Title: Cued reactivation during slow-wave sleep induces connectivity changes related to memory stabilization.

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Abstract

Memory reprocessing following acquisition enhances memory consolidation. Specifically, neural activity during encoding is thought to be 'replayed' during subsequent slow-wave sleep (SWS). This natural tendency of memory replay can be induced by external cueing, known as "targeted memory reactivation". Here, we analyzed data from a published study (van Dongen et al. 2012), where auditory cues reactivated learned visual object-location memories during SWS. Memory replay during sleep presumably involves a shift in connectivity across the brain. Therefore, we characterized the effects of memory reactivation on brain network connectivity using graph-theory. We found that cue presentation during SWS introduced dynamic changes in network integration of the occipital cortex, a visual region that was also active during the object retrieval task. Importantly, enhanced network integration of the occipital cortex showed a behavioural benefit and predicted overnight memory stabilization. Furthermore, occipital cortex displayed enhanced connectivity with mnemonic regions, namely the hippocampus, parahippocampal gyrus, thalamus and medial prefrontal cortex during cue versus control sound presentation. Finally, network integration of early occipital cortex during cueing in SWS was related to increased activation of the bilateral parahippocampal gyrus, a region involved in coding for spatial associative information, at the post-sleep test. Together, these results support a neural mechanism where cue-induced replay during sleep promotes memory consolidation by increased integration of task-relevant perceptual regions with mnemonic regions.

Introduction

The neural representations of recently acquired declarative memories are thought to be selectively reactivated during ensuing rest periods, particularly during slow-wave sleep (Diekelmann and Born 2010; Diekelmann et al. 2011). For instance, hippocampal place cells in rats that co-activated during active behavior were found to be reactivated in concert during ensuing slow-wave sleep (Wilson and McNaughton 1994). Later studies found that this coordinated reactivation, also dubbed replay, extended to the perceptual cortex involved in the initial processing of the stimulus, such as the visual cortex (Ji and Wilson 2007). Furthermore, evidence of neural replay has also been found in the medial prefrontal cortex (Euston et al. 2007; Peyrache et al. 2009; Genzel and Battaglia 2017). These results demonstrate the occurrence of neural memory replay in rodents, and have been shown to be behaviorally relevant for the retention of associative memories (Dupret et al. 2010).

Systems-level consolidation theory posits that neural memory traces are initially hippocampus-dependent, and subsequently integrate into neocortical networks (Squire et al. 1984; McClelland et al. 1995; Nadel and Moscovitch 1997; Frankland and Bontempi 2005). The neural trace is presumed to initially involve the hippocampus, which acts like a hub binding elements into a coherent memory trace, and the cortical regions that were involved in the initial processing of the stimulus at encoding (Nyberg et al. 2000; Polyn et al. 2005; Danker and Anderson 2010). During sleep, especially when the brain is in a highly synchronous state (i.e. slow-wave sleep stage), specific tightly coupled oscillatory patterns are observed, including slow-oscillations, thalamic spindles, and hippocampal ripples (Siapas and Wilson 1998; Mölle et

82 al. 2002, 2006; Staresina et al. 2015) that have been related to memory consolidation (Schabus
83 et al. 2004; Marshall et al. 2006; Axmacher et al. 2008; Girardeau et al. 2009; Cox et al. 2012).
84 Thus, it is plausible to posit that memory reactivation during sleep facilitates information
85 exchange between neocortex and hippocampus, enabling the strengthening of connections
86 between these areas as well as amongst neocortical modules, thereby contributing to systems-
87 level memory consolidation (Marshall and Born 2007).

88 Neural memory reactivation is difficult to observe in humans, as its timing is generally
89 unknown. However, reactivation can also be induced using cues associated with information
90 learned previously. For example, presenting an odor during slow-wave sleep induces activation
91 of the hippocampus and stabilizes associated memory traces (Rasch et al. 2007; Diekelmann et
92 al. 2010, 2011). Furthermore, whereas studies have found that audio-visual-spatial associations
93 can be stabilized in a targeted manner using specific auditory cues (Rudoy et al. 2009; Creery et
94 al. 2015), these cueing effects can also affect all associations acquired within the same learning
95 context (Oudiette et al. 2013). Van Dongen and colleagues (2012) performed targeted memory
96 reactivation in the magnetic resonance (MR) scanner using auditory cues that were paired with
97 specific object-location associations. Even though no consistent effect of cueing on memory
98 stabilization was found, individual differences in the effect of cueing positively correlated with
99 activity in the hippocampus, thalamus and cerebellum during slow-wave sleep. Given repeated
100 earlier findings of memory improvement from auditory cues presented during slow-wave sleep
101 (Oudiette et al. 2013; Ong et al. 2016; Schouten et al. 2017), it is likely that the noisy scanner
102 environment contributed to excessive variability here, with some individuals effectively tuning

103 out all auditory input, or alternatively that cueing affected all associations acquired within the
104 same learning context (Oudiette et al. 2013).

105 Here, we re-analyzed data acquired by van Dongen and colleagues (2012) using a principled
106 graph theoretical analysis approach. Triggering reactivation of memory traces with auditory
107 cues, could induce a process akin to spontaneous replay, which likely involves whole-brain
108 connectivity changes facilitated by synchronous brain states during SWS. Conventional analysis
109 methods are often unable to capture the coordinated whole-brain connectivity changes
110 expected during memory replay. Thus, a network perspective can be leveraged using methods
111 developed within the framework of graph theory (Bullmore and Sporns 2009). The participation
112 coefficient is a metric that captures the extent to which a region integrates and distributes
113 information as a result of the number and positioning of their contacts in the network (Power
114 et al. 2013). Specifically, it quantifies network integration by looking at the importance of a
115 given node for interactions between subnetworks. The participation coefficient can be used to
116 index the integration of a given voxel (node) in the wider brain (network) during cued
117 reactivation of visuospatial associations during SWS.

118 If a cue induces memory reactivation akin to replay, and this indeed plays a role in memory
119 consolidation involving a hippocampal-neocortical trace shift, then one should expect a
120 coordinated neural activation of multiple regions, including the sensory features of the cued
121 memory (in this case visual object features in the occipital cortex and the spatial layout in the
122 parahippocampal gyrus), as well as the key mnemonic regions (such as the hippocampus,
123 thalamus, and medial prefrontal cortex). This increased integration of these and other regions

would be expected to be beneficial for memory stabilization across sleep and result in an increased involvement of the neocortex in the memory trace.

Materials and Methods

The Materials and Methods have been described extensively in the original report (van Dongen et al. 2012). Here, we summarize relevant details about the experimental setup, supplemented with details about the data analysis performed for this report.

Participants

This re-analysis includes data from all the 22 participants included in the original report (for complete description, see van Dongen et al., 2012). Briefly, 56 participants were initially recruited from the online research participant system of the Radboud University Nijmegen (age range 18–27; 14 males). Twenty-two participants in the sample reached a sufficient amount of slow-wave sleep in the scanner to allow at least 80 % of the cueing protocol to be completed whilst in the slow-wave sleep stage. Furthermore, these participants did not display micro-arousals in response to sound presentations, nor did they report explicit knowledge of the sounds having been presented while they slept. The experiment was conducted in accordance with national legislation for the protection of human volunteers in nonclinical research settings and the Helsinki Declaration and approved by the local ethics committee. Participants were compensated for participation with course credits or a monetary fee.

143 *Procedures*

144 The experiment started between 7 and 8 PM, and participants went to sleep between 10 PM
145 and midnight (see figure 1). Participants were placed in the MR scanner, and the sound volume
146 was individually calibrated to a level at which the participant could distinguish individual sounds
147 while the scanner was operating. Participants learned 50 object-location associations, each
148 object-location pair being presented together with a unique sound associated with the object,
149 inside the scanner. When all associations were learned to criterion (all objects placed within 4
150 cm from the correct location on two subsequent rounds), a pre-test was performed on all
151 associations (Test 1). Next, participants were prepared outside the scanner for
152 polysomnographic recordings, and placed in the scanner again for a 2 hr. rest/sleep period,
153 while EEG was monitored online and fMRI data was continuously acquired. When participants
154 had entered stable slow-wave sleep for a period of time as visible on the ongoing
155 polysomnographic EEG, sounds were presented to the participants using an MR-compatible
156 headphone. Specifically, half of the sounds that were associated with specific object-location
157 associations during learning were presented as 'cue sounds' during sleep (25 sounds presented
158 twice, for a total of 50 cue sound presentations). Moreover, control sounds were presented
159 that had not been previously associated with learning materials (5 sounds presented five times,
160 for a total of 25 control sound presentations). After a 2 hr sleep, participants were awoken
161 before they performed the post-test with all object-location associations (Test 2).

162 *Object-location test*

163 The object-location task was performed in three stages: learning, Test 1 and Test 2. Here, we
164 focus on the behavioral data from Test 1 and Test 2 for further analysis. Participants learned

the location of 50 object pictures. For each participant, 50 objects were randomly assigned to 50 screen locations (screen size: 47 × 35 cm, resolution: 1024 × 768, viewing distance: 60 cm). In the learning phase, participants first passively viewed all 50 objects in their respective screen locations (duration: 3s presentation followed by a 1s inter-stimulus interval), paired with hearing the object related sound (e.g. a cat's 'meow' when the object was a cat, sound duration: 500 ms). Next, the learning phase continued with several iterative rounds of active learning, where an object was presented at the center of the screen simultaneously with the auditory stimulus. Participants were then required to place the object in its original location using an MR-compatible joystick, and to press a button to confirm the object placement (self-paced timing). Feedback was given in every round of learning by displaying the object in the correct location for 3s. Objects were presented in a random order, and with every new round those objects were excluded that had been placed within 4 cm from the correct location on the two preceding rounds. Upon reaching criterion performance on all objects, participants performed the pre-sleep test (Test 1) for all objects without feedback. An identical post-sleep test was performed after sleep (Test 2). During tests, object placement was followed by an inter-stimulus period of fixation (duration jittered between 3 and 5s).

181 *MRI Data Acquisition*

182 Participants were scanned using a reduced-noise EPI sequence with sinusoidal gradients to
183 avoid acoustic resonances of the scanner (Schmitter et al. 2008). Functional (T2*) images were
184 acquired with whole-brain coverage (28 axial slices, ascending slice acquisition, TR = 2,511 ms,
185 TE = 38 ms, 90° flip angle, matrix = 64 x 64, bandwidth = 1,502 Hz per voxel, slice thickness = 3.5
186 mm, slice gap = 15%, field of view (FOV) = 244 mm). Structural (T1) images were acquired with a
187 MPRAGE sequence (176 sagittal slices, TR = 2,250 ms, TE = 2.95 ms, 15° flip angle, matrix = 256
188 x 256, slice thickness = 1.0 mm, FOV = 256 mm).

189 *MRI Data Preprocessing*

190 The fMRI data acquired during Test 1, the sleeping period and Test 2 were preprocessed using
191 standard routines implemented in SPM8 (www.fil.ion.ucl.ac.uk). The first five volumes of each
192 functional EPI run were discarded, and an outlier algorithm was used to check for corrupted
193 slices from each image and replaced using between-volume interpolation. The functional
194 images were then realigned, and coregistered to the structural image, spatially normalized to
195 MNI-space (resampled at voxel size 2 x 2 x 2 mm), and smoothed using a Gaussian kernel (8 mm
196 FWHM). The structural images were segmented into gray matter, white matter, cerebrospinal
197 fluid, and residual compartments (outside brain and skull) using the unified segmentation
198 algorithm as implemented in SPM8. These compartments were used as masks to extract the
199 mean intensity level across the whole time-series, and entered as compartment regressors to
200 account for effects related to non-specific signal fluctuations.

Network analysis during cueing

We employed a graph theoretic framework (Bullmore and Sporns 2009; Ekman et al. 2012) to analyze connectivity patterns during the presentation of sound cues in the scanned sleep period. The aim was to measure dynamic changes in the whole-brain network properties during memory reactivation, and specifically changes in integration of certain brain regions with the whole brain network. The metric of interest was defined as the participation coefficient (Guimera et al. 2005; Power et al. 2013; Backus et al. 2016), which quantifies for each node (i.e. voxel) the diversity of its inter-modular connections (Rubinov and Sporns 2010), specified as the amount of connections with nodes in other submodules relative to the total amount of connections. It therefore is a measure of the importance of a given node for global inter-modular integration across the brain.

A high-pass filter with a cutoff period of 128s was applied to the functional time series. Sound presentation during sleep was modelled using a separate regressor for each sound presentation (to obtain trial-specific beta-estimates for the 50 cue sound presentations and 25 control sound presentations) with a duration of 5 seconds each (the duration of the individual sound presentations). The design matrix also included six regressors of no interest to account for head movement, and the three compartment regressors to account for non-specific signal fluctuations. The resulting beta estimates were concatenated separately for cue sound and control sound trials. This procedure resulted in two separate beta time-series (i.e., cue sounds and control sounds) for all voxels in the brain. Next, voxel-wise correlation coefficients of beta time-series were computed to quantify pairwise functional connectivity (Rissman et al. 2004) for each condition separately (i.e., cue sounds and control sounds). The connectivity matrices

223 were thresholded by preserving only significant connections ($p < 0.05$ FDR corrected) and
224 setting all other connections, as well as all negative correlations to zero. Following the
225 procedure used by Power and co-workers (2011), modular network structure was first derived
226 across all sound presentations using a connectivity matrix based on the regions defined by the
227 AAL (Automated Anatomical Labeling) atlas (Tzourio-Mazoyer et al. 2002). Here, beta time-
228 series were averaged across voxels of the region, and a 116 x 116 region-by-region connectivity
229 matrix was constructed. After thresholding this connectivity matrix (edges > 0, $p < 0.05$ FDR
230 corrected), the matrix was parcellated into subnetworks using modularity detection according
231 to the Louvain method (Blondel et al. 2008). Next, each voxel in the whole-brain voxel-wise
232 connectivity matrix was assigned to a module, allowing the calculation of their participation
233 coefficient ranging from 0 (provincial hub: connections are only present within its module) and
234 1 (connector hub: connections are only present with other modules; Power et al., 2013). The
235 resulting participant coefficient maps for cue sounds and control sounds were contrasted on an
236 individual subject level and this contrast was subsequently tested at the second level using a
237 one-sample t-test.

238 *Psychophysiological interaction (PPI) analysis during cueing*

239 The previous analysis determined which of the regions increased in global inter-modular
240 network integration. To determine which regions in other modules of the brain were connected
241 with the 'hub' (the region found to display increased network integration during cueing), we
242 performed a follow-up seed-based functional connectivity analysis. This psychophysiological
243 interaction (PPI) analysis served to determine which brain regions were more connected with

244 the hub during the presentation of cue versus control sounds. Therefore, we extracted the
245 physiological signal time series from the seed region found in the network analysis (cluster-
246 forming threshold at Z > 2.33) to serve as a physiological regressor. This was entered into a
247 general linear model (GLM) along with the psychological variable (cue versus control sounds)
248 and the psychophysiological interaction regressor. Small-volume correction was used for the
249 bilateral parahippocampal gyri, as this region was activated by the object-location retrieval task
250 (van Dongen et al. 2012), and thalamus and hippocampus, as these are smaller structures
251 hypothesized to be involved in memory reactivation. Here, anatomical masks implemented in
252 the IBASPM 71 Atlas of the WFU Pick Atlas Tool in SPM were used (Iturria-Medina et al. 2007).
253
254 *Activity analysis during pre- and post-sleep test*
255 We next assessed whether network integration of visual cortex during cueing in slow-wave
256 sleep results in increased involvement of cortical associative regions at retrieval. For this
257 reason, we contrasted the neural activations observed during the pre-sleep and post-sleep test,
258 and related this contrast to the participation coefficient of the occipital cortex as observed in
259 the graph theory analysis (see Results-section). The fMRI data for Test 1 and Test 2 were
260 analyzed in one GLM. Similar to van Dongen and colleagues (2012), this model included
261 regressors for cued and uncued associations, which were modeled as delta functions to be
262 convolved with the canonical hemodynamic response function (HRF), along with temporal
263 derivatives provided by SPM8. The design matrix included six regressors of no interest per test
264 session to account for head movement using the realignment parameters, as well as
265 compartment regressors accounting for non-specific signal fluctuations. Furthermore, a high-

266 pass filter with a cutoff period of 128s was implemented to remove low-frequency fluctuations
267 from the time series. Parameter images were generated on the first level (for each individual
268 participant) based on a contrast between all trials of Test 1 and all trials of Test 2, to assess
269 changes in retrieval activity from the pre- to the post-sleep test. We collapsed across cued and
270 uncued associations because network participation of the occipital cortex was not specifically
271 related to only the cued associations in the learned set (see 'Results'). These contrast images
272 were entered into a one-sample t-test on the second level, with the participation coefficient of
273 the occipital cortex during cue versus control sound presentation entered as a covariate. Small-
274 volume correction was used for the bilateral parahippocampal gyri, as this region was activated
275 by the object-location retrieval task and cue-sound presentation (van Dongen et al. 2012). Here,
276 an anatomical mask of the parahippocampal gyrus implemented in the IBASPM 71 Atlas of the
277 WFU Pick Atlas Tool in SPM was used (Iturria-Medina et al. 2007).
278
279 **Results**

280 *Network changes following cueing*

281 We probed changes in network dynamics in response to the presentation of cue sounds versus
282 control sounds. The presentation of cue sounds was hypothesized to induce reactivation of the
283 corresponding memory trace, which would presumably be reflected in increased integration of
284 areas that represent sensory features of the cued memory representation, e.g. occipital cortex.
285 Indeed, we found a specific increase in the participation coefficient of early visual (occipital)
286 cortex (peak MNI coordinates: x,y,z = [4, -70, 6], z_{21} = 3.12, cluster-forming threshold at Z>2.33,
287 corrected for the whole brain at $p < 0.05$ using Gaussian Random Field Theory), indicating that

288 occipital cortex displays an increased inter-modular connectivity when presented with cue
289 sounds during deep sleep. The region observed overlaps with regions that were activated in
290 response to the object-location task during Test 1 (see figure 2b). This overlap may be indicative
291 of replay of the original visuo-spatial neural memory trace in response to auditory cues
292 presented during sleep, in the absence of any external visual input.
293 To determine what brain regions this area in the occipital cortex preferentially connects to
294 when recruited in the network by the auditory cues, we performed a psychophysiological
295 connectivity analysis. The seed was defined as the region in cortex that displayed an increase in
296 participation coefficient during cueing (cluster-forming threshold at $z > 2.33$). The task contrast
297 was formed by the presentation of cue sounds versus control sounds. The occipital cortex was
298 found to be connected to the left hippocampus (peak MNI coordinates: x,y,z = [-32, -16, -18],
299 z_{21} = 3.36), right hippocampus (peak MNI coordinates: x,y,z = [22, -2, -22], z_{21} = 2.98), left
300 parahippocampal gyrus (peak MNI coordinates: x,y,z = [-26, -22, -26], z_{21} = 3.10), left thalamus
301 (peak MNI coordinates: x,y,z = [-4, -2, -0], z_{21} = 3.11), and right thalamus (peak MNI coordinates:
302 x,y,z = [2, -16, -12], z_{21} = 2.67), and a medial prefrontal region extending from premotor cortex
303 to anterior cingulate cortex and into dorsomedial prefrontal cortex (see figure 3, all cluster-
304 forming threshold at $z > 2.33$, cluster-size corrected for the whole brain, or reduced search
305 regions based on predefined anatomical areas for the bilateral hippocampus, parahippocampal
306 gyrus and thalamus, thresholded at $p < 0.05$ using Gaussian Random Field Theory). Thus, during
307 cueing, the occipital cortex was connected to several regions where memory replay has been
308 shown to occur (hippocampus, medial prefrontal cortex), and that are involved in oscillatory

309 patterns of activity (spindles, ripples, slow-waves) that allow for systematic interactions
310 between brain regions (e.g., the hippocampus, medial prefrontal cortex and thalamus).

311 *Network dynamics related to behavioral cueing effect*

312 As expected, memory performance decreased on average (Test 1 performance: error = 2.74 ±
313 0.12 cm; Test 2 performance: error = 3.12 ± 0.14: Δerror = 0.37 ± 0.08; t_{21} = 4.48, p < 0.001),
314 both for cued (Δerror = -0.44 ± 0.11 cm, t_{21}(3.86, P = 0.001)), and uncued associations (Δerror =
315 −0.33 ± 0.11 cm, t_{21}(2.56, P = 0.018)). There was no effect of cueing on participants' memory
316 accuracy as tested after sleep ($F_{1,21}$ = 0.83; P = 0.374). Thus, there was forgetting across the
317 sleep/rest session, and this forgetting appeared to be unspecific across cued and uncued
318 object-location associations.

319 However, there was substantial variability in the effect of cueing on forgetting. Therefore,
320 different neural responses to cueing might explain individual differences in the effect of cueing.
321 Particularly, increased network participation of the occipital cortex could be an index of how
322 much the visual-spatial memory trace became reactivated in a given subject when auditory
323 cues were presented. Indeed, the increased participation coefficient during cue sound
324 presentation was found to be associated (see figure 2) with reduced overnight forgetting of
325 visual-spatial object locations (τ_{21} = -0.311, p = 0.045). This relationship was specifically present
326 for the visual-spatial locations that were associated with sound cues (τ_{21} = -0.338, p = 0.029),
327 but not for other locations (τ_{21} = -0.211, p = 0.174), although the difference in these correlations
328 did not reach significance (Williams-Hotelling test, t = 0.433, p = 0.670).

329 The distribution of the increase in participation coefficient followed a non-normal positively
330 skewed distribution, with about half of participants displaying only a minimal increase in
331 participation coefficient during cueing (PC < 0.1). Therefore, we performed a median split to
332 better characterize the group that experienced an increase in network participation of the
333 occipital cortex (PC+), versus the group with no increase (PC=). The PC= group displayed
334 significant forgetting (Δerror = 0.68 ± 0.32; t_{10} = 6.042, p < 0.001), for both cued (Δerror = 0.69 ±
335 0.41; t_{10} = 5.620, p < 0.001) and uncued (Δerror = 0.48 ± 0.44; t_{10} = 3.636, p = 0.005)
336 associations. In contrast, the PC+ group did not display any forgetting (Δerror = 0.16 ± 0.35; t_{10} =
337 1.551, p = 0.152), for both cued (Δerror = 0.20 ± 0.57; t_{10} = 1.186, p = 0.263) and uncued (Δerror
338 = 0.12 ± 0.61; t_{10} = 0.653, p = 0.529) associations. Note that we do not report the statistical test
339 to compare performance differences between the two groups, as this comparison would not be
340 statistically independent from the across-subject correlation reported earlier. This result
341 indicates that in the group where there was an increased network participation of occipital
342 cortex, overnight forgetting was reduced, but the effect was not specific to the cued items,
343 extending to other object-location associations encoded within the same spatial layout and
344 temporal learning context (see figure 4).

345 *Overnight activity changes related to network integration during cueing*

346 We then considered whether the increased network integration of the occipital cortex during
347 sleep had subsequent effects on neural activity when retrieving object-locations during the
348 post-sleep test. Here, we found that the participation coefficient was related to a positive
349 increase in retrieval-related activity from Test 1 to Test 2 in both the posterior left

350 parahippocampal gyrus (peak MNI coordinates: *x,y,z* = [-16, -32, -18], z_{21} = 4.14), and posterior
351 right parahippocampal gyrus (peak MNI coordinates: *x,y,z* = [16, -32, -18], z_{21} = 3.26, cluster-
352 forming threshold at Z>2.33, corrected for a reduced search volume based on anatomical masks
353 of the left and right parahippocampal gyrus at a threshold of $p < 0.05$ using Gaussian Random
354 Field Theory). Thus, increased network integration of the occipital cortex during cueing had a
355 subsequent effect on the neural regions activated during the retrieval of object-locations at
356 Test 2, thereby increasingly recruiting the bilateral parahippocampal gyrus.
357

358 **Discussion**

359 Here, we investigated the whole-brain neural network reorganization induced by cued
360 reactivation of memory traces during deep sleep, and its behavioral and neural effects on the
361 subsequent retrieval of those memories. For that purpose, we re-analyzed fMRI data reported
362 in study by van Dongen and colleagues (2012) using whole-brain connectivity measures. We
363 demonstrate that when participants were in a state of slow-wave sleep, absent of visual input,
364 and were cued with sounds associated with previously learned visuospatial information, the
365 occipital cortex displayed increased network integration as measured with the participation
366 coefficient. Furthermore, subjects who displayed an increase in cue-induced network
367 integration of the occipital cortex showed increased memory stabilization. This finding is
368 indicative of a process in which the sound cue induces a global reprocessing of the learning
369 episode, akin to memory replay, which is conducive to subsequent memory retention. Note
370 that while the occipital cortex is not directly related to mnemonic processes, previous studies
371 have shown that cue-triggered reactivation can lead to instantiations of previously experienced

372 episodes in this region (Xu et al. 2012; Ekman et al. 2017). It is likely that this reinstitution is
373 coordinated with other mnemonic regions. Indeed, various regions displayed increased
374 connectivity with the occipital cortex during the presentation of cue sounds: memory and
375 replay-related regions, such as the hippocampus, thalamus and medial prefrontal cortex; and
376 higher-order associative cortices implicated in the encoding of the memory trace, such as the
377 parahippocampal gyrus. These results are in line with a study by Ji & Wilson (2007) that shows
378 coordinated replay between the hippocampus and a perceptual region that was involved in the
379 initial processing of the stimulus (here: the occipital cortex). Cueing during slow-wave sleep also
380 showed an enduring neural effect during memory retrieval as measured during the post-sleep
381 retrieval test. Specifically, subjects with greater network integration of the occipital cortex
382 during SWS recruited the parahippocampal gyrus more at subsequent retrieval.

383 It should be noted that the network reorganization found during the presentation of cue
384 sounds was not specifically related to memory stabilization of those object-location
385 associations associated with the cues. The association of network participation with memory
386 stabilization was numerically larger for cued, versus uncued material, but the interaction was
387 not statistically significant. There could be various explanations for this unspecific effect. First,
388 the MRI scanner is a suboptimal environment for auditory presentation during sleep. Therefore,
389 it is conceivable that the acoustic stimuli presented during deep sleep in a noisy environment
390 effectively modulated ongoing brain processes in some individuals, but not others, contributing
391 to large inter-individual differences and lower power of demonstrating this effect than in other
392 studies conducted outside of the MR-scanner environment (Rudoy et al. 2009). Indeed, we
393 observed a non-normal positively skewed distribution of increase in participation coefficient

394 during cueing. There was an overall effect on memory retention for participants who showed
395 increased participation coefficient during cueing – but there did not appear to be a cue-specific
396 memory benefit even when only considering this sub group. It is also possible that the study
397 was underpowered to observe an interaction effect, and that a larger sample would have
398 rendered a significant interaction. Furthermore, it is possible that the cueing had a non-specific
399 effect on all material acquired within the same temporal and spatial learning context (as also
400 suggested by the results reported in Oudiette et al., 2013). Indeed, all object-location
401 associations were acquired within the same learning context; the same scanning session and
402 temporal learning sequence, and most importantly, all objects were located on the same two-
403 dimensional spatial grid. Therefore, it is plausible that the various object-locations became
404 integrated into a unitary map, or spatial schema (Tse et al. 2007; van Buuren et al. 2014) at
405 initial learning and/or during replay (Durrant et al. 2015; Hennies et al. 2016). As such, object
406 locations could have been stored not only with reference to the two-dimensional grid, but also
407 directly or indirectly in spatial reference to the other objects. As such, a sound cue could have
408 reactivated certain object-location associations, but also objects located within the objects'
409 vicinity. In fact, it has been proposed that sleep-dependent memory replay is instrumental in
410 the integration of memories into a cognitive schema (Lewis and Durrant 2011). Thus, while
411 network integration of the occipital cortex during cueing is demonstrated to be positively
412 related to overall memory stabilization of a set of object-location associations, the fact that an
413 integrated spatial schema was cued may have contributed to non-specific reactivation of all
414 object-locations within this set.

415 Bearing in mind this caveat, our results show a network reorganization in response to cues
416 presented during SWS that is consistent with an active model of sleep-dependent memory
417 consolidation. Previous research has shown that whole-brain connectivity patterns display a
418 general reduction in thalamo-cortical and cortico-cortical connectivity and an increase in local
419 clustering during slow-wave sleep (Spoormaker et al. 2010). Against this backdrop, a
420 hippocampal-neocortical dialogue takes place facilitating systems-level consolidation by
421 integrating hippocampal-dependent memories into neocortical storage sites (Buzsáki 1996;
422 Diekelmann and Born 2010; Mitra et al. 2016). This hippocampal-neocortical dialogue is
423 orchestrated through oscillatory electrophysiological patterns characteristic of slow-wave sleep
424 and related to memory replay (Diba and Buzsáki 2007; Davidson et al. 2009). Specifically, slow
425 (delta wave) oscillations propagate across the brain and to the medial temporal lobes, including
426 the hippocampus, exerting a global control over spiking activity (Massimini et al., 2004; Nir et
427 al., 2011). Mechanistically, the up-state of slow oscillations has been found to enable thalamic
428 sleep spindles in the sigma band, which in turn cluster high-frequency hippocampal ripples in
429 their troughs (Staresina et al., 2015). These hippocampal ripples then are proposed to allow for
430 a precisely timed exchange of mnemonic information between hippocampus and neocortex,
431 potentiating and strengthening the neocortical memory trace. Indeed, the importance of slow
432 oscillations and spindles for memory consolidation has been demonstrated with behavioral
433 correlations (Schabus et al., 2004; Holz et al., 2012). Moreover, intracranial recordings in
434 humans have found time-locked ripple events during sleep in both hippocampus and
435 neocortex, the latter being related to memory consolidation (Axmacher et al., 2008).
436 Experimental disruption of hippocampal ripples in rats impaired consolidation of hippocampal-

437 dependent spatial memories (Girardeau et al., 2009). Therefore, during slow-wave sleep,
438 hierarchically nested oscillations presumably serve to consolidate memories by tightly coupling
439 the neocortex (especially medial prefrontal cortex), thalamus and medial temporal lobes, which
440 are the regions that are displaying an increased interconnectivity during cueing here.

441 Thus, during slow-wave sleep, there is increased cross-talk between the hippocampus,
442 thalamus and neocortex. If external auditory cueing would induce a reactivation of the visuo-
443 spatial memory traces, one would expect that cortical representation areas corresponding to
444 the modality of the memory trace would be recruited to participate in this cross-talk. Indeed,
445 the occipital cortex displayed an increase in global network integration during cueing, and when
446 probing the neural connectivity changes paired with this increase, we found that this region
447 increases its connectivity with the regions involved in active cross-talk during slow-wave sleep,
448 namely the hippocampus, thalamus and medial prefrontal cortex. These regions resemble those
449 that are involved in the oscillatory patterns that orchestrate hippocampal-neocortical cross-talk
450 (Mölle et al. 2006; Staresina et al. 2015). It might at first sight be surprising that the auditory
451 cortex is not recruited in response to auditory cueing. However, it should be noted that a
452 contrast was made between two conditions in which sounds were presented. The critical
453 difference in the contrast is that cue sounds were associated with specific visuo-spatial
454 memories, but not control sounds. It remains the case that one could still expect increased
455 connectivity of the auditory cortex with visual, spatial, and memory regions during cueing, the
456 sounds being associated with the object-locations. As psychophysiological interaction analyses
457 were informed by results of the graph theory analyses, we did not further probe connectivity
458 from an anatomically defined seed in the auditory cortex here. Despite this caveat, it remains to

459 be noted that cueing recruited the occipital cortex to engage with mnemonic regions that have
460 been shown to display increased cross-talk during slow-wave sleep, and this promoted the
461 stabilization of the associated memory traces in neocortical storage sites.

462 Sleep supports memory retention in a selective manner, and information is prioritized based on
463 perceived future relevance at encoding (Wilhelm et al. 2011; Van Dongen et al. 2012) emotional
464 salience (Hu et al. 2006; Wagner et al. 2006; Payne et al. 2007) and consistency across episodes
465 and with prior knowledge (Tamminen et al. 2013; Durrant et al. 2015). Possibly, relevant
466 information is selectively prioritized for active processing during sleep, whereas non-prioritized
467 memory traces are forgotten, perhaps aided by homeostatic regulation of synaptic plasticity
468 through global downscaling (Tononi and Cirelli 2003, 2006; Genzel et al. 2014). The net result is
469 an increase in signal-to-noise for the prioritized memory traces in the neocortex, contributing
470 to memory stabilization. Indeed, this appears to be the case in the parahippocampal gyrus,
471 where an increase in retrieval-related activation of the posterior parahippocampal gyrus at
472 post-sleep test versus pre-sleep test was related to an increase in network participation of the
473 occipital cortex during cueing. The parahippocampal gyrus is known to be involved in
474 representing the local visual environment (Epstein and Kanwisher 1998) and learning spatial
475 layouts (Aguirre et al. 1996; Epstein et al. 1999). The increased involvement of the
476 parahippocampal gyrus during post-sleep retrieval is consistent with a role of the
477 parahippocampal gyrus in binding together low-level visual information from downstream
478 occipital cortex. Notably, the parahippocampal gyrus was activated already during retrieval at
479 the pre-sleep test, showed selective increased activation in response to cueing, and displayed
480 increased connectivity with the occipital cortex during cued reactivation, as originally reported

481 in van Dongen & colleagues (2012). The results reported here further elucidate the mechanisms
482 involved in targeted memory reactivation by taking a network perspective.

483 In sum, we show here that inducing the reactivation of object-location memories with
484 associated auditory cues during slow-wave sleep increases the integration of occipital cortex
485 with the global brain network, specifically those regions involved in memory replay, such as the
486 medial temporal lobe, thalamus and medial prefrontal cortex. Furthermore, global network
487 integration of occipital cortex during cueing is predictive of overnight memory stabilization of
488 learned materials and an increased involvement of parahippocampal gyrus during the post-
489 sleep retrieval. These findings highlight how graph theory analysis can be used to assess whole-
490 brain connectivity patterns during targeted memory reactivation in SWS, and contribute to a
491 better understanding of sleep-related memory consolidation.

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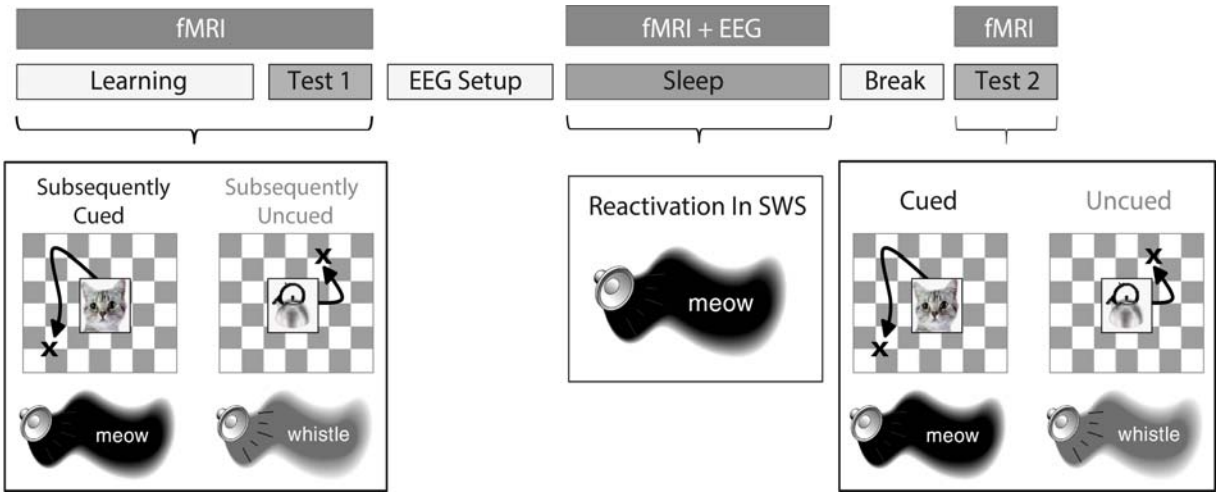
645 **Figures**

646 Figure 1



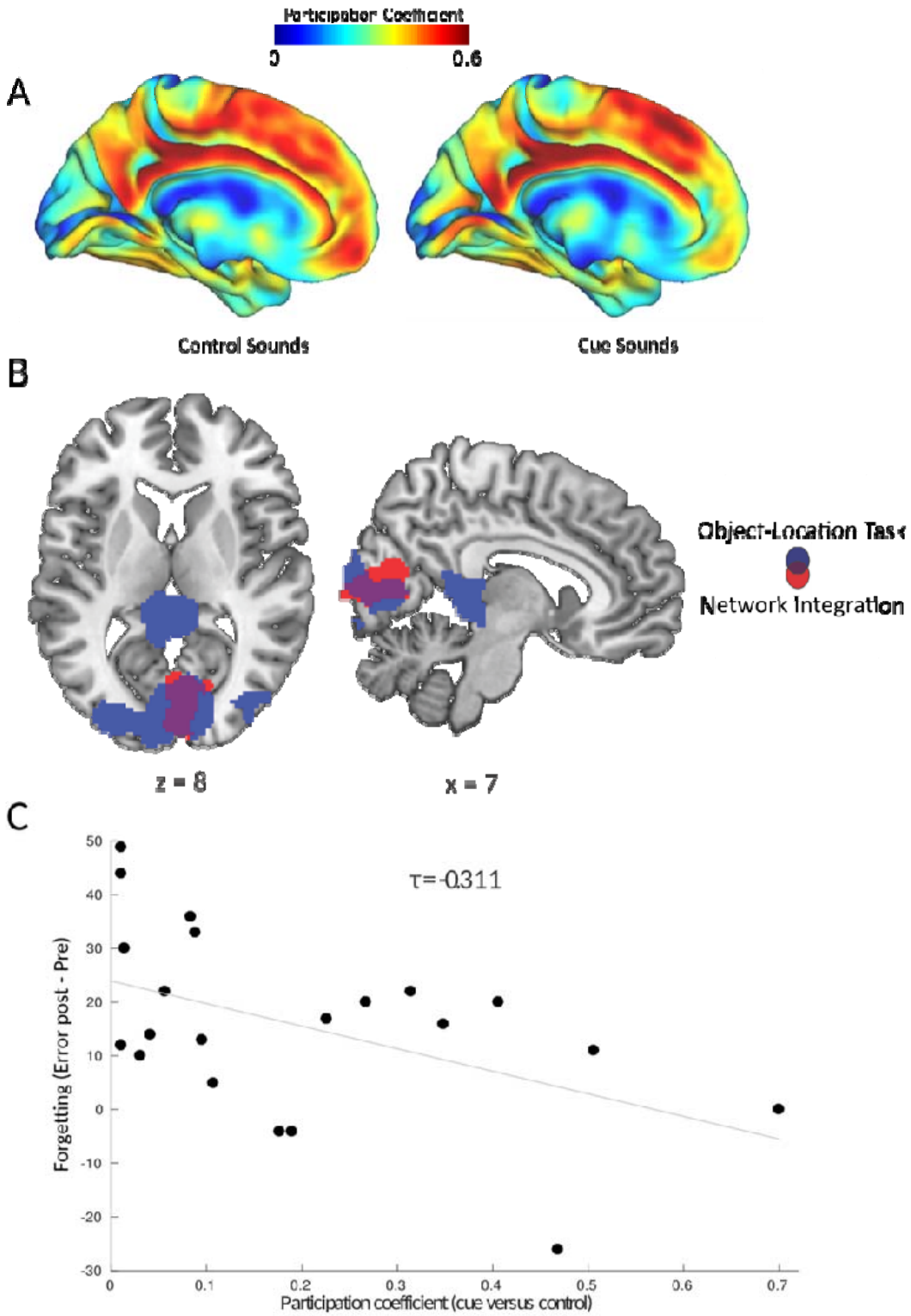
647

648 ***Schematic depiction of the experimental procedure.***

649 *Participants learned 50 object-location associations, presented simultaneously with object-related*
650 *sounds, inside the MR-scanner. After a baseline test (Test 1), participants were set up for*
651 *polysomnographic recordings and went to sleep inside the MR-scanner. Half of the learned associations*
652 *were cued with sounds during slow-wave sleep (SWS). After awaking and a short break, participants*
653 *performed another post-sleep test (Test 2). Figure adapted with permission from the initial report (van*
654 *Dongen et al., 2012).*

655

656 Figure 2



657

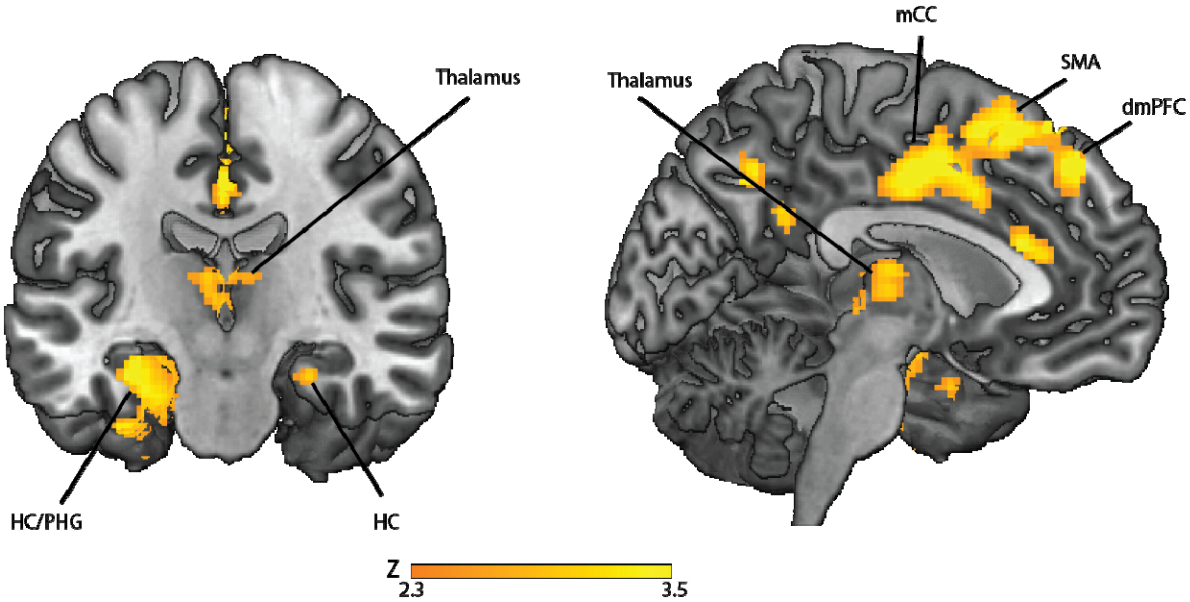
Replay-related changes in network integration predict behavioral memory stabilization.

A) Participation Coefficient mapped for every voxel in the brain during the presentation of control sounds (not previously paired with object-location information, left panel) and cue sounds (previously paired with object-location associations, right panel).

B) Increased network integration was found only in the occipital cortex during the presentation of cue sounds versus control sounds during SWS. This region overlapped with the set of regions activated in response to the object-location association task. Parametric maps were superimposed onto a template brain, using the cluster-forming threshold of Z>2.33.

C) The increase in participation coefficient of the occipital cortex during cueing predicted memory stabilization, indicated by reduced forgetting (expressed as the difference in error distances) between test 1 (pre) and test 2 (post).

671 Figure 3



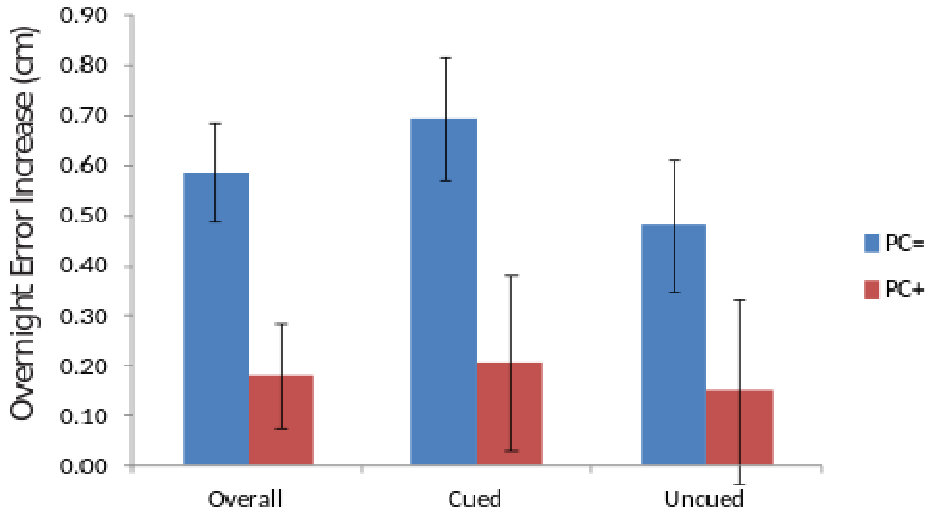
672

673 ***Regions displaying greater coupling with occipital cortex during cueing in SWS***

674 *Regions displaying stronger coupling with the occipital cortex for cue sounds compared to control*
675 *sounds. Parametric maps were superimposed onto a template brain, using the cluster-forming threshold*
676 *of Z>2.33. HC = Hippocampus, PHG = Parahippocampal Gyrus, mCC = Middle Cingulate Cortex, SMA =*
677 *Supplementary Motor Area, dmPFC = Dorsomedial Prefrontal Cortex.*

678

679 Figure 4



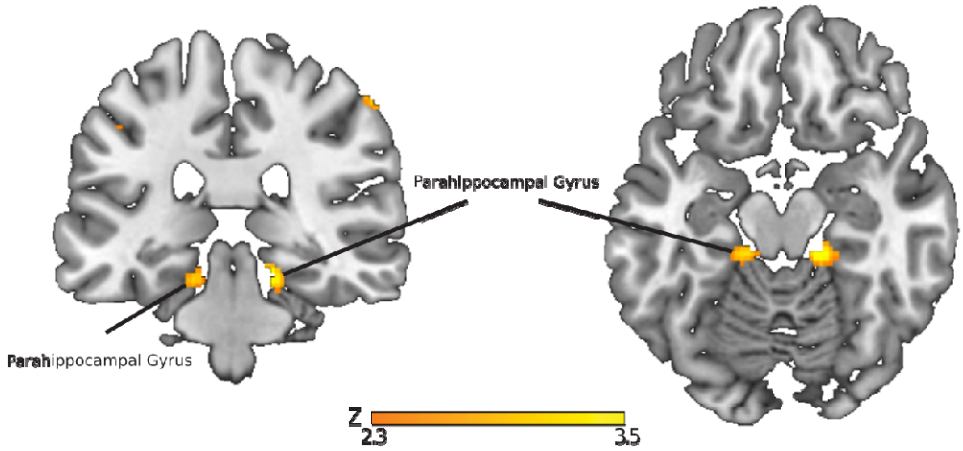
680

681 ***Overnight forgetting, split by network integration in occipital cortex***

682 *The bar graph depicts the change in error distance between the pre-sleep Test 1 and the post-sleep Test*
683 *2 for a group where there was an increase in network integration (as measured with the participation*
684 *coefficient) in occipital cortex (PC+) and a group where no such increase was found (PC=). The groups*
685 *were defined using a median split on the participation coefficient values acquired from the occipital*
686 *cortex in the cue sounds > control sounds comparison. The error distance is operationalized as the*
687 *distance between the location where the object is placed at test, and the actual object location as*
688 *presented during the learning phase. A higher overnight error increase indicates a reduction in accuracy*
689 *of placement across sleep, indicative of increased forgetting. The group where cueing induced increasing*
690 *network integration in the occipital cortex (PC+) displayed reduced forgetting overall, indicating that, in*
691 *this group, cueing stabilizes memories overnight.*

692

693 Figure 5



694

695 ***Overnight activity increases related to network integration of occipital cortex***

696 *Regions where there is a relation between on the one hand, an activity increase between the pre-sleep*

697 *and post-sleep test and, on the other hand, increased network integration of occipital cortex during*

698 *cueing in the SWS between Test1 and Test 2. Parametric maps were superimposed onto a template*

699 *brain, using the cluster-forming threshold of Z>2.33.*