rolls et al. 1993). The finding of neurons with these response patterns has been interpreted—both by Brown and colleagues and by Rolls and colleagues—as related to recognition memory processes. It has also been proposed that this effect leads to a tuning of the neural population towards a sparse representation of the stimuli (Rainer and Miller 2000).

It has to be mentioned that the neurons described in all these studies were not stimulus-selective. On the contrary, the responses of the MTL neurons we record from are highly selective (Quian Quiroga et al. 2007) and they do not simply reflect the familiarity or novelty of the pictures, as it was the case for the previous two single cell studies in humans and the abovementioned studies in monkeys. Interestingly, the nonselective novelty-dependent neurons reported in the MTL (both in monkeys and humans) were obtained during explicit memory tasks. Our results are more reminiscent of studies in IT cortex in monkeys, which showed a decreased firing of single neurons when a stimulus was shown repeatedly while performing a DMS task (Desimone 1996; Li et al. 1993; Miller et al. 1991). In the later case, because responses were selective to specific novel stimuli, it has been claimed that these are not just novelty detectors—i.e., they do not respond to just any novel

Downloaded from jn.physiology.org on January 12, 2010

stimuli—and instead, they act as adaptive mnemonic filters providing a signal of a novel stimulus deserving attention. In addition to this, recent single cell studies in monkey IT while monkeys performed repeated visual fixation and stimulus classification tasks (Anderson et al. 2008 and Freedman et al. 2006, respectively) have shown a long-term decrease of neuronal firing. Furthermore, two other studies in monkey IT during a visual fixation task reported neurons showing a time-localized response suppression without a change in response onset, as in our case (Liu et al. 2009; Sawamura et al. 2006).

Even though response patterns in these studies are similar to the ones reported here, the main difference is that the neurons reported here were recorded in the human MTL. In contrast to monkey IT cortex, converging evidence has shown that the MTL is part of the declarative memory system and is not necessary for perception (Gazzaniga et al. 1998; Squire et al. 2004). Given this role of MTL neurons, it is plausible to infer that the novelty effect reported here is correlated to memory formation processes, in agreement with our previous claim that these neurons are making the link between perception and memory (Quian Quiroga et al. 2005, 2008a). In particular, decreased firing with stimulus repetition may reflect the decrease of relevant information to be stored into memory after each presentation, because the amount of information is larger the first time the picture is seen than after several presentations. In other words, subjects may remember seeing a particular picture during the experiments, but after several repetitions, not much relevant information that could be stored in memory is added by any further presentation of the same image. A mechanistic explanation of how these neurons know what the relevant information is goes beyond what can be inferred with current data. However, it is in principle possible that the relevant information is selected by attention mechanisms in upstream areas or that the MTL neurons described here interact with the less selective MTL neurons described by Rutishauser et al. (2006) and Viskontas et al. (2006) to assess stimulus novelty or familiarity.

Our experimental design did not include control conditions—like showing some of the pictures for the first time half way through the experiment—to rule out arousal effects. It is therefore possible that the actual arousal state of the patients may have contributed to the repetition effects described in our study. However, it seems not likely that such pattern of responses can be attributed to an overall effect of tiredness within a recording session because *1*) we found stronger repetition effects for the first sessions compared with the ones observed in sessions performed on following days and *2*) decreases in firing were not uniform for the different MTL areas. In particular, there was no stimulus repetition effect for the neurons in the parahippocampal cortex, thus rendering a general "lack of arousal" explanation less plausible. In line with these results showing different response patterns for different MTL areas, we previously reported a lower selectivity (i.e., neurons fired to more images) and earlier responses in parahippocampal cortex compared with the other MTL areas (Mormann et al. 2008).

The role of different MTL areas in memory formation is still under discussion (Eichenbaum 2000; Gazzaniga et al. 1998; Moscovitch et al. 2006; Squire et al. 2004). For example, it has been argued that the system formed by hippocampus and entorhinal cortex could provide support for establishing the link between the different components of episodic memories (Brown and Aggleton 2001; Eichenbaum et al. 2007). Meanwhile, the amygdala has been correlated to emotional memories (McGaugh 2004; Phelps and LeDoux 2005). Adding to this evidence of functional specialization within the MTL, our results show a dissociation in the response pattern of neurons in the parahippocampal cortex compared to the other MTL areas. In particular, parahippocampal neurons were the only ones that did not show a decrease in firing with stimulus repetition. Interestingly, the parahippocampal cortex has been identified as part of a system supporting stimulus familiarity (Brown and Aggleton 2001; Eichenbaum et al. 2007), a finding that it is not in disagreement with our results because we used familiar pictures—i.e., the persons or objects used were well known to the patients before the experiment. Considering the abovementioned explanation that MTL neurons may be providing the link between perception and long-term memory formation, the lack of a stimulus repetition effect in parahippocampal neurons may be showing that this area is not involved in such a process. However, this claim should be further validated with future experiments analyzing the firing of this population of selective neurons with memory related tasks.

ACKNOWLEDGMENTS

We thank all the patients for participating in our study and M. J. Ison, J. Martinez, and S. Thill for discussions.

GRANTS

This work was supported by grants from the Engineering and Physical Sciences Research Council, Medical Research Council, National Institute of Neurological Disorders and Stroke, National Institute of Mental Health, Defense Advanced Research Projects Agency, and the Mathers Foundation. F. Mormann acknowledges support from Marie Curie Outgoing International Fellowship from the European Commission.

REFERENCES

Anderson B, Mruczek REB, Kawasaki K, Sheinberg D. Effects of familiarity on neural activity in monkey inferior temporal lobe. *Cereb Cortex* 18: 2540–2552, 2008.

Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, Strauss MM, Hyman SE, Rosen BR. Response and habituation of the human amygdala during visual processing of facial expression. *Neuron* 17: 875–887, 1996.

Brown MW, Aggleton JP. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* 2: 51–61, 2001.

Buckley MJ, Gaffan D. Perirhinal cortical contributions to object perception. *Trends Cogn Sci* 10: 100–107, 2006.

Corkin S. What's new with the amnesic patient H.M.? *Nat Rev Neurosci* 3: 153–160, 2002.

Desimone R. Neural mechanisms for visual memory and their role in attention. *Proc Natl Acad Sci USA* 93: 13494–13499, 1996.

Eichenbaum H. A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci* 1: 41–50, 2000.

Eichenbaum H, Yonelinas AP, Ranganath C. The medial temporal lobe and recognition memory. *Annu Rev Neurosci* 30: 123–152, 2007.

Fahy FL, Riches IP, Brown MW. Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Exp Brain Res* 96: 457–472, 1993.

Freedman DJ, Riesenhuber M, Poggio T, Miller EK. Experience-dependent sharpening of visual shape selectivity in inferior temporal cortex. *Cereb Cortex* 16: 1631–1644, 2006.

Fried I, MacDonald KA, Wilson CL. Single neuron activity in human hippocampus and amygdala during recognition of faces and objects. *Neuron* 18: 753–765, 1997.

Gazzaniga MS, Ivry RB, Mangun GR. *Cognitive Neuroscience: The Biology of the Mind.* New York: "WW" Norton, 1998.

Gelbard-Sagiv H, Mukamel R, Harel M, Malach R, Fried I. Internally generated reactivation of single neurons in human hippocampus during free recall. *Science* 322: 96–101, 2008.

Downloaded from jn.physiology.org on January 12, 2010

Grill-Spector K, Henson R, Martin A. Repetition and the brain: neural models of stimulus-specific effects. *Trends Cogn Sci* 10: 14–23, 2006.
Henson RNA, Rugg MD. Neural response suppression, haemodynamic repetition effects, and behavioural priming. *Neuropsychologia* 41: 263–270, 2003.
Hung CP, Kreiman G, Poggio T, DiCarlo JJ. Fast readout of object identity from macaque inferior temporal cortex. *Science* 310: 863–866, 2005.
Kreiman G, Fried I, Koch C. Single-neuron correlates of subjective vision in the human medial temporal lobe. *Proc Natl Acad Sci USA* 99: 8378–8383, 2002.
Kreiman G, Koch C, Fried I. Category-specific visual responses of single neurons in the human medial temporal lobe. *Nat Neurosci* 3: 946–953, 2000a.
Kreiman G, Koch C, Fried I. Imagery neurons in the human brain. *Nature* 408: 357–361, 2000b.
Lavenex P, Amaral DG. Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus* 10: 420–430, 2000.
Li L, Miller EK, Desimone R. The representation of stimulus familiarity in anterior inferior temporal cortex. *J Neurophysiol* 69: 1918–1929, 1993.
Liu Y, Murray SO, Jagadeesh B. Time course and stimulus dependence of repetition-induced response suppression in inferotemporal cortex. *J Neurophysiol* 101: 418–436, 2009.
Logothetis NK. What we can do and what we cannot do with fMRI. *Nature* 453: 869–878, 2008.
Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412: 150–157, 2001.
Logothetis NK, Sheinberg DL. Visual object recognition. *Annu Rev Neurosci* 19: 577–621, 1996.
McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci* 27: 1–28, 2004.
Miller EK, Li L, Desimone R. A neural mechanism for working and recognition memory in inferior temporal cortex. *Science* 254: 1377–1379, 1991.
Mormann F, Kornblith S, Quian Quiroga R, Kraskov A, Cerf M, Fried I, Koch C. Latency and selectivity of single neurons indicate hierarchical processing in the human medial temporal lobe. *J Neurosci* 28: 8865–8872, 2008.
Moscovitch M, Nadel L, Winocur G, Gilboa A, Rosenbaum RS. The cognitive neuroscience of remote episodic, semantic and spatial memory. *Curr Opin Neurobiol* 16: 179–190, 2006.
Naya Y, Yoshida M, Miyashita Y. Backward spreading of memory-retrieval signal in the primate temporal cortex. *Science* 291: 661–664, 2001.
Olshausen BA, Field DJ. Sparse coding of sensory inputs. *Curr Opin Neurobiol* 14: 481–487, 2004.
Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 48: 175–187, 2005.
Quian Quiroga R, Kraskov A, Koch C, Fried I. Explicit encoding of multimodal percepts by single neurons in the human brain. *Curr Biol* 19: 1308–1313, 2009.
Quian Quiroga R, Kreiman G, Koch C, Fried I. Sparse but not 'Grandmother-cell' coding in the medial temporal lobe. *Trends Cogn Sci* 12: 87–91, 2008a.
Quian Quiroga R, Mukamel R, Isham EA, Malach R, Fried I. Human single-neuron responses at the threshold of conscious recognition. *Proc Natl Acad Sci USA* 105: 3599–3604, 2008b.
Quian Quiroga R, Nadasdy Z, Ben-Shaul Y. Unsupervised spike detection and sorting with wavelets and super-paramagnetic clustering. *Neural Comput* 16: 1661–1687, 2004.
Quian Quiroga R, Reddy L, Koch C, Fried I. Decoding visual inputs from multiple neurons in the human temporal lobe. *J Neurophysiol* 98: 1997, 2007.
Quian Quiroga R, Reddy L, Kreiman G, Koch C, Fried I. Invariant visual representation by single neurons in the human brain. *Nature* 435: 1102–1107, 2005.
Rainer G, Miller EK. Effects of visual experience on the representation of objects in the prefrontal cortex. *Neuron* 27: 179–189, 2000.
Ranganath C, Rainer G. Neural mechanisms for detecting and remembering novel events. *Nat Rev Neurosci* 4: 193–202, 2003.
Riches IP, Wilson FAW, Brown MW. The effects of visual stimulation and memory on neurons of the hippocampal formation and the neighboring parahippocampal gyrus and inferior temporal cortex of the primate. *J Neurosci* 11: 1763–1779, 1991.
Rolls ET, Cahusac PMB, Feigenbaum JD, Miyashita Y. Responses of single neurons in the hippocampus of the macaque related to recognition memory. *Exp Brain Res* 93: 299–306, 1993.
Rolls ET, Perrett DI, Caan AW, Wilson FAW. Neuronal responses related to visual recognition. *Brain* 105: 611–646, 1982.
Rosenbaum RS, Kohler S, Schacter DL, Moscovitch M, Westmacott R, Black SE, Gao F, Tulving E. The case of K.C.: contributions of a memory-impaired person to memory theory. *Neuropsychologia* 43: 989–1021, 2005.
Rutishauser U, Mamelak AN, Schuman EM. Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. *Neuron* 49: 805–813, 2006.
Sakai K, Miyashita Y. Neural organization for the long-term memory of paired associates. *Nature* 354: 152–155, 1991.
Saleem KS, Tanaka K. Divergent projections from the anterior inferotemporal area TE to the perirhinal and entorhinal cortices in the macaque monkey. *J Neurosci* 16: 4757–4775, 1996.
Sawamura H, Orban GA, Vogels R. Selectivity of neuronal adaptation does not match response selectivity: a single-cell study of the fMRI adaptation paradigm. *Neuron* 49: 307–318, 2006.
Scoville W, Milner B. Loss of recent memory after bilateral lesions. *J Neurol Neurosurg Psychiatr* 20: 11–21, 1957.
Shoham S, O'Connor DH, Segev R. How silent is the brain: is there a "dark matter" problem in neuroscience? *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 192: 777–784, 2006.
Squire LR, Stark CEL, Clark RE. The medial temporal lobe. *Annu Rev Neurosci* 27: 279–306, 2004.
Suzuki WA. The anatomy, physiology and functions of the perirhinal cortex. *Curr Opin Neurobiol* 6: 179–186, 1996.
Tanaka K. Inferotemporal cortex and object vision. *Annu Rev Neurosci* 19: 109–139, 1996.
Viskontas IV, Knowlton BJ, Steinmetz PN, Fried I. Differences in mnemonic processing by neurons in the human hippocampus and parahippocampal regions. *J Cogn Neurosci* 18: 1654–1662, 2006.
Viskontas I, Quian Quiroga R, Fried I. Human medial temporal lobe neurons respond preferentially to personally-relevant images. *Proc Natl Acad Sci USA* In press.
Wirth S, Yanike M, Frank LM, Smith AC, Brown EN, Suzuki WA. Single neurons in the monkey hippocampus and learning of new associations. *Science* 300: 1578–1581, 2003.
Xiang J, Brown MW. Differential neuronal encoding of novelty, familiarity and recency in regions of the anterior temporal lobe. *Neuropharmacology* 37: 657–676, 1998.

Downloaded from jn.physiology.org on January 12, 2010

Supplementary material

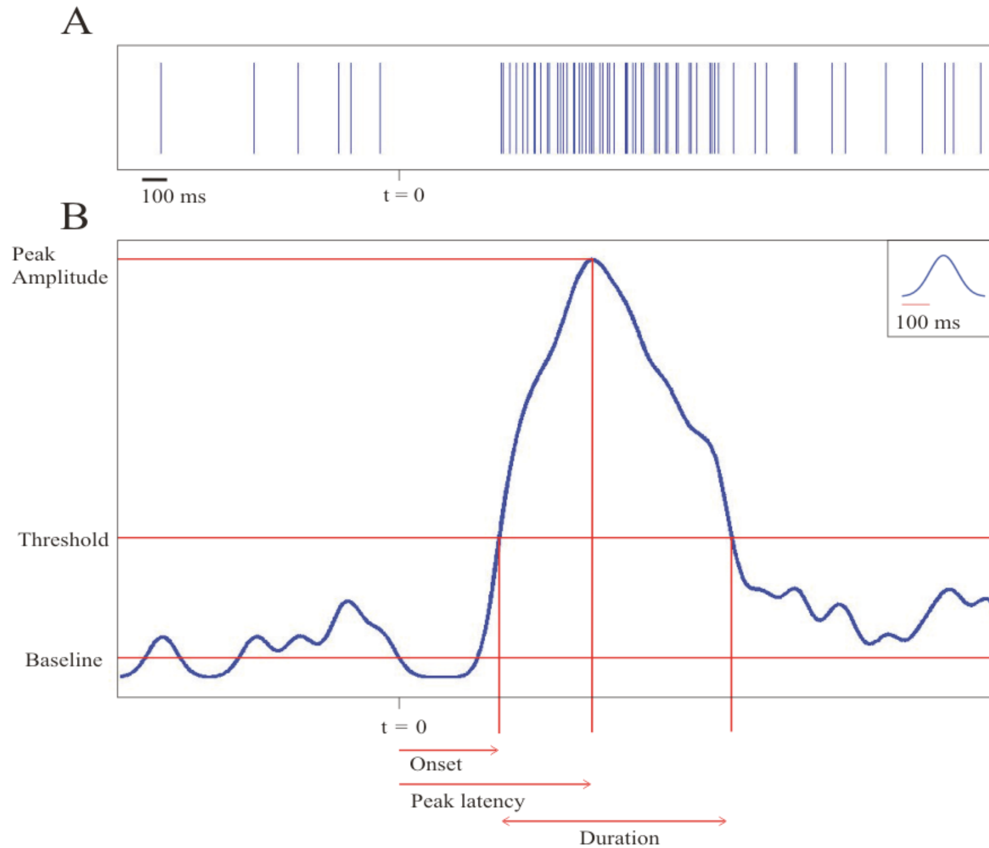

Figure S1: Parameters used for timecourse statistical analysis. **A:** Raster plot of a response from an idealized neuron. **B:** Instantaneous firing rate corresponding to the raster plot in A obtained by convolving the spike train with a Gaussian kernel with σ = 100ms. A threshold was defined as the mean of the baseline plus 4 s.d. The onset of the response was defined as the time when the firing rate curve crossed this threshold and stayed above it for at least 100 ms. The offset was defined similarly, but with the firing rate curve crossing the threshold downward for at least 100 ms. Duration was the difference between the offset and the onset times. Time t=0 ms symbolizes the onset of the picture.

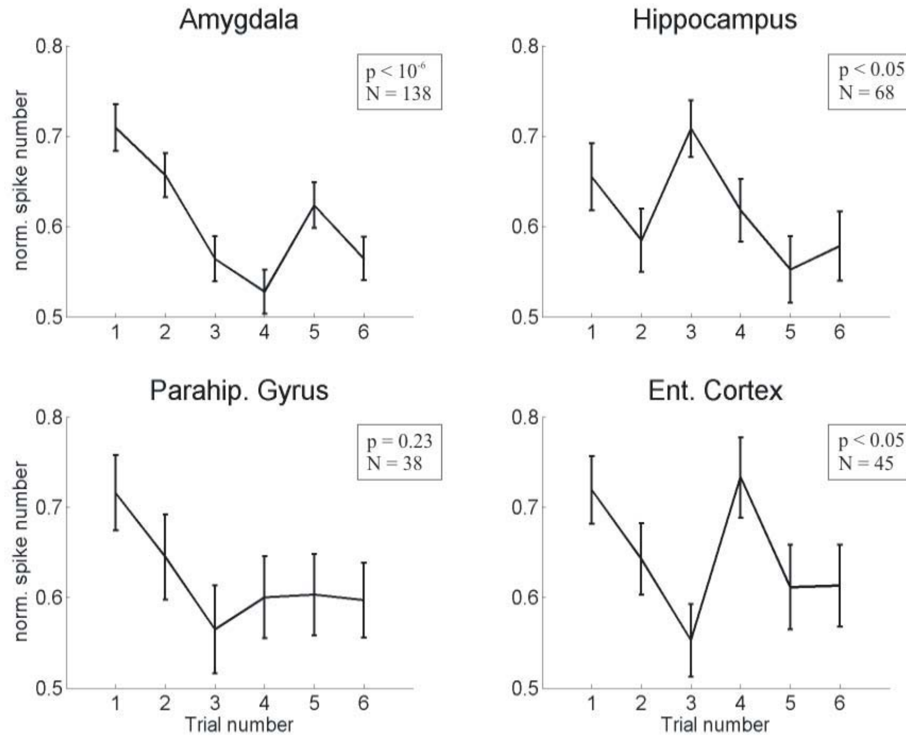

Figure S2: Mean number of spikes per trial for different MTL regions in the second session. Responses had a significant decay with picture repetition for amygdala, hippocampus and entorhinal cortex ($F(5,754) = 7.55$, $p < 10^{-6}$; $F(5,373) = 2.5$, $p < 0.05$; $F(5,249) = 2.68$, $p < 0.05$, respectively) but not for parahippocampal gyrus ($F(5,219) = 1.38$, $p = 0.23$). N refers to the number of responses for each particular region. Note that the patterns for the second session were not as clear as the ones found in the first experimental session, in agreement with data shown in Figure 4. Bars denote s.e.m.

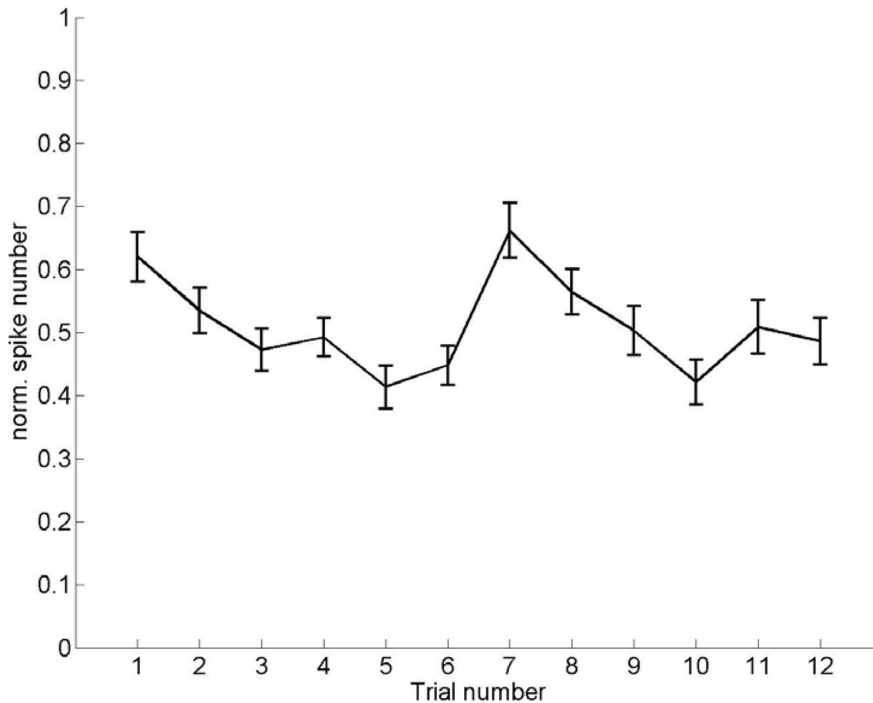

Figure S3: Normalized spike responses for 51 responses from 18 neurons traceable along the 2 sessions. Trials 1-6 correspond to the first experiment, and trials 7-12 to the second one. The normalization for all 12 trials is made using the same value. Given that the 51 responses come from traceable neurons we divided the response of each trial by the maximum response within the 12 of them. The difference in firing between the 12 trials was highly significant ($F(11,592) = 4.21$, $p < 10^{-5}$, ANOVA test with trial number as the independent variable and the repeated measures were the normalized number of spikes per response). The difference between trial 6 and 7 was also significant ($t = -4.15$, d.f. = 100, $p < 10^{-4}$, paired T-Test between both trials). Comparison between the mean of all the responses corresponding to each of the sessions did not show a significant difference between the experiments ($t = -1.39$, d.f. = 610, p = 0.166 for a paired T-Test between all the normalized responses from the corresponding experiment). With the analysis for the unnormalized value of the

responses, a significant difference between the 12 trials was still present, although due to a higher variability, corresponding to different firing rates of the responsive neurons, the value was slightly lower ($F(11,592) = 2.35$, $p < 0.01$). Similarly for the unnormalized responses, a statistical difference was found between trials 6 and 7 ($t = -2.93$, d.f. = 100, $p < 0.005$), but not between all the mean of the different experiments ($t = -0.72$, d.f. = 610, $p = 0.47$). Bars denote s.e.m.

Supplementary material

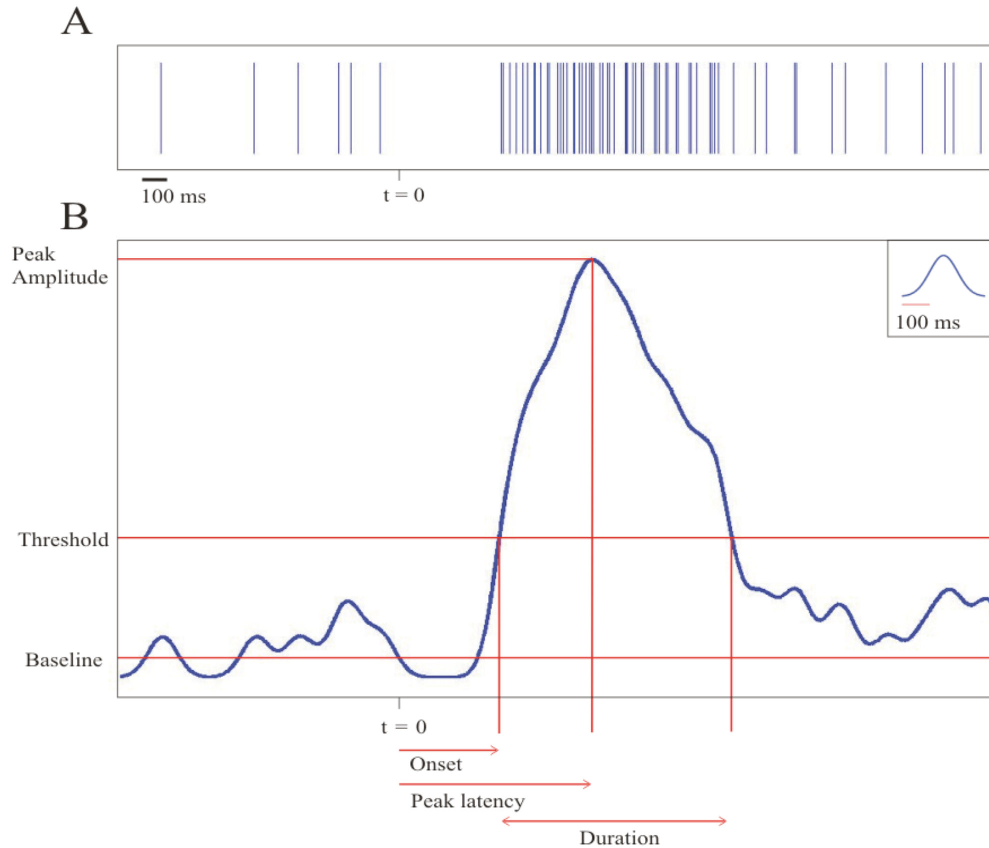

Figure S1: Parameters used for timecourse statistical analysis. **A:** Raster plot of a response from an idealized neuron. **B:** Instantaneous firing rate corresponding to the raster plot in A obtained by convolving the spike train with a Gaussian kernel with σ = 100ms. A threshold was defined as the mean of the baseline plus 4 s.d. The onset of the response was defined as the time when the firing rate curve crossed this threshold and stayed above it for at least 100 ms. The offset was defined similarly, but with the firing rate curve crossing the threshold downward for at least 100 ms. Duration was the difference between the offset and the onset times. Time t=0 ms symbolizes the onset of the picture.

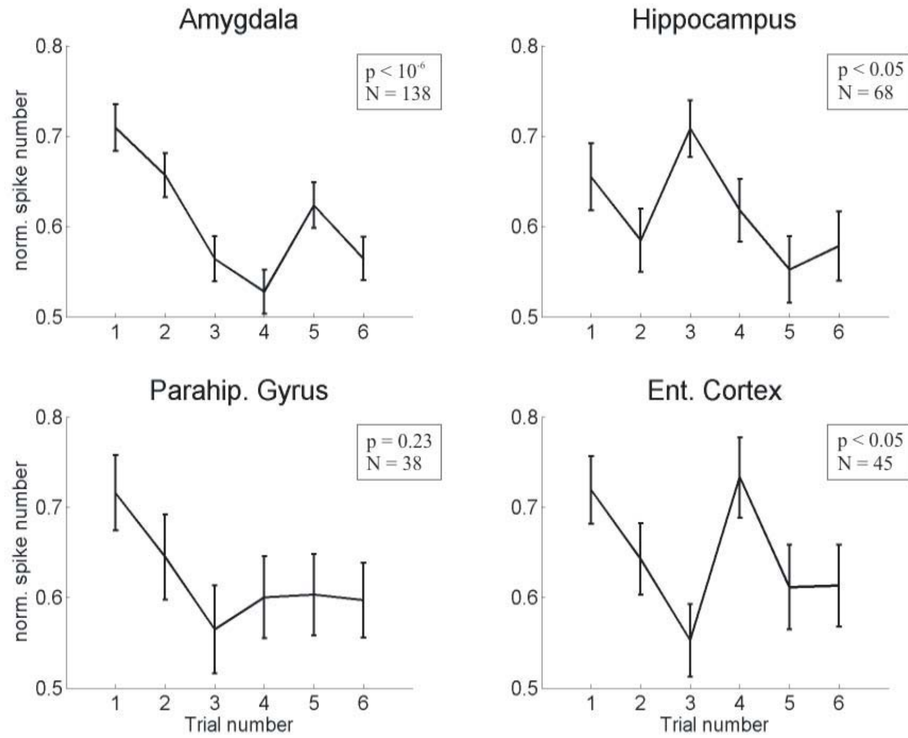

Figure S2: Mean number of spikes per trial for different MTL regions in the second session. Responses had a significant decay with picture repetition for amygdala, hippocampus and entorhinal cortex ($F(5,754) = 7.55$, $p < 10^{-6}$; $F(5,373) = 2.5$, $p < 0.05$; $F(5,249) = 2.68$, $p < 0.05$, respectively) but not for parahippocampal gyrus ($F(5,219) = 1.38$, $p = 0.23$). N refers to the number of responses for each particular region. Note that the patterns for the second session were not as clear as the ones found in the first experimental session, in agreement with data shown in Figure 4. Bars denote s.e.m.

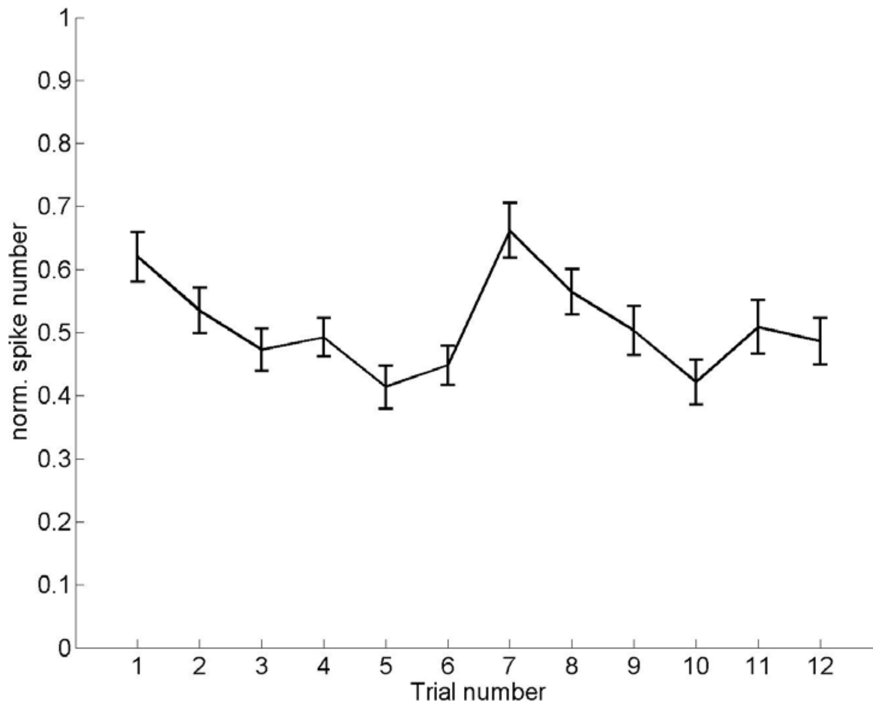

Figure S3: Normalized spike responses for 51 responses from 18 neurons traceable along the 2 sessions. Trials 1-6 correspond to the first experiment, and trials 7-12 to the second one. The normalization for all 12 trials is made using the same value. Given that the 51 responses come from traceable neurons we divided the response of each trial by the maximum response within the 12 of them. The difference in firing between the 12 trials was highly significant ($F(11,592) = 4.21$, $p < 10^{-5}$, ANOVA test with trial number as the independent variable and the repeated measures were the normalized number of spikes per response). The difference between trial 6 and 7 was also significant ($t = -4.15$, d.f. = 100, $p < 10^{-4}$, paired T-Test between both trials). Comparison between the mean of all the responses corresponding to each of the sessions did not show a significant difference between the experiments ($t = -1.39$, d.f. = 610, $p = 0.166$ for a paired T-Test between all the normalized responses from the corresponding experiment). With the analysis for the unnormalized value of the

responses, a significant difference between the 12 trials was still present, although due to a higher variability, corresponding to different firing rates of the responsive neurons, the value was slightly lower ($F(11,592) = 2.35$, $p < 0.01$). Similarly for the unnormalized responses, a statistical difference was found between trials 6 and 7 ($t = -2.93$, d.f. = 100, $p < 0.005$), but not between all the mean of the different experiments ($t = -0.72$, d.f. = 610, $p = 0.47$). Bars denote s.e.m.