Responses of human medial temporal lobe neurons are modulated by stimulus repetition.

Carlos Pedreira$^1$, Florian Mormann$^{2,4}$, Alexander Kraskov$^3$, Moran Cerf$^{2,4}$, Itzhak Fried$^{4,5}$, Christof Koch$^2$ and Rodrigo Quian Quiroga$^{1,2,4}$

$^1$Department of Engineering, University of Leicester, UK.

$^2$Computation and Neural Systems, California Institute of Technology, Pasadena, California.

$^3$UCL Institute of Neurology, Queen Square, London WC 1N 3BG, London, UK.

$^4$Department of Neurosurgery, David Geffen School of Medicine, and Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, California.

$^5$Functional Neurosurgery Unit, Tel-Aviv Medical Center and Sackler Faculty of Medicine, Tel-Aviv University

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#Corresponding author: Dept. of Engineering. Univ. of Leicester, UK.

Tel: +44 116 252 2314 Fax: +44 116 252 2619. Email: rqqg1@le.ac.uk

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Abstract

Recent studies have reported the presence of single neurons with strong responses to visual inputs in the human medial temporal lobe. Here we demonstrate 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 novel stimuli was not the same for all medial temporal lobe structures, expressing differences among hippocampus, entorhinal cortex, amygdala and parahippocampal cortex. These findings are consistent with the view that MTL neurons link visual percepts to declarative memory.
Introduction

Visual object recognition occurs along the ventral visual pathway, extending from primary visual areas (V1) to the inferotemporal cortex (IT) (Logothetis and Sheinberg 1996; Tanaka 1996), which in turn has direct projections to the medial temporal lobe (MTL) (Lavenex and Amaral 2000; Saleem and Tanaka 1996; Suzuki 1996). Single cell studies in the monkey MTL reported stimulus selective neurons which 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). 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 i) the well-established findings about the role of the MTL in memory storage, ii) the relatively long latency of MTL responses – of 300 ms or longer after stimulus onset (Mormann et al. 2008; Quian Quiroga et al. 2005) –, compared to about 120 ms in monkey IT (Hung et al. 2005) and iii) 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 the activity elicited by stimulus repetition in humans showed a decrease in activity for different areas, including the MTL, which has been related to processes supporting functions 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 paradigms with stimulus repetition induced response suppression patterns in monkey inferotemporal (IT) cortex 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 investigate whether a similar response pattern was also present in the visual responses in the human MTL and hypothesize – considering the abovementioned studies about the function of this particular area – that such a finding with MTL neurons may be due to its role in declarative memory.

Materials and Methods

Subjects and recordings

Subjects were 26 patients with pharmacologically intractable epilepsy (15 men; 22 right handed; 17-54 years old). Extensive non-invasive 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 by computed tomography co-registered to preoperative MRI. Here we report data from sites in the hippocampus, amygdala, entorhinal and
parahippocampal cortexes. Each electrode probe had a total of nine micro-wires at its end, eight active recordings channels and one reference (Fried et al. 1997). The differential signal from the micro-wires was amplified using a 64 channel Neuralynx system, filtered between 1 and 9,000 Hz and sampled at 28 kHz. Each recording session lasted about 30 minutes.

The data reported here was recorded during 44 experimental sessions in these 26 patients. Subjects lay in bed facing a laptop computer on which pictures of animals, objects, landmark buildings, as well as 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). Images covered about 1.5° of the visual angle and were presented for 1 second at the center of the screen, 6 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 sec presentation sessions since 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. Due to 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 standard deviations (s.d.) of the baseline periods (1000 to 300 ms before stimulus onset) for all stimuli, and had at least two spikes in the time interval between 300 and 1000 ms after the stimulus onset. For those pictures eliciting significant responses, we computed the total number of spikes from 300 ms to 2000 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 analysis of variance (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 as well as for each of the four subregions separately.

For a further characterization of the differences in firing across stimulus repetition, for each of the significant responses we calculated a linear regression of the number of spikes with trial number. Then, 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, \( \sigma = 100 \) ms). From the average instantaneous firing rate (across all responses) for each trial we defined: i) the peak amplitude; ii) its latency; iii) the onset of the response, as the point where the instantaneous firing rate crossed 4 s.d. above baseline and stayed above for at least 100 ms, and iv) the duration, as the time interval between response onset and offset (Figure S1). Offset was defined, similarly to onset, but crossing the 4 s.d. line downwards and staying below it for at least 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 1210 MTL units (515 single units and 695 multi-units), with an average of 46.6 units per session. Of these 1210 units, 262 (22%; 132 single units and 130 multi-units) 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 multi-units), with an average of 41.3 units per session. Out of these 745 units, 110 (15%; 57 single units and 53 multi-units) 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 first session against 15% for the second one) was significant (chi-square, p < 0.001).

**Single cell responses**

Figure 1 presents 4 example significant responses recorded in four different patients. For each response we display the raster plots (first trial at the top), the number of spikes per trial and the peri-stimulus time histograms. The neuron in panel A was located in the entorhinal cortex. Its average baseline activity was 2Hz and it fired with up to 20Hz to the patient’s own picture. The neuron in panel B was located in the amygdala and from a mean baseline activity of about 7Hz, it responded with up to 50Hz to the picture of a monkey. The neuron in panel C was located in the hippocampus and from a baseline of about 3Hz it responded with 30Hz to the picture of a squirrel. Finally, the neuron in panel D was in the parahippocampal cortex and it responded to a picture of the World Trade Center with about 45 Hz from a baseline of 10Hz. All these units increased their firing at least 3 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**

Figure 2a shows the mean normalized number of spikes for each trial of the 725 responses recorded in the first sessions, 238 from neurons located in amygdala, 311 form hippocampus, 105 from entorhinal cortex and 71 from parahippocampal cortex. As shown in the single cell examples of Figure 1, there was a significant
difference of the normalized number of spikes with trial number (F(5,4084) = 19.34, p < 10^{-15}, Test 1, see Materials and Methods). Note that the normalized spike number does not reach 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 panels B to E of Figure 2. 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), while 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 Materials and Methods). As shown in Figure 3, there was a significant difference between areas (F(3,721) = 7.1, p < 10^{-3}), which was mainly due to the smaller slope values in the responses 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, d.f. = 104, p < 0.005; t = -5.77, d.f. = 310, p < 10^{-7}; t = -8.11, d.f. = 237, p < 10^{-13}; respectively), while the ones from parahippocampal cortex were not (t = -0.26, d.f. = 70, p = 0.8).

**Time profile of the responses**

Figure 4a shows the instantaneous firing rates for each trial (see Materials and 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 Figure 2 seems to be due to differences in the duration of the responses as well as differences in the peak amplitudes. Moreover, it is apparent that for all trials the onset of the responses does not differ significantly. Panels B to E of Figure 4 show the firing rate of the responses divided by areas. Higher amplitude for the first trial can be seen in responses from amygdala and delayed maximum can be seen in the first trial for hippocampus and entorhinal cortex. To verify these observations, we assessed and statistically compared the onset, duration, peak amplitude and latency of the responses, as defined in Figure S1 (Test 4).

Figure 5a presents the average peak latency over the entire population of neurons for each of the 6 trials. There was a statistically significant difference of the peak latencies with trial number (F(5,3998) = 6.41, p < 10^{-5}) for the last trials. The same statistical analysis performed for each area separately, presented in panels B to 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 can be seen in panels B, C and E). Figure 6a shows the analysis of the peak amplitude value of all responses As in the case of the peak latency, there were significant differences with trial number (F(5,3998) = 2.83, p < 0.05). However, as can be seen in panels B to E, in this case a separate analysis for each area showed that this effect was significant only for the responses from the amygdala (F(5,1442) = 2.92, p < 0.05), while it was not significant for the responses in 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 Figure 4, panel A of Figure 7 showed a significant decrease with trial number for the duration of the responses (F(5,3531) =
3.09, \( p < 0.01 \)). In a separate analysis for each area this effect was only significant for the responses in amygdala \((F(5,1205) = 2.43, p < 0.05\), panel B of Figure 7). Figure 8 shows that there were no significant differences for the onset latencies of the responses either for the entire population of responses \((F(5,3531) = 0.26, p = 0.9\), see panel A) or for data divided by area (panels B to E).

Results for the second experimental sessions

Next, we compared the results of the first sessions with those obtained in 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 these sessions, being recorded from amygdala (138), hippocampus (68), entorhinal (45) and parahippocampal cortexes (38). The slope values for the different locations are presented in Figure 3 (normalized responses analysis divided by area as reported in Figure 2 for the first session are available in the supplementary material, Figure 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 \((t = -4.01, \text{ d.f.} = 137, p < 0.001 \text{ and } t = -2.16, \text{ d.f.} = 44, p < 0.05\), respectively;
Test 3). Note that in general, the responses in the second session had overall lower slope values compared to the ones of the first session.

Statistical differences in the slope of decay of firing with trial number 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 of the slope values across regions ($F(3,1006) = 8.28$, $p < 10^{-4}$). In this first analysis, the comparison between the first and the second sessions showed a general trend, with smaller slope values for the second sessions ($F(1,1006) = 3.55$, $p = 0.06$). Since 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 ($F(1,899) = 4.68$, $p < 0.05$).

Discussion

In this study we have shown 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 in the responses in the amygdala, hippocampus and enthorinal cortex (but not parahippocampal cortex). Given that the onset of responses was not different for the different trials (see Figure 8), the decrease in peak latency can be attributed to a ‘time-sharpening’ of the responses – 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 Figure 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 due to the less accurate estimation of the response duration, which accumulates 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 responses 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 amygdala and parahippocampal cortex, the latter one (together with the perirhinal cortex) giving the main inputs 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 non-invasive techniques such as EEG and fMRI (e.g. 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 activity of single neurons, since they only measure the activity of large populations (Logothetis et al. 2001, 2008). 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 and colleagues, neurons from the hippocampal and parahippocampal regions showed a higher firing rate for novel pictures. Moreover, neurons from hippocampus presented a decrease of their firing below the baseline activity for subsequent presentations. Rutishauser and colleagues described two subsets of cells in hippocampus and amygdala: one group of cells that increased their firing when the stimulus presented was new and another one that increased their firing when it was shown few moments before (Rutishauser et al. 2006). Although related, there were two main differences between these two studies and the one presented here. First, the neurons described by Rutishauser and colleagues (2006) and by Viskontas and colleagues (2006) were not stimulus selective, as their behaviour was independent of the particular picture shown. This lack of selectivity compared to our study can be attributed to the fact that: i) we used familiar stimuli, which are more likely to elicit responses (Viskontas et al, in press). ii) we used an optimal spike sorting algorithm that is particularly suited to detect sparsely firing neurons, which typically have very low baseline firing rates (see e.g. Quian Quiroga et al, 2008b, 2009); iii) we recorded continuous data and used optimal offline analysis. Note that many acquisition systems detect spikes online based on amplitude thresholds set by hand. These thresholds may be set to non-optimal values, especially if the experimenter is dealing with a relatively large number of channels. In particular, this approach may miss or non-optimally 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 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 with 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 with stimulus repetition effects 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 infero-temporal (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 stimulus (Fahy et al. 1993; Riches et al. 1991; Xiang and Brown 1998). Rolls and colleagues described neurons in the anterior border of the macaque thalamus that responded only
to familiar stimuli in a recognition task (Rolls et al. 1982) and in another study with a
similar task, they found that about 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 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, both
studies in monkeys and humans showing these non-selective novelty dependent
neurons in MTL were recording 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, since these
responses were selective to specific novel stimuli, it has been claimed that they are
not just novelty detectors – i.e. they do not respond to any novel 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 recordings in monkey IT while
performing repeated visual fixation and stimulus classification tasks (Anderson et al.
2008; Freedman et al. 2006, respectively) have shown decreasing firing pattern in
neurons in long term basis. Even more, closer to our studies, two other studies in
monkey IT during a mere visual fixation task reported neurons showing response
suppression patterns localized in time, affecting mostly the firing pattern of the responses after reaching the maximum, but not the latency of the responses (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 with these studies is that the neurons in our study were recorded in the human MTL. 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) – in contrast to monkey IT cortex. 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, a decrease in firing rate with repetition may reflect the decrease of relevant information to be stored into memory after each presentation since 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 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: i) we found stronger repetition effects for the first sessions compared to the ones observed in sessions performed on following days; ii) 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 the current results showing different responses patterns for different MTL areas, we previously reported lower selectivity (i.e. neurons fired to more images) and earlier responses in parahippocampal cortex compared to 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 failed to 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 using memory-related tasks in this population of selective neurons.

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**Figure captions**

**Figure 1: Single cell responses.** The 4 panels (A-D) correspond to single cell responses to the preferred stimulus for 4 different patients. Responsive neurons were located in entorhinal cortex, amygdala, hippocampus and parahippocampal cortex, respectively. For each response we display the raster plots (first trial at the top), the number of spikes within a 300-2000 ms time window (right) and the peri-stimulus time histograms (bottom). A decrease in the number of spikes with trial number can be observed for each picture. The onset (t=0ms) and offset (t=1000ms) of the pictures are marked by dotted lines.

**Figure 2: Mean number of spikes per trial** from 26 patients. Panel A shows the result for all 725 responses. There was a significant effect of the trial number on the mean firing rate (ANOVA; F(5,4084) = 19.34, p < 10^{-15}). The mean number of spikes is reduced by an average of 17% from the first trial to the last one of the session. Panels B-E show the responses divided by area. Responses had a significant decay with picture repetition for amygdala, hippocampus and entorhinal cortex (F(5,1345) = 16.87, p < 10^{-15}; F(5,1726) = 6.03, p < 10^{-4}; F(5,588) = 2.52, p < 0.05, respectively) but not for parahippocampal cortex (F(5,407) = 0.98, p = 0.43). N refers to the number of responses for each graphic. Bars denote s.e.m.
Figure 3: Mean slopes of the decay in response magnitude with trial number. Slopes of the responses grouped by location. The first and second 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 s.e.m.

Figure 4: Mean instantaneous firing rate for each trial. Panel A shows the average over the whole set of responses (725). Note that all 6 trials have similar onset latency, but for the first trials, specially for trial number 1, in dark blue, there was a larger duration of the responses, together with later and slightly higher peaks. Panels B-E show the data broken down by areas. In panel B, responses from amygdala (238 responses) showed a clear higher rate and later peak for the first presentation, diminishing for the second one and again for the rest of the trials. In panel C responses from hippocampus (311) showed a later peak in the first (later than 500 ms) compared to the rest of the trials. Panel E presents responses from entorhinal cortex (105) that showed the same later peak for first and second presentations. No effect can be seen in responses from parahippocampal cortex (71) in panel D.

Figure 5: Average peak latency of the significant responses. Panel A shows the averaged peak latency for all the 725 responses. The peak was significantly earlier for later trials \((F(5,3998) = 6.41, p < 10^{-5})\). Panels B-E show the same analysis for the different areas. Significant decrease of the time the maximum of the response was reached with trial number can be seen in responses from amygdala (B), hippocampus
(C) and entorhinal cortex (E) \( \text{F}(5,1422) = 7.82, \ p < 10^{-6}; \text{F}(5,1860) = 7, \ p < 10^{-5} \) and \( \text{F}(5,624) = 3.36, \ p < 0.01 \) respectively), while responses from parahippocampal cortex did not reach a significant difference between trials \( \text{F}(5,246) = 2.03, \ p = 0.07 \). N refers to the number of responses for each graphic. Bars denote s.e.m.

**Figure 6: Average peak amplitude of the significant responses.** Panel A shows the averaged peak amplitude for all the 725 responses. The peak was significantly higher for the first trials \( \text{F}(5,3998) = 2.83, \ p < 0.05 \). Panels B to E show the data broken down by areas. Responses from amygdala shown a significant decrease in the maximum value reached with trial number \( \text{F}(5,1422) = 2.92, \ p < 0.05 \). Non significant differences were found for the recordings from the rest of MTL areas \( \text{F}(5,1860) = 0.98, \ p = 0.43; \text{F}(5,624) = 0.91, \ p = 0.47; \text{F}(5,246) = 0.48, \ p = 0.79 \) for hippocampus, entorhinal cortex and parahippocampal cortex, respectively). N refers to the number of responses for each graphic. Bars denote s.e.m.

**Figure 7: Average duration of the significant responses.** Panel A shows the averaged response duration for all the 725 responses. The duration decreased significantly for later trials \( \text{F}(5,3531) = 3.09, \ p < 0.01 \). Panels B to E show the data broken down by areas. A significant decrease in trial number can be appreciated for the amygdala responses \( \text{F}(5,1205) = 2.43, \ p < 0.05 \). Responses from hippocampus showed a similar behaviour, but the difference between trials did not reach a significant value \( \text{F}(5,1505) = 1.62, \ p = 0.15 \). Entorhinal and parahippocampal cortices responses showed a similar duration for all trials \( \text{F}(5,469) = 0.23, \ p = 0.95 \).
and $F(5,334) = 0.48$, $p = 0.79$, respectively. N refers to the number of responses for each graphic. Bars denote s.e.m.

Figure 8: Average onset latency of the significant responses. Panel A shows the averaged onset latency for the 725 responses. No significant difference on time was found between trials ($F(5,3531) = 0.26$, $p = 0.9$). Panels B-E present the data broken down by areas. No differences with trial number were found for the latency of the responses in any of the MTL areas ($F(5,1205) = 0.35$, $p = 0.88$; $F(5,1505) = 0.66$, $p = 0.65$; $F(5,469) = 0.25$, $p = 0.94$; $F(5,334) = 0.13$, $p = 0.99$; respectively). N refers to the number of responses for each graphic. Bars denote s.e.m.
References


