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Internally Generated Reactivation of Single Neurons in Human Hippocampus During Free Recall

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The emergence of memory, a trace of things past, into human consciousness is one of the greatest mysteries of the human mind. Whereas the neuronal basis of recognition memory can be probed experimentally in human and nonhuman primates, the study of free recall requires that the mind declare the occurrence of a recalled memory (an event intrinsic to the organism and invisible to an observer). Here, we report the activity of single neurons in the human hippocampus and surrounding areas when subjects first view television episodes consisting of audiovisual sequences and again later when they freely recall these episodes. A subset of these neurons exhibited selective firing, which often persisted throughout and following specific episodes for as long as 12 s. Verbal reports of memories of these specific episodes at the time of free recall were preceded by selective reactivation of the same hippocampal and entorhinal cortex neurons. We suggest that this reactivation is an internally generated neuronal correlate of the subjective experience of spontaneous emergence of human recollection.

The human hippocampus and its associated structures in the medial temporal lobe (MTL) transform present experience into future conscious recollections (*1*–*4*). Human MTL neurons respond in a highly specific manner to complex stimulus features (*5*), to complex stimulus categories (*5*, *6*), to individual persons or landmarks (*7*–*9*), and to previously seen and novel stimuli (*5*, *10*, *11*). These responses have been demonstrated for stationary stimuli and are usually brief, often lasting between 300 and 600 ms following stimulus onset and rarely persist beyond 1-2 s (*8*). However, the human experience is seldom that of stationary stimuli; rather we live and operate in a constantly changing environment. In this environment we encounter complex stimuli constituting episodes, series of variant multimodal representations linked in temporal succession. It is such temporally sequenced information that is processed by the human MTL (*12*, *13*), and later becomes available for conscious recollection. For this reason we set out to examine how neurons in the MTL respond to cinematic sequences depicting specific episodes, and later when these episodes spontaneously come to mind in the absence of external stimuli, in a free recall situation that can be reported by individual subjects

Subjects were patients with pharmacologically intractable epilepsy with implanted depth electrodes to localize the focus of seizure onset. For each patient, the placement of the depth electrodes was determined exclusively by clinical criteria (*14*, *15*). Patients first participated in a *viewing session* in which they viewed a series of audiovisual clips lasting 5 to 10 s each. Each clip depicted an "episode" featuring famous people, characters or animals engaged in activity, or landmarks described from various views, and was presented 5-10 times in a pseudorandomized order (*15*). After the viewing session, patients performed an intervening task (1-5 min; (*15*)) after which they were asked to freely recall the clips they had seen and verbally report immediately when a specific clip "comes to mind" (*free recall session*). Patients spontaneously recalled a mean of 83.2% (±5% SEM) of the clips presented.

Thirteen patients participated in a total of 43 viewing and recall sessions. We recorded from a total of 857 units (441 single units and 416 multi units; (*15*)) in the MTL and the medial frontal cortex (table S1). A unit was considered responsive to a specific clip if it showed a consistent elevated pattern of firing in all trials of that clip. Overall, the majority of recorded neurons, 475 units (54.9%), showed a significant response to one or more of the clips, i.e., consistently increased firing rate in at least one 500 ms segment of clip presentation (*15*). There were no differences in proportion of responsive units among the various regions sampled in this study (Table S1, P = N.S., $\chi^2(5) = 7.6$). Of the responsive units, 46 (9.7%) showed a sustained response to at least one clip, i.e., a significant elevation of firing rate through most of the clip duration (though not necessarily at a fixed level;

(*15*)). Interestingly, 44 of these cells were in MTL and only two in medial frontal lobe ($P < 0.03$, $\chi^2(1) = 5.2$; table S1, fig. S1). Twenty of these cells maintained their elevated firing rate at least 1 s beyond clip offset, and in some cases up to 5 s beyond clip offset. Responses observed were as long as 12 s and were usually attenuated only by the onset of the next clip.

For example, a single unit in the right entorhinal cortex of a patient, presented with a selection of 48 different clips, responded in a sustained manner to an episode from the animated TV series *The Simpsons* (Fig. 1A). Each time this clip was shown, the firing rate was elevated to an average of 15.57 Hz, compared with 2.11 and 2.23 Hz during other clips and blank periods respectively ($P < 10^{-9}$, two sample t-test). The response persisted for the entire 5 s duration of the clip, continued in some of the trials up to 5 s following clip offset, and appears to be silenced only by the onset of a different clip (Fig. 1B; see also movie S1a).

The neuron did not respond exclusively to this *Simpsons* episode. Even within the limited selection of 48 clips there was a considerably weaker, yet significant response to another clip, an episode from the TV sitcom *Seinfeld*. Of the 46 units with sustained responses, a unit responded in a sustained manner to an average of 1.4 ± 0.1 SEM clips (or average of 5.7% ± 0.5 SEM of clips presented). For another example see fig. S2.

In a second example, a neuron in left anterior hippocampus responded with elevated firing rate throughout a single clip from a choice of 48 clips - that of the actor Tom Cruise during an interview (Fig. 2, A and B; movie S2a). Note that this cell also exhibits shorter, transient (*15*) neuronal responses to other clips, i.e., consistent elevation of firing rate above baseline only during *particular segments* of the clip (green boxes in Fig. 2A), possibly reflecting the preference of the cell to a specific feature of the clip or to an episode within the clip. Additional examples are shown in figs. S3 and S4. Overall, responsive units significantly responded in a sustained or transient manner to an average of 17.7% ± 0.7 SEM of the clips presented (or 4.0 ± 0.2 SEM clips) (fig. S5).

We next examined the neuronal firing at the time of free recall when no external representation of the stimulus is present, no external cue is provided, and no external constraint is placed upon the recall process. We found that the neurons that responded during viewing of a particular clip also responded during recall of that clip, with a robust elevation of firing rate for several seconds which could be detected during a *single* recall trial. This is illustrated for the entorhinal cortex neuron which responded selectively during viewing of a 5 s video clip from the cartoon *The Simpsons* (Fig. 1, A and B): when all 16 clips were freely recalled, the maximal firing rate was obtained in conjunction with recall of *The Simpsons* episode (Fig. 1C). The unit's firing rate rose to more than 3 SD above baseline (*15*) about 1500 ms *prior* to onset of the verbal report of recall, peaked about 100 ms prior to verbal report onset, but returned to baseline only after 10 s or more (see also Supplementary Movie S1b). Similar examples are illustrated in Figure 2 and movie S2b, and in figures S6 to S10.

This recurrence of selective activity during recall was not an isolated observation found in a few neurons, but was also evident when the population of responsive units was examined as a whole. We calculated the average ratio of firing rate during viewing of clips to baseline firing rate (FR-ratio; (*15*)), for all responsive entorhinal cortex and hippocampal units (Fig. 3A, upper panel). The FR-ratio during viewing of the "preferred clip" ((*15*); red) is compared to the average FR-ratio across all other clips with nonsignificant responses in the same viewing session (grey). The composite graph shows a marked elevation of the ratio following onset of the preferred clip, during viewing, and notably also 3 s after clip offset. The FR-ratio histogram for the recall is then shown in the lower panel of Fig. 3A, averaged across the same units with respect to the onset time of verbal report of recall (*15*). During recall of the preferred clip (red), the averaged firing rate of the neurons increased significantly above baseline in the 3 s *prior* to onset of verbal report of recall and remained significantly above baseline in the ensuing 2 s ($P < 0.05$, Student's t-test; (*15*)). These neurons remained at baseline firing during recall of clips which did not elicit significant responses during viewing (grey). This recurrence during free recall, of the same selective neuronal responses present during viewing, was found in the population of hippocampal and entorhinal units but *not* in medial frontal units (compare bottom panels of Fig. 3, A and B). These frontal units exhibited a significant selective increase in firing rate during viewing but not during recall (Fig. 3B, upper and bottom panel, respectively). This episode-specific reactivation phenomenon was weak in amygdala and absent from parahippocampal gyrus (fig. S11), but was particularly striking for hippocampal and entorhinal neurons with sustained responses (Fig. 3C). We also noted reactivation of inhibitory responses, but because of the low baseline firing rate, these inhibitory responses were evident only at the population level (fig. S12). This phenomenon was most prominent in entorhinal cortex cells. For further details see Supporting Text.

In conclusion, we report here a subset of neurons in the human hippocampus and entorhinal cortex that exhibited highly reliable and specific responses during viewing of video episodes. These responses persisted throughout an episode or appear during specific segments. The same neurons showed an increased firing rate again with free conscious recall, *prior* to the verbal report, when the sequence of physical sensory stimuli is absent and no external cues are provided. This

recurrence during recall of specific past neuronal activity was not observed in medial frontal cortex sites. However, it is possible that top-down early recall signals do originate in other frontal or temporal lobe regions not sampled in this study (*16–20*).

Could the findings reported here be attributed to the neurological pathology of the patients? Although these results should be viewed with caution, such interpretation is unlikely for various reasons. Epileptic activity is characterized by highly correlated activity in large groups of neighboring neurons. The neuronal responses reported here were extremely sparse and seen selectively in individual neurons out of dozens of non responsive neurons that were recorded in their immediate vicinity. Furthermore, only 27% of the units were recorded from within the epileptogenic seizure foci. No significant difference was detected when these units were excluded from the analysis (see Supporting Text for more details).

The responses to episodes observed here were often remarkably selective and relatively sparse; yet it is clear, even by a simple statistical reasoning, that these neurons must display selective responses to multiple other clips which had not been presented, as only a minute fraction of the vast set of possible episodes was tested in our study (*9*). Whether multiple clips to which a neuron responds may be related by some abstract association rule is not clear at present. However, some intriguing examples in our data suggest that such rules may exist (see Supporting Text and figs. S2, S9, S13 to S15). It is also important to exercise caution in claims as to what exact aspect of the clips the cell responded to. The critical point however is that the same selective responses recur during free recall.

Several neuroimaging studies show that brain activity present during the learning of information, as indirectly measured by the BOLD signal, is reinstated during cued or free recall (*21–24*), although spatio-temporal limits of fMRI restrict the possible conclusions. Selective increases in single unit activation during mental imagery of stationary stimuli has been reported (*25*). However, unlike the situation in our study, subjects were cued by an external, content specific, sensory stimulus.

The sparse neuronal responses rising from a very low baseline to robust firing during a specific episode is reminiscent of the responses of hippocampal place cells in rodents (*26*), in which, a cell responds whenever the animal is in a particular place in the environment. Internally generated replay of previous firing sequences by hippocampal neurons has been reported in rodents, mostly during sleep and rest states following locomotion (*27–29*), but also during the awake state (*30*, *31*) and at decision points of a spatial memory task (*32*) where it might be predictive of the animal's future choice (*33*). However the relationship of such replay in rodents to recall of past navigation events has been merely conjectural. Our results from conscious human patients, who can spontaneously declare their memories, now directly link free recall and neuronal replay in hippocampus and entorhinal cortex. The hippocampal and entorhinal machinery used in spatial navigation in rodents may have been preserved in humans but put to a more elaborate and abstract use (*5*, *34–36*).

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1164685/DC1
Materials and Methods
Figs. S1 to S15
Table S1
References
Movies S1 and S2

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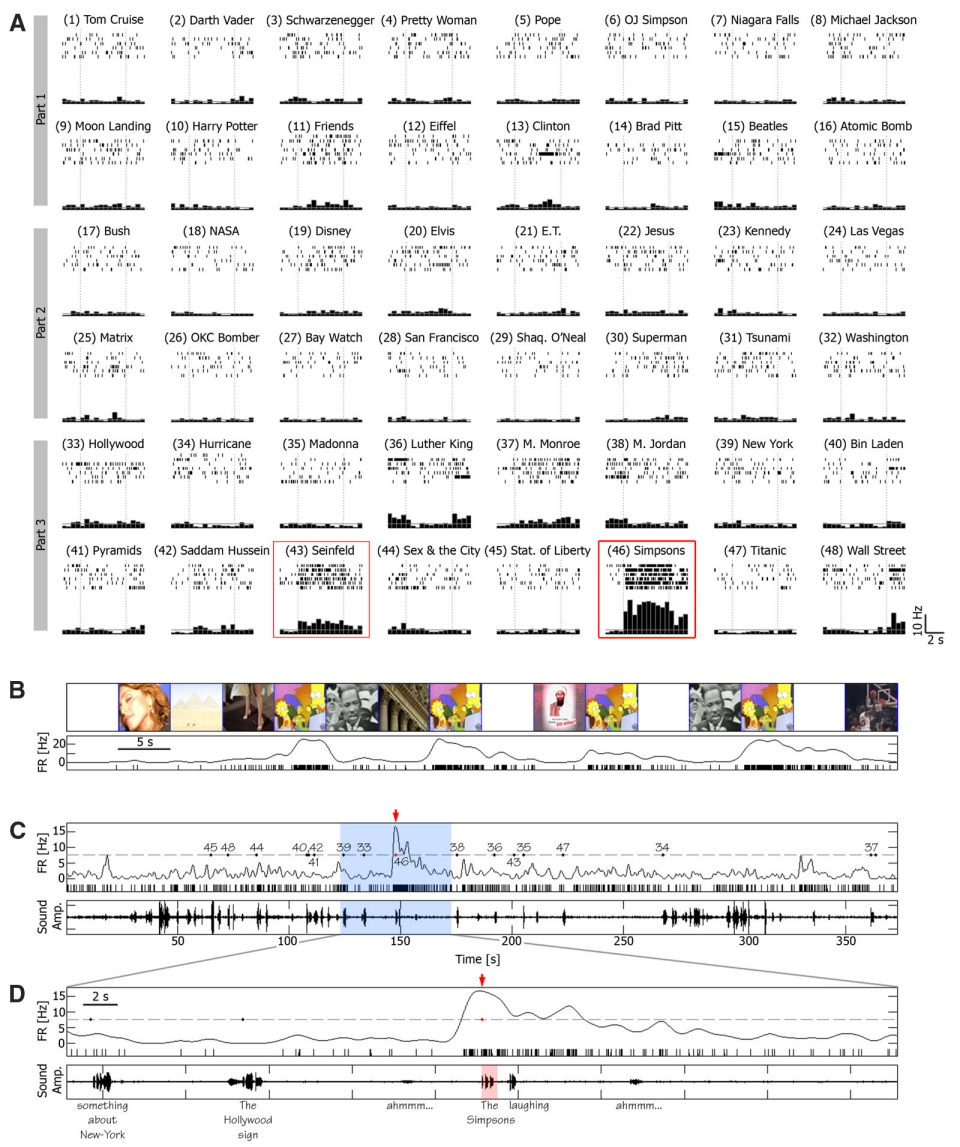
Fig. 1. A single-unit in the right entorhinal cortex is activated during viewing and recall of an episode from the TV series *The Simpsons*. (**A**) Cell responses to a selection of 48 different episodes (movie-clips) presented to the patient in three different viewing sessions (parts 1-3). For each clip, the corresponding raster plots (6 trials, order of trial number from top to bottom) and post-stimulus time histogram (500 ms bins) are given. Vertical dashed lines indicate clip onset and offset (5 s apart); 5 s blank periods were presented occasionally within groups of successive clips and were used to calculate the baseline firing rate, denoted by a grey horizontal line. Red boxes indicate sustained responses. (**B**) Trial by trial response of the neuron. Order of clips is for illustration purpose; more intervening clips separated successive *Simpsons* clips in the actual experiment. Spike raster plot and instantaneous firing rate (spike train convolved with a Gaussian of FWHM = 1200 ms) are displayed together. (**C**) Free recall session that followed the third viewing session (part 3). Sound amplitude of patient voice is shown in bottom panel; a spike raster plot and instantaneous firing rate are shown in upper panel; grey dashed line denotes the average firing rate during the recall session + 3 SD; numbered dots denote onset time of verbal report of recall events, corresponding to clip numbers in (A). Note the distinct elevation of firing rate just before the patient reported the recall of the *Simpsons* clip (red arrow). (**D**) A50 s window around the *Simpsons* recall event (blue area in (C)). Patient words are denoted below the bottom panel. Note that the cell's firing rate rose significantly above baseline already 1500 ms prior to onset of verbal report of the *Simpsons* clip, and returned to baseline after more than 10 s.

Fig. 2. A single-unit in the left anterior hippocampus is activated during viewing and recall of an episode (conventions as in Fig. 1) (**A** and **B**) Note the sustained elevation of firing rate during the episode depicting actor Tom Cruise on an interview on the *Oprah Winfrey Show* (red box). Note also the transient responses to various clips (green boxes). (**C** and **D**) Free recall session that followed the first viewing session (part 1). Note that the burst of spikes that accompanied the recall of the Tom Cruise clip began 1500 ms before onset of verbal report ("Tom Cruise…On Oprah").

Fig. 3. Average FR-ratio histograms during viewing and during free-recall. (**A**) (upper panel) Ratio of firing rate during viewing of clips to baseline firing rate is averaged across all responsive hippocampal and entorhinal cortex cells (n=154). Vertical dashed lines denote clip onset and offset. Cells increased their firing rate significantly above baseline during and following viewing of their preferred clip ((*15*); red bars; note small elevation prior to clip onset, probably attributable to anticipatory effect). These cells remained at baseline firing rate (FR-ratio = 1) during viewing of other clips ((*15*); grey bars). (**A**) (bottom panel) FR-ratio during recall events is averaged across the same cells from upper panel. Traces were aligned on the onset time of verbal report of recall (zero time, vertical dashed line). Note that the same cells increased their firing rate significantly above baseline in the 3 s *prior* to onset of verbal report of their preferred clips (red bars) and maintained this elevated firing rate in the ensuing 2 s. These cells remained however at baseline during recall of clips that did *not* elicit significant responses during viewing (grey bars). Stars denote statistical significance of $p<0.05$ (t-test, (*15*)). (**B**) Same as (A) but for cells from

anterior cingulate (n=56). Note that in contrast to hippocampal and entorhinal cortex cells, although these cells exhibited selectivity during viewing (upper panel), this selectivity was *not* maintained during free recall (bottom panel). (**C** and **D**) same as (A) but for sustained and transient responses separately. H – hippocampus; EC – entorhinal cortex; AC – anterior cingulate.


A
Part 1
Part 2
Part 3
(1) Tom Cruise
(2) Darth Vader
(3) Schwarzenegger
(4) Pretty Woman
(5) Pope
(6) OJ Simpson
(7) Niagara Falls
(8) Michael Jackson
(9) Moon Landing
(10) Harry Potter
(11) Friends
(12) Eiffel
(13) Clinton
(14) Brad Pitt
(15) Beatles
(16) Atomic Bomb
(17) Bush
(18) NASA
(19) Disney
(20) Elvis
(21) E.T.
(22) Jesus
(23) Kennedy
(24) Las Vegas
(25) Matrix
(26) OKC Bomber
(27) Bay Watch
(28) San Francisco
(29) Shaq. O'Neal
(30) Superman
(31) Tsunami
(32) Washington
(33) Hollywood
(34) Hurricane
(35) Madonna
(36) Luther King
(37) M. Monroe
(38) M. Jordan
(39) New York
(40) Bin Laden
(41) Pyramids
(42) Saddam Hussein
(43) Seinfeld
(44) Sex & the City
(45) Stat. of Liberty
(46) Simpsons
(47) Titanic
(48) Wall Street
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Sound Amp.
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The Hollywood sign
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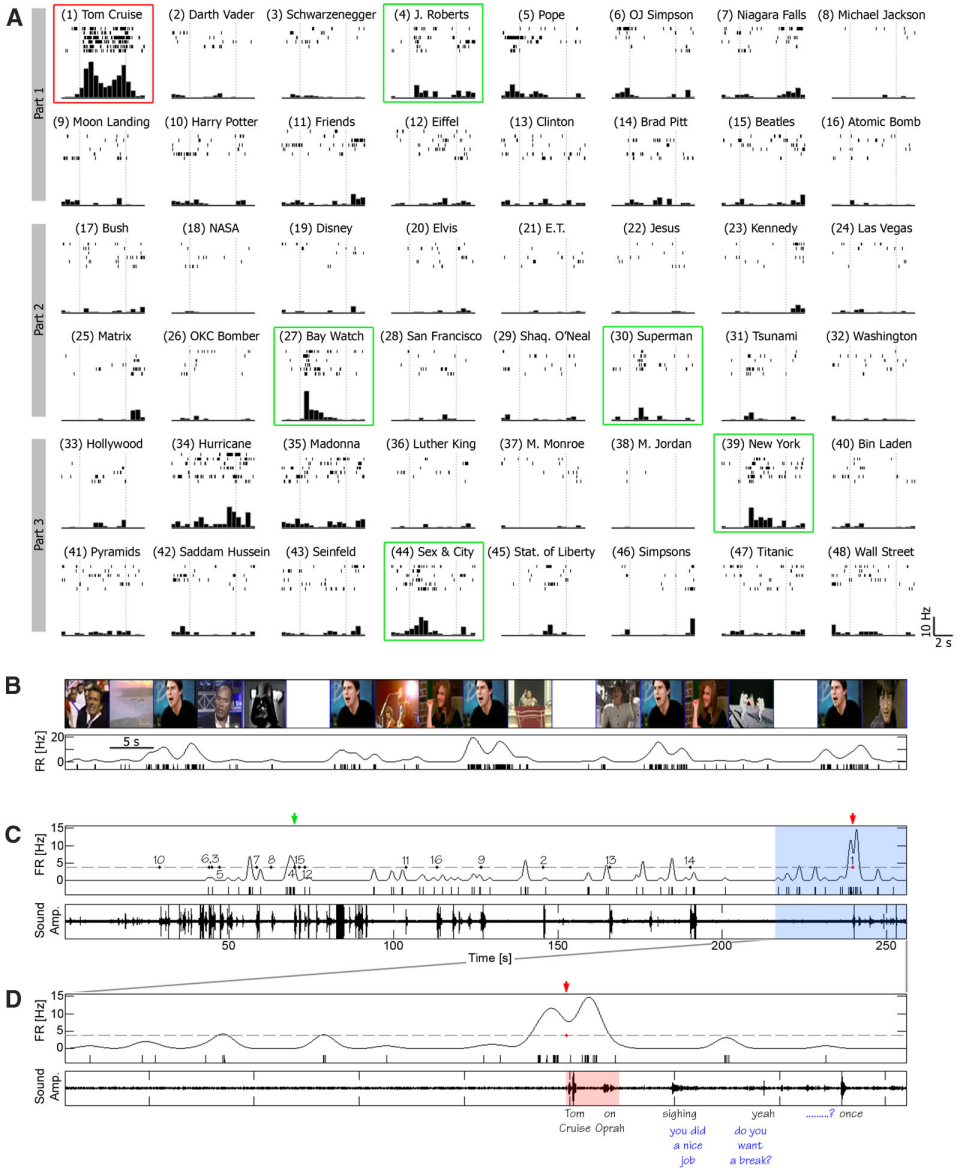
The Simpsons
laughing
ahmmm...


A
Part 1
(1) Tom Cruise
(2) Darth Vader
(3) Schwarzenegger
(4) J. Roberts
(5) Pope
(6) OJ Simpson
(7) Niagara Falls
(8) Michael Jackson
(9) Moon Landing
(10) Harry Potter
(11) Friends
(12) Eiffel
(13) Clinton
(14) Brad Pitt
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(16) Atomic Bomb
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(22) Jesus
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(24) Las Vegas
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(32) Washington
Part 3
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(36) Luther King
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(38) M. Jordan
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(40) Bin Laden
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(44) Sex & City
(45) Stat. of Liberty
(46) Simpsons
(47) Titanic
(48) Wall Street
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on Oprah
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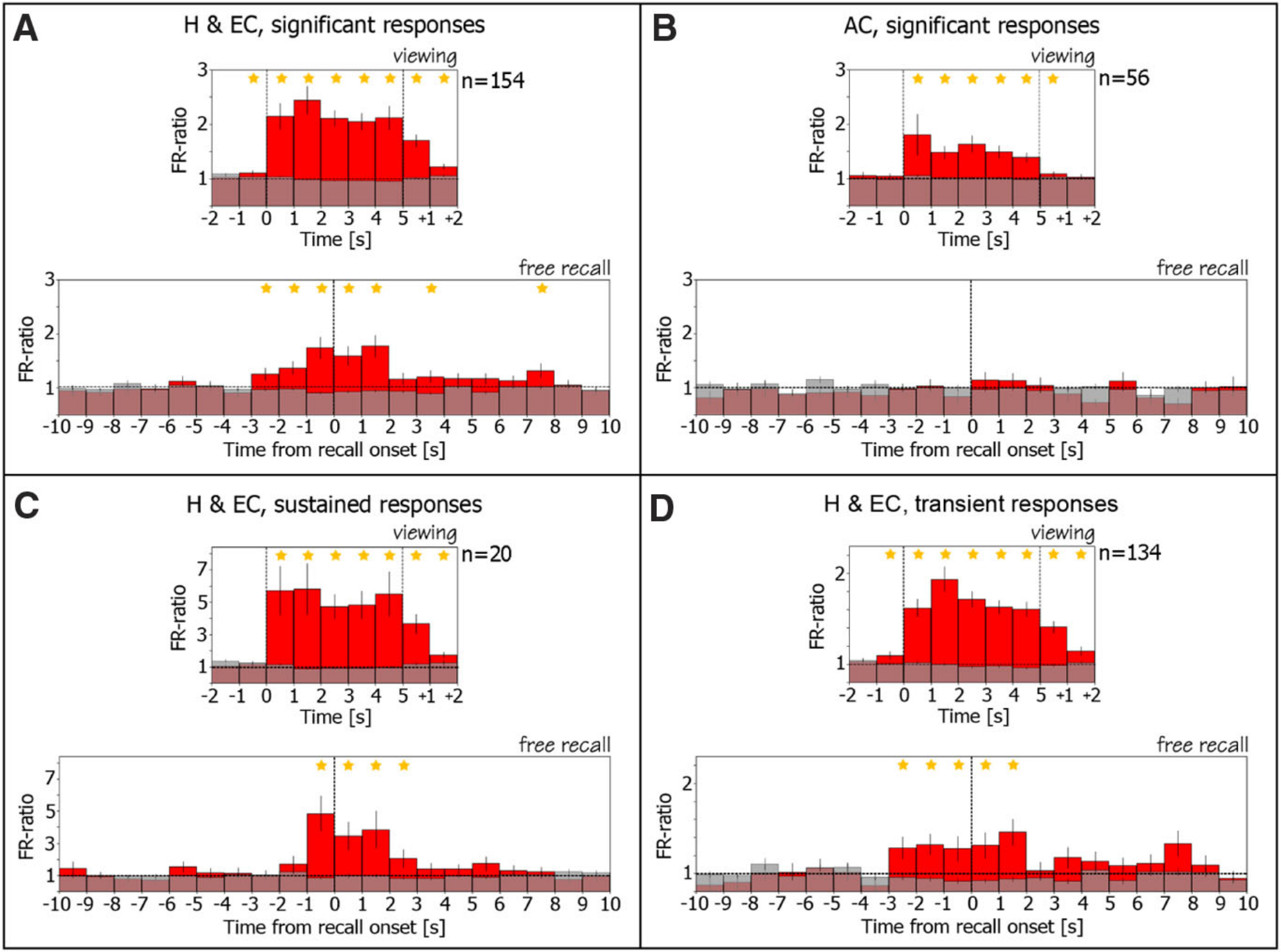
you did a nice job
yeah
do you want a break?
.....? once


A
H & EC, significant responses
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FR-ratio
Time [s]
free recall
Time from recall onset [s]
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AC, significant responses
viewing
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free recall
C
H & EC, sustained responses
viewing
n=20
free recall
D
H & EC, transient responses
viewing
n=134
free recall
preferred clip
all nonsignificant clips
overlap
p<0.05