Reactivation of Complex Events and Preactivation in Humans

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Abstract: The hippocampus' role in memory integration is starting to be understood. However, the exact mechanisms by which existing memories affect newly acquired information remains a matter of debate. We present two approaches to investigate this topic. It has been demonstrated that reactivation during off-line periods is important for memory integration. In previous studies, reactivation of relatively simple stimuli had been investigated but evidence for reactivation of complex events has been lacking. For our first experiment, we use multivariate pattern analyses to assess reactivation of complex events. Analyses of the resting-state blocks of an fMRI memory integration paradigm with complex life-like stimuli showed no evidence for reactivation. We discuss methodological limitations that could explain these results.

In rodents it has been found that not only reactivation of previously encoded information, but also *pre*activation of to be encountered information can be beneficial for memory integration. Contrary to reactivation which refers to experiences in the past, preactivation refers to activation corresponding to events in the future. To date, preactivation had not been assessed in humans. In our second experiment, we present a novel behavioral reaction time paradigm to assess preactivation. The results strongly suggest that humans show preactivation of to be encountered events. For future research, we suggest an fMRI version of the preactivation paradigm used here to shed light on the hippocampus' role in preactivation.

Keywords: hippocampus, memory integration, reactivation, preactivation

Decades of research have shown that the hippocampus is the core brain hub supporting memory formation (Burgess, Maguire, & O'Keefe, 2002; Squire, 1992). Only recently, we began to understand the hippocampus' role in *memory integration*, but key questions are still unanswered (e.g. Collin, Milivojevic, & Doeller, 2015; Milivojevic, Vicente-Grabovetsky, & Doeller, 2015; Schlichting & Preston, 2015; Shohamy & Wagner, 2008; Zeithamova, Schlichting, & Preston, 2012). Memory integration reflects the ability to make inferences about the relationship between events that are not experienced together and it is seen as one of the hallmarks of higher-level cognition. To illustrate, you might decide on a sunny morning to leave your bedroom window open before you leave the house. Later that morning, while listening to the weather forecast on the radio, you learn that heavy rain is predicted for that afternoon. Due to your ability to integrate these two events, you may decide to go home to close your windows in order to prevent your room from getting wet.

The above example demonstrates that memory integration is required continuously throughout daily life, in order for us to connect events that are not experienced together. Theories suggest that when we experience a novel event (i.e. hearing the weather forecast), related memories become reactivated (i.e. leaving the bedroom window open) through pattern completion mechanisms in the hippocampus (Preston & Eichenbaum, 2013; Zeithamova et al., 2012). The reactivation of related memories is thought to facilitate inferences about the relationship between events (Schlichting, Zeithamova, & Preston, 2014), which in turn facilitates memory integration and learning (Schlichting & Preston, 2014). Schlichting and Preston (2015) argue that reactivation of related memories is subserved by the hippocampus, while the medial prefrontal cortex (mPFC) may bias the reactivation to behaviorally relevant memories by selecting specific task-relevant memories for reactivation (Schlichting & Preston, 2015). Empirical evidence supporting the idea that hippocampal-mPFC circuits underlie our ability to link related events was recently obtained in our lab. While in an fMRI scanner, participants viewed seemingly unrelated events during the first phase of a memory integration paradigm. In the next phase, these events could either be linked or not. By adopting a multivariate approach representations of brain activity could be compared between the two conditions. For events that had been linked, increased neural similarity in the hippocampus and the medial prefrontal cortex (mPFC) was found, while neural activity for events that had not been linked became more dissimilar (Milivojevic et al., 2015).

Reactivation does not only seem to be relevant for relating different events to one another, but also for relating different places to one another, for example, when learning a certain route. Evidence from single-cell recordings in the rat hippocampus revealed that specific cells become active only at certain positions in the environment, thereby representing the animal's position (O'Keefe & Dostrovsky, 1971). When the rat covers a certain trajectory, the 'place cells' representing different positions across that trajectory become active in a corresponding temporal order (Lee & Wilson, 2002). Importantly, the same place cells that were activated while the rats crossed the trajectories, also became active in the exact same order during subsequent periods of sleep (Lee & Wilson, 2002; Wilson & McNaughton, 1993) and this reactivation was behaviorally relevant for subsequent memory recall (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010). These findings support the idea that memory reactivation not only important for memory *integration*, but also for memory *consolidation*; the process through which memories become stabilized for later retrieval (Tambini & Davachi, 2013). Reactivation is called 'replay' in animal research, where single-cell recording are commonly used. Replay occurs not only during sleep (Ji & Wilson, 2007; Skaggs & Mcnaughton, 1996), but also during periods of wakeful rest (Carr, Jadhav, & Frank, 2011; Gupta, van der Meer, Touretzky, & Redish, 2010; Karlsson & Frank, 2009).

There are only a limited amount of studies investigating reactivation in humans, and only relatively simple stimuli have been used in these studies (Deuker et al., 2013; M. L. Schlichting & Preston, 2014; Tambini, Ketz, & Davachi, 2010). Deuker and colleagues used pictures of mostly objects and faces on a plain black background and showed that reactivation of stimulus-specific activity patterns occurred during periods of sleep and awake rest. Furthermore, they showed that the amount of reactivation predicted performance on a subsequent associative memory task (Deuker et al., 2013). This shows that - at least for simple stimuli – reactivation occurs in humans and that this is a behaviorally relevant process.

A process similar to reactivation also seems to be important in generating predictions about how events are related and thereby for integrating related events. Empirical evidence from animal research using single-cell recordings in the rat hippocampus, demonstrated that during rest periods place cells do not only replay previously experienced trajectories, but also show activation corresponding to novel trajectories that were never experienced (Dragoi & Tonegawa, 2012; Dragoi & Tonegawa 2013). In this process, called 'preplay' in animals, the hippocampus seems to combine previously encoded information to come up with hypotheses about possible trajectories in the environment, which enables fast learning and integration thereafter. For example, if an animal learned a certain route from A to B, preplay may enable the animal to combine learned information to come up with a more efficient trajectory (i.e., a shortcut). The activation pattern corresponding to this shortcut is observed during rest periods, *before* the animal experiences this shortcut. Thus, preplay seems to be relevant when integrating information to form

hypotheses about possible trajectories in the environment. We expect a similar mechanism to be involved in forming predictions about the relationship between events. To illustrate, suppose you are at a party and you are introduced to a man named Mr. Johnson. Later that evening you are introduced to a woman with the same surname. Based on that information you may hypothesize that the man and woman are married, or alternatively that they are siblings. Here, we argue that specific patterns of neural activity in the hippocampus underlie your ability to come up with predictions about how the man and woman are related to each other, *before* finding out what their actual relation is. The imaging methods used in humans do not allow us to look at the sequences of cell activation directly, and therefore we will refer to this process as preactivation rather than preplay. Contrary to *re*activation that refers to experiences in the past, *pre*activation refers to activation corresponding to events in the future. To our knowledge, the current study is the first to assess preactivation in humans.

Taken together, the above findings open up the exciting possibility that the hippocampus' role in memory is not a passive reactivation of earlier experiences, but instead an active linking process in order to come up with predictions about the future. In this view memories become the building blocks for predicting the future.

In the current study we present two approaches to test the idea of the hippocampus as an active 'brainstormer' about the future. Human experiences are rather complex. We therefore expect that reactivation observed for simple events, also should be present for complex events. To test this first hypothesis, we will use multivariate pattern analyses to establish whether complex events are indeed reactivated in the hippocampus. This will be accomplished by analyzing resting-state brain data collected in a separate study from our group that used a memory integration paradigm consisting of complex real-life stimuli as mentioned above (Milivojevic et al., 2015). Specifically, we expect to find higher correlations between the task-related activity pattern and the *post* resting-state block and lower correlations between the task-related activity pattern and the *pre* resting-state block. In addition, we expect that preactivations can be used to form novel predictions about the relationships between events that were not yet experienced. To test this second hypothesis, we developed a novel behavioral reaction time paradigm. This paradigm enables us to test people's ability to form hypotheses about relationships between events, before such relationships are actually experienced. If there is indeed preactivation, we expect to see faster responses for stimuli that fit people's initial believes about the relationships between events, as compared to stimuli that do not fit with people's believes.

Experiment 1

Method

The resting-state data presented here, used to test if there is reactivation of complex events, were acquired in a study with a separate research question that was published elsewhere (see Milivojevic et al., 2015). This section is a summary of the method of that study, including only information that was relevant for the current research question.

Participants.

The final sample for this study consisted of nineteen right-handed participants (6 male) between 18 and 29 years old ($M = 22.95$, $SD = 3.12$) with normal or corrected-to-normal vision. All participants gave written informed consent according to the local ethics committee (CMO region Arnhem-Nijmegen, NL).

Procedure and Materials.

Six animated stories were created using The Sims 3 life-simulation game. Each story was comprised of three 5 second events that formed a narrative. In a typical example, event "A" showed a grandfather eating soup, event "B" showed a child playing with a doll and in event "L" (labeled L for "link") the grandfather was putting the child to bed. In addition, there was an unrelated control event "X" to control for effects due to temporal co-occurrence. In the typical example, event X depicted a young person watching television. Event L was presented only during the second phase of the experiment and it could link event A and B, but not event X, together into a coherent narrative. Participants were not aware of assignment of events to conditions.

To control for nonspecific stimulus effects and spurious visual similarity, there were two versions of every narrative. Event A was always the same, but events B and L were different. Event X in the first version was event B in the second version and vice versa. In the example, the alternative event L showed the youngster feeding the child, thereby linking the playing child (event A) and the youngster watching television (here, event B) together, whereas the grandfather eating soup was now control event X. Thus, all participants saw the same events A, B and X, but half of the participants saw one version of event L (e.g. grandfather putting the child to bed) and the other half saw the alternative version of event L (e.g. youngster feeding the child). See figure 1 for an overview of the experimental procedure (adapted with permission from Milivojevic et al., 2015).

Figure 1

In the fMRI scanner, each narrative was presented in three phases: pre-insight, linking and post-insight. In the pre-insight phase participants saw events A, B and X six times in a pseudorandom order. In the linking-phase, event L was presented six times. The post-insight phase was the same as the pre-insight phase, but events were presented in a new pseudorandom order. After participants completed the three phases of one narrative, the experiment moved on to the next narrative that was presented in the same fashion, until all six narratives had been shown.

Crucial for the current study, inside the scanner the experiment was preceded with a pre resting-state block of seven minutes before the beginning of the task. During this block, participants were instructed to lie still and focus on the fixation cross that was presented in the middle of the screen. At the end of the experiment there was a post-resting state block of equal length with the same instructions. In the current study, we will analyze these resting-state blocks in search of reactivation of complex events.

Data analysis.

Image acquisition.

Imaging data was acquired on a 3T Siemens TIM Trio scanner using a 32-channel head coil. A custom 3D echo-planar imaging (EPI) pulse sequence was used with the following parameters: volume TR = 1800 ms; time echo (TE) = 25 ms; flip angle = 15°; volume resolution = 2 mm3 ; field of view (FOV) = 224x224x112 mm; slab orientation = -25° pitch rotation; 3D acceleration factor = 2. The pre and post resting-state blocks consisted of 234 volumes (7.02 minutes) each. The structural T1-weighted image was acquired using an MPRAGEgrappa sequence with the following parameters: TR = 2300 ms; TE = 3.03 ms; flip angle = 8°; inplane resolution = 256x256 mm; number of slices = 192; acceleration factor PE = 2; voxel resolution = 1 mm3 , duration = 321 seconds.

Preprocessing.

Image preprocessing and analysis were performed using Automatic Analysis Toolbox (https://github.com/rhodricusack/automaticanalysis), which uses custom scripts combined with core functions from SPM8 (www.fil.ion.ucl.ac.uk/spm), FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) and FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). To improve image quality we bias-corrected the structural images (Ashburner & Friston, 2005) and then de-noised the structural images with an optimized non-local means filter (Manjon, Coupe, Marti-Bonmati, Collins, & Robles, 2010) before segmentation into grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF). Next, we realigned the functional images and co-registered them to the structural images with standard procedures in SPM 8. Subsequently, normalization parameters were calculated. We then used these parameters to create subject-specific regions of interest (ROIs) GM masks in native space.

Representational Similarity Analysis.

We used representational similarity analysis (RSA) to analyze the multivoxel pattern of neural activation during the pre and post resting-state blocks in four ROIs. Two ROIs were structurally defined and corresponded to the left and right hippocampi, based on the AAL template (Tzourio-Mazoyer et al., 2002). These areas were selected on the basis of our theoretical framework. The two remaining ROIs were functionally defined and corresponded to the posterior hippocampus (pHPC) and the medial prefrontal cortex (mPFC). We selected these functional ROIs because the strongest task effects in the study by Milivojevic and colleagues (2015) were found in these two regions. We used a sphere of 8 mm around the peak voxels. We used the beta images corresponding to our regressors of interest (events A, B, and X before and after the link was presented, and event L, i.e. seven regressors for each story) from a GLM on the data acquired while participants performed the task described above. For every ROI, we then calculated Fisher Z-transformed Pearson's correlation coefficients of multivoxel patterns as a proxy of

neural similarity between the regressors that represented the task activation pattern and each resting-state volume separately for both the pre and the post resting-state block. We then calculated the mean correlation for the seven event types (Apre, Bpre, Xpre, L, Apost, Bpost, and Xpost). These mean correlations were used in analyses of variance with IBM SPSS Statistics version 23. Based on our main hypothesis, we expected to find stronger reactivation and therefore higher correlations between the *post* block resting-state volumes and the task activation pattern as compared to the *pre* resting-state block. In addition, we expected to find higher correlations for integrated events A and B as compared to the non-integrated event X.

Since the reactivation effects are typically very subtle, we additionally analyzed the data in an analysis potentially more suitable to pick up on these subtle effects. Here, we sought the highest correlation for each post block resting-state volume separately and coded to which regressor (A, B or X) this maximum correlation belonged. With this binary coding of the data (maximum correlation: 1 = yes, 0 = no) any subtle effects in the data are magnified. Subsequently, we used an analysis of variance to test if the total number of maximum correlations (the total number of 1's) was higher for events A and B as compared to X.

Results

RSA in left and right Hippocampi (structurally defined).

We examined the correlations in the structurally defined ROIs – the left and right hippocampi – with a repeated measures ANOVA. We included resting-state block (pre, post), event type (A, B, X) and insight (pre, post) as within-subject variables. The effects of most interest to our research question are the main effect of resting-state block and the resting-state block x event type interaction. Both of these effects were non-significant in the left Hippocampus (F (1,15) = 0.290, Bonferoni corrected $p > 1$ and F (1.5,22.5) = 0.361, Bonferoni corrected $p > 1$, respectively) and in the right Hippocampus (F (1,15) = 2.112, Bonferoni corrected $p = .334$ and F (1.5,22.7) = 1.020, Bonferoni corrected $p = .712$, respectively). See figure 2 for a visual overview of these results. All other effects in this repeated measures ANOVA were also non-significant.

Figure 2

Binary analysis in left and right Hippocampi.

We used a repeated-measures ANOVA on the post-resting state data to test if the total number of maximum correlations was different for events A, B and X (see methods for detailed description of analysis logic). In the left Hippocampus, there was a significant difference between the events (F (2,30) = .021, Bonferroni corrected p = .042, η_p^2 .226). However, pairwise comparisons and the corresponding means indicated that on average there were significantly less maximal correlations for event B (M = 12.16) as compared to event A (M = 13.25, p = .050) and X (M = 13.59, p = .020). This is not in line with what we hypothesized. The difference between A and X was not significant (p = .461). Next, we repeated the same analysis for the right Hipocampus and found no significant difference in average maximal correlations for events A, B and X (M = 12.98, 13.19 and 12.83, respectively). See figure 3 for a visual overview of these results.

Figure 3

RSA in Posterior Hippocampus and mPFC (functionally defined).

We examined the correlations in the functionally defined ROIs – posterior hippocampus and mPFC – in a similar fashion. We conducted a repeated measures ANOVA with resting-state block (pre, post), event type (A, B, X) and insight (pre, post) as within-subject variables. Again, the effects of most interest for our study are the main effect of resting-state block and the resting-state block x event type interaction. Both of these effects were also non-significant in the posterior hippocampus (F (1,15) = 0.935, Bonferoni corrected p = .6981 and F (1,15.5) = 1.082, Bonferoni corrected p = .634, respectively) and in the mPFC (F (1,15) = 0.453, Bonferoni corrected p > 1 and F (1.7,26.1) = .828, Bonferoni corrected p = .866, respectively). See figure 4 for a visual overview of the results. In this analysis, all other effects were also non-significant.

Figure 4

Binary analysis in Posterior Hippocampus and mPFC.

Here, we again used a repeated-measures ANOVA to test if the total number of maximum correlations in the post-resting state data was different for events A, B and X. We found no significant differences in the posterior hippocampus (F (1.8,27.5) = 0.296, Bonferroni corrected p > 1) between events A, B and X (M = 13.23, 12.84, 12.96, respectively) or in the medial prefrontal cortex (F (2,30) = 0.006, Bonferroni corrected p > 1, M = 13.01, 13.03 and 12.96, respectively). See figure 5 for an overview of these results.

Figure 5

Discussion Experiment 1

In the first experiment we investigated whether the previous finding of reactivation for simple events, also could be observed for complex events. To our knowledge, this was the first study using complex events when investigating reactivation. Based on our results described, it can be concluded that we were not able to demonstrate reactivation for the complex life-like events used in this experiment.

There are two possible explanations for the absence of reactivation in the current experiment: either reactivation does not occur for complex events, or reactivation does occur for complex events but we failed to demonstrate this with the current design. We will first discuss the first possibility that there is no reactivation of complex events. Several studies have now demonstrated reactivation of simple stimuli (Deuker et al., 2013; Schlichting & Preston, 2014; Tambini et al., 2010). In those studies reactivation also seems to be behaviorally relevant because it enhances learning and memory processes. Given the complexity of human's daily life, it could be argued that it is even more relevant to learn and memorize complex events, as compared to simple stimuli in isolation. From this perspective it seems rather unlikely that only simple but not complex events are reactivated.

The second possibility is that there is reactivation of complex events but we were not able to demonstrate it. Complex events might be reactivated only under specific circumstances, which did not coincide with the resting-state blocks investigated here. It has been demonstrated that memory tends to decrease when people are tired (for a review see e.g. Maquet, 2001). The decrease in memory could be explained by a decreased availability of resources for reactivation, which in turn could lead to decreased consolidation. Participants sometimes find experiments, and fMRI experiments in particular, to be exhausting, and this could be a potential explanation to why we did not observe reactivation. It should be noted however, that immediate memory of the events was good, as indicated by high accuracy on a subsequent memory task (see supplementary material in Milivojevic et al., 2015). The memory scores could be considered as evidence against the idea that there were limited resources left for reactivation of the events.

In contrast to the idea that participants were possibly exhausted, another reason we failed to observe reactivation, could be the amount of times the events were repeated in the experiment; thus, no reactivation was necessary to facilitate integration and memory processes. This explanation is in line with the high memory performance.

Lastly, to date reactivation has only been observed during periods of sleep and rest (Deuker et al., 2013; Schlichting & Preston, 2014; Tambini et al., 2010). The resting-state blocks examined here could potentially be regarded as active states rather than resting states. Thus, since the study was not originally designed to assess reactivation, the task design might not have been optimal for this aim. Participants were instructed that they should lie still and look at a fixation cross on the screen during the resting-state blocks, which does not necessarily mean that participants were resting. In Schlichting and Preston's (2014) reactivation study participants were specifically instructed to keep their eyes closed and think about whatever they liked. Similarly, in the reactivation study by Deuker and colleagues (2013) participants were instructed to try falling asleep. Crucially, these instructions are rather different from the instructions given in our experiment. For future reactivation studies it may be important to specifically instruct participants to keep their eyes closed and relax.

Experiment 2

In the first experiment, we examined if reactivation of complex events could be demonstrated empirically, since identifying such activity patterns would strengthen the basis for our second hypothesis, that preactivation of events could be observed in humans. As discussed above, the design of the first experiment showed to be suboptimal to test reactivation, and the paradigm cannot be used to test preactivation processes. Therefore, we developed a novel behavioral paradigm specifically targeted at investigating preactivation, which will be described in the current section.

Method

Participants.

For this study, we recruited sixteen participants (1 male) between 18 and 33 years of age (M = 22.06, SD = 3.44). Two participants were excluded from further analyses due to poor behavioral performance (see data analysis section below). The final sample of seventeen participants were all right-handed and between 18 and 33 years old (M_{age} = 22.14, SD = 3.55). Participation in this study was voluntary and was compensated with money or study credit points. All participants gave written informed consent according to the local ethics committee (CMO region Arnhem-Nijmegen, NL).

Stimuli.

We used life-simulation video game *The Sims 3* to create screenshots of life-like events. Every story consisted of two events (event "A" and "B") that were presented on the screen simultaneously. Crucially, these events could be linked with a specific item, called the "link item". In a typical example, event A showed a man sitting on a sofa in front of a television and in event B the same event was shown but with the television turned on. The crucial link item in this case is a remote control. There were eight stories in total that were selected from a larger set that was used in the pilot phase. We selected the final stories because they yielded correct link item responses in more than 70 percent of individuals that participated in the pilot.

Unbeknown to the participants, we created an alternative version for every story in which the same event was depicted, but with a different person in a different context. Participants were randomly assigned to one of the two versions of the task. The different context was used during the behavioral task in Phase 2 to assess integration effects (see below).

To rule out semantic similarity as an explanation for our results, we included items that could semantically be linked to the story, but that could not serve as a link item. Such items will be referred to as control items. In the aforementioned example, the control item was a DVD player. This item is clearly related to the example story, but it cannot be used to turn on the television from the sofa. Based on our second hypothesis, we expected faster responses for link items because these items where *preactivated* during the first phase of the experiment. One could argue that faster responses for link items are not due to *preactivation*, but to mere semantic relatedness of the items with the scenes. Crucially, since both the link item and the control item are semantically related to the scenes, but only the link item can be used to link the scenes together, finding an effect on the link items but not on the control items, would rule out semantic relatedness as an alternative explanation.

Procedure.

Participants were seated in front of a computer on which we ran the experiment with Presentation software (version 18). Instructions were presented on screen in black letters on a light grey background. After initial instructions, participant saw two example stories to illustrate the task in Phase 1 of the experiment. The example stories were different from the stories that were used for the task. After the examples, participants were presented with two rounds in which event A and B were presented on screen simultaneously for 9 seconds, with 1 second break between each story. This was used to familiarize participants with the stimuli; no response was required. In the third and final round, participants were instructed to call out loud after each story which item they believed would best link event A and B presented on screen. See figure 6 for an overview of Phase 1.

Figure 6

On average participants indicated the correct linking item in seven out of eight stories (range 6-8). If an incorrect response was given, we excluded trials corresponding to those stories in the second phase (see below for explanation of this task) from further analyses.

Between Phase 1 and 2 there was a three minute break in which participants were instructed to relax and think of nothing special to enhance reactivation processes.

After the break Phase 2 two started, which consisted of a reaction time task. Here, participants were randomly presented with all the link items and the corresponding control items from Phase 1, one item at a time. Participants used their left and right index finger on a button box to indicate whether the item on screen had been a link item during Phase 1 or not. The meaning of the buttons i.e. yes or no, was written on a paper that was placed below the button boxes to avoid confusion. Left and right hand

responses were randomized across participants. Participants were instructed to respond as fast and as accurate as possible. After a response was made there was a 1 second break before the experiment automatically moved on to the next item.

Each item could be presented on one of three backgrounds. The first possibility was on a congruent background which was the same as the scenes that were used to elicit *preactivation* processes in Phase 1. In the aforementioned example the item would be presented in the same room, with the same sofa and same television as was used during the first phase. The second possibility was a neutral background which showed the item on a plain white background. Finally, the item could be presented on an incongruent background that corresponded to the alternative version of the task that participants had not seen before. In the example, the item would be presented in a room of similar size, but with different colors on the walls, floors and ceiling and with different furniture. See figure 7 for an overview of Phase 2. There were 10 repetitions for each story. Thus, participants responded 480 times in total (8 stories x 3 backgrounds (congruent, neutral, incongruent) x 2 item types (link, control) x 10 repetitions).

Figure 7

We concluded the experiment with a questionnaire in which participants indicated to what extend the items that were shown in Phase 2 corresponded to the items they imagined as the correct link items in Phase 1. Participants used a ten-point scale to indicate their answer. The minimum score "1" meant "this picture does not at all look like what I imagined during Phase 1", whereas the maximum score "10" meant "this picture matches exactly what I imagined during Phase 1". For the questions representing the link items we expected high scores. We also included the control items and on those questions, scores were expected to be low. The order of the items in the questionnaire was randomized. See figure 8 for an example question from that questionnaire.

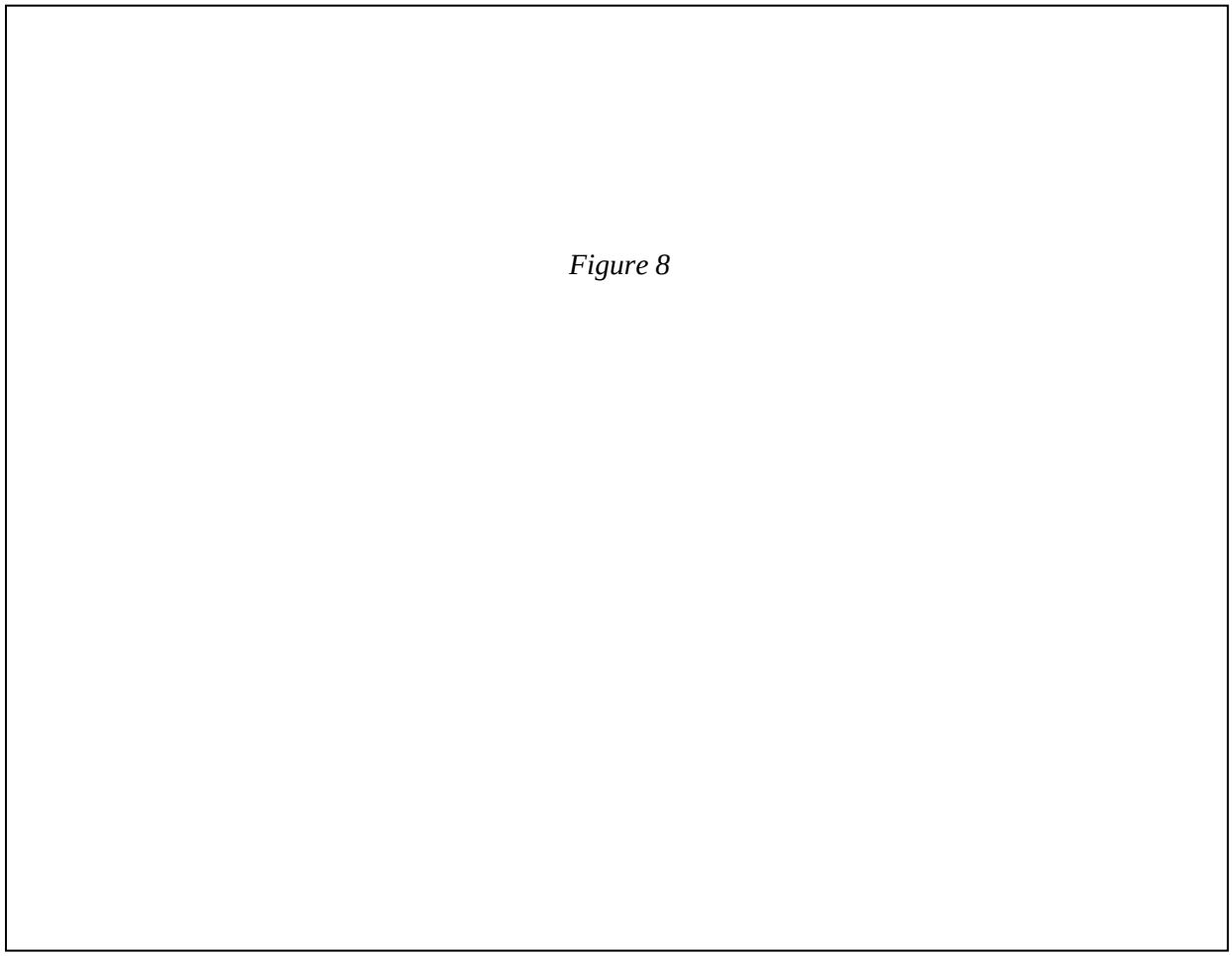

Figure 8

Data analysis.

Due to technical problems only seven out of ten repetitions could be used for analysis, which brought the total to 336 responses per participant. In addition, we included only accurate responses i.e. when participants pressed "yes" when presented with a link item and "no" when presented with a control item (M = 314.94, SD = 22.84 or 93.73 % correct). Reaction times shorter than 200 ms or longer than 2000 ms were also excluded (M = 6.69, SD = 14.41). This absolute cut-off was chosen before analyzing the data (Ratcliff, 1993). We then calculated six mean reaction times per participant: three for the link items in either one of the three contexts (congruent, neutral, incongruent) and the same for the control items. These means were subsequently used in a repeated measures analysis of variance. We tested if responses were faster on link items as compared to control items, which would be an indication of preactivation. In addition, we tested if responses were even faster for congruent contexts than for incongruent contexts (i.e. an interaction effect), which would indicate integration. We used IBM SPSS Statistics version 23 to analyze our results.

Two participants had to be excluded due to poor behavioral performance. We could not calculate mean response times for one of these participants due to a combination of a high number of inaccurate responses during both Phase 1 and Phase 2. This could indicate that this person did not understand the task. The second participant we had to exclude because her average response times were extremely long (>1 SD on half of the variables and >2 SDs on the other half of the variables).

Results

Phase 1.

After Phase 1, participants indicated the correct linking item on average on 7 (range 6 – 8) out of 8 trials in total. In the analyses below, only correct trials were included (see Method for additional information).

Phase 2 reaction times.

Repeated measures ANOVA.

We conducted a repeated measures ANOVA with item type (link, control) and context (congruent, neutral, incongruent) as within-subject factors, and reaction time as dependent variable. There was a significant main effect of item type ($F(1,13) = 6.830$, $p = .021$, $\eta_p^2 = .344$). The mean values showed that responses were faster for the link items ($M = 579.23$ ms) than for the control items ($M = 599.60$ ms), which is an indication for preactivation. In addition, there was a significant main effect of context ($F(1.7,21.7) = 14.084$, $p < .001$, $\eta_p^2 = .520$). Pairwise comparisons and the corresponding means indicated that this effect was driven by significantly shorter reaction times for the neutral context ($M = 565.37$ ms) as compared to both the congruent context ($M = 597.62$, $p < .001$) and the incongruent context ($M = 605.25$, $p = .001$). The difference between the congruent and the incongruent context was not significant ($p = .345$). Finally, the item type x context interaction effect was not significant ($p = .717$). See figure 9 for a visual overview of the results.

Figure 9

Non-parametric tests.

The sample size was rather small (N = 14) and the assumption of normality was somewhat violated. Therefore, we used non-parametric tests to reanalyze our results. We used a Wilcoxon signed ranks test to analyze the effect of item type (link, control). This effect was significant (Z = -2.103, p = .035). The median for the link items (Mdn = 563.28) was lower than for the control items (Mdn = 589.48). There is no non-parametric way of testing multiple effects in a single model. Therefore, the p-values for individual tests should be corrected for multiple comparisons. It should be noted that the effect of item type did not survive this correction (Bonferroni corrected p =.105). Subsequently, we conducted a Friedman test to assess the effect of context (congruent, neutral, incongruent). This effect was significant ($\chi^2(2) = 18.143$, Bonferoni corrected $p < .001$). Post hoc Wilcoxon tests indicated that the differences between the neutral and the congruent context (Z = -3.233, p = .001) and between the neutral and the incongruent context were significant (Z = -3.296, p = .001). The difference between the congruent and incongruent context was not significant (Z = -0.471, p = .638). Here, the median reaction times were as follows: Congruent: 582.52 ms, Neutral: 554.83 ms, Incongruent: 584.56 ms. Finally, we conducted a Friedman test on the link – control difference scores for the three contexts to analyze the interaction effect. This effect was not significant ($\chi^2(2) = 0.143$, Bonferoni corrected $p > 1$). Aside from the effect of item type not surviving the correction, these results are generally the same as those from the parametric analysis described above.

Questionnaire.

We used a paired samples T-Test to compare the scores on the questionnaire for link items and control items. The difference was significant (t = 38.879, p <.001, Cohen's d = 12.28). The mean score for the link items was 8.97, whereas the mean score for control items was 1.50. This indicated that the pictures of items we used during Phase 2 were a good representation of what people predict naturally as link items during Phase 1. Subsequently, we used separate T-Tests to compare corresponding link and control item pairs. All differences were significant (Bonferroni corrected p <.01 for all comparisons). For an overview of these results, see figure 10.

Figure 10

Control analyses.

We calculated the total number of good responses on the questionnaire as an indication of participant's performance level. Good responses were defined as scores 1-3 for link items and scores 8-10 for control items. Repetition of the repeated measures ANOVA as described above including performance level as a standardized covariate did not affect the results. This means all effects that were significant before were significant now and the effect that was not significant remained non-significant now. In addition, no significant interactions with performance level were observed. This demonstrates that our effects are consistent across different performance levels.

Discussion Experiment 2

In the second experiment we used a novel behavioral paradigm to assess preactivation processes. To our knowledge, this was the first study to assess preactivation in humans. Both the link and the control item were related to the scenes, but only the link item could be used to link the scenes together. The link item was therefore hypothesized to become preactivated, which in turn would lead to shorter reaction times. We found faster responses for the link items than for the control items. This result supports our hypothesis that that the link items were preactivated during the first phase of the experiment, that is, *before* participants actually saw the link items. This demonstrates that preactivation occurs in humans, at least as reflected in behavior. This finding not only translates the preplay findings observed in rats to humans, but may also provide an explanatory mechanism for the longstanding evidence that pre-existing memories are important for how new memories become represented thereafter (Squire, 1992).

The control items in our experiment were not only used to test preactivation; they we carefully chosen to rule out semantic relatedness as an alternative explanation for our results. Both the link and control items were semantically related to the scenes, thus any difference observed between link and control items cannot be attributed to semantic relatedness. Since reaction times on the link items were faster than on the control items, we can rule out semantic relatedness as an alternative explanation for our results.

We further hypothesized that preactivation would be closely related to memory integration. We postulated that the link items would become integrated with the stories eliciting the reactivation. However, the absence of interaction effects demonstrates that there was no facilitating effect from presenting the link item on the supposedly integrated scene. In fact, we observed no differences overall between supposedly integrated backgrounds as compared to supposedly non-integrated control backgrounds. One explanation could be that preactivation and integration are related but behaviorally independent process. However, there is compelling evidence suggesting that reactivation is an essential part of memory integration in humans (e.g. Kuhl, Bainbridge, & Chun, 2012; Rasch & Born, 2007, for a review see Schlichting & Preston, 2015) , and additional evidence suggesting that preplay is beneficial for relating places and behaviorally relevant paths in rats (Dragoi & Tonegawa, 2012; Dragoi & Tonegawa, 2013; Pfeiffer & Foster, 2013). Taken together, these findings suggest that it is likely that preactivation is important for the integration of events in humans. Future investigation of the preactivation paradigm from a neural perspective could shed more light on the question to what extend these processes are related.

An alternative methodological explanation for the absence of an integration effect could be due to that participants concentrated on the item and actively ignored the background in order to respond as fast as possible. Thus, the background could have been regarded as a distracter. Our finding that responses were fastest in the neutral condition, where items were presented on a white screen, is in line with this idea. Either presenting the background first and overlaying the item after a delay or adapting the stimuli such that the items are placed within the scenes (e.g. a remote control lying on the salon table) instead of presenting the item overlaid on top of the background as was done here, could circumvent this issue in future experiments.

The current study has a few limitations. As stated above, the stimuli could be optimized to better be able to assess integration in this experiment. It could also be argued that the stimuli used here were relatively simple, and not complex enough. However, to our knowledge this is the first paradigm developed to assess preactivation which motivated us to start with relatively simple stimuli, before progressing to more complex events. In the future, the stimuli could include even more complex stimuli such as the videos used in the experiment by Milivojevic and colleagues (2015). Another suggestion for future research is to decrease the amount of instructions to investigate to what extend preactivation emerges naturally. Finally, another limitation is the sample size, which was relatively small. Importantly, we were still able to find significant results, indicating that the sample size was big enough to assess most of the effect sizes observed here.

Our behavioral preactivation paradigm obviously does not allow us to investigate whether preactivation is indeed mediated by the hippocampus as hypothesized. We believe that question is best answered in an fMRI study adopting multivariate analyses techniques. We will describe our ideas for the future study in the concluding discussion below.

Summary and Concluding Discussion

In this study, the question whether there is preactivation of future complex events in humans was put central. We investigated two sub questions in order to answer our main question: (1) is there reactivation of complex life-like events during post resting-state blocks, and (2) can preactivation of events be demonstrated by adopting a novel behavioral reaction time task. In short, we found no evidence for our first hypothesis that reactivation occurs for complex life-like events. However, we argued that the absence of evidence could be explained by methodological shortcomings. Therefore, we developed a novel behavioral paradigm in order to be able to assess preactivation. The preactivation paradigm results provided strong evidence for our second hypothesis, that events are indeed preactivated in humans.

The two approaches we presented here serve as groundwork for a future fMRI experiment in which an adapted version of the preactivation paradigm will be used to assess the neural correlates of preactivation in humans. In this fMRI experiment. At the very end of the experiment, while still in the scanner, we would present both the link and the control items to train a neural classifier. The classifier will be used to investigate whether neural representations of the link - and not the control items - are already present during the resting-state blocks, *before* people get to see the items. If people indeed preactivate the link items, we expect to find stronger representations of the link items as compared to the control items. We expect to find strongest preactivation effects in the hippocampus. Such findings would support our general idea that the hippocampus acts as an active brainstormer, generating predictions about the future.

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