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, Takashima, 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 increased 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 (SWS) (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 SWS (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. SWS stage), specific tightly coupled oscillatory patterns are observed, including slow-oscillations, thalamic spindles, and hippocampal ripples (Siapas and Wilson 1998; Mölle et al.

82 2002, 2006; Staresina et al. 2015) that have been related to memory consolidation (Schabus et
83 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 the 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, termed "targeted memory reactivation". For example, presenting an odor
91 during SWS induces activation of the hippocampus and stabilizes associated memory traces
92 (Rasch et al. 2007; Diekelmann et al. 2010, 2011). Furthermore, whereas studies have found
93 that audio-visual-spatial associations can be stabilized in a targeted manner using specific
94 auditory cues (Rudoy et al. 2009; Creery et al. 2015), these cueing effects can also affect all
95 associations acquired within the same learning context (Oudiette et al. 2013). Van Dongen and
96 colleagues (2012) performed targeted memory reactivation in the magnetic resonance (MR)
97 scanner using auditory cues that were paired with specific object-location associations. Even
98 though no consistent effect of cueing on memory stabilization was found, individual differences
99 in the effect of cueing positively correlated with activity in the hippocampus, thalamus and
100 cerebellum during SWS. Given repeated earlier findings of memory improvement from auditory
101 cues presented during SWS (Oudiette et al. 2013; Ong et al. 2016; Schouten et al. 2017), it is
102 likely that the noisy scanner environment contributed to excessive variability here, with some

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

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

If a cue induces memory reactivation akin to replay, and this indeed plays a role in memory consolidation involving a hippocampal-neocortical trace shift, then one should expect a coordinated neural activation of multiple regions, including the sensory features of the cued memory (in this case visual object features in the occipital cortex (DeYoe et al. 1996) and the spatial layout in the parahippocampal gyrus (Aminoff et al. 2007)), as well as the key mnemonic

124 regions (such as the hippocampus, thalamus, and medial prefrontal cortex). This increased
125 integration of these and other regions would be expected to be beneficial for memory
126 stabilization across sleep and result in an increased involvement of the neocortex in the
127 memory trace.

128

129 **Materials and Methods**

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

133 *Participants*

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

143 and approved by the local ethics committee. Participants were compensated for participation
144 with course credits or a monetary fee.

145 *Procedures*

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

Object-location test

The object-location task was performed in three stages: learning, Test 1 and Test 2. Here, we 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).

183 *MRI Data Acquisition*

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

192 *MRI Data Preprocessing*

193 The fMRI data acquired during Test 1, the sleeping period and Test 2 were preprocessed using
194 standard routines implemented in SPM8 (www.fil.ion.ucl.ac.uk). The first five volumes of each
195 functional EPI run were discarded, and an outlier algorithm was used to check for corrupted
196 slices from each image and replaced using between-volume interpolation. The functional
197 images were then realigned, and coregistered to the structural image, spatially normalized to
198 the Montreal Neurological Institute (MNI) EPI template (resampled at voxel size 2 x 2 x 2 mm),
199 and smoothed using a Gaussian kernel (8 mm full-width at half maximum, FWHM). The
200 structural images were segmented into gray matter, white matter, cerebrospinal fluid, and
201 residual compartments (outside brain and skull) using the unified segmentation algorithm as
202 implemented in SPM8. These compartments were used as masks to extract the mean intensity

203 level across the whole time-series, and entered as compartment regressors to account for
204 effects related to non-specific signal fluctuations.

205 *Network analysis during cueing*

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

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

224 correlation coefficients of beta time-series were computed to quantify pairwise functional
225 connectivity (Rissman et al. 2004) for each condition separately (i.e., cue sounds and control
226 sounds). The connectivity matrices were thresholded by preserving only significant connections
227 ($p < 0.05$ False discovery rate (FDR) corrected) and setting all other connections, as well as all
228 negative correlations to zero. Following the procedure used by Power and co-workers (2011),
229 modular network structure was first derived across all sound presentations using a connectivity
230 matrix based on the 116 anatomical regions defined by the AAL (Automated Anatomical
231 Labeling) atlas (Tzourio-Mazoyer et al. 2002). Here, beta time-series were averaged across
232 voxels of the region, and a 116 x 116 region-by-region connectivity matrix was constructed.
233 After thresholding this connectivity matrix (edges > 0, $p < 0.05$ FDR corrected), the matrix was
234 parcellated into subnetworks using modularity detection according to the Louvain method
235 (Blondel et al. 2008). Next, each voxel in the whole-brain voxel-wise connectivity matrix was
236 assigned to a module, allowing the calculation of their participation coefficient ranging from 0
237 (provincial hub: connections are only present within its module) and 1 (connector hub:
238 connections are only present with other modules; Power et al., 2013). The resulting
239 participation coefficient maps for cue sounds and control sounds were contrasted on an
240 individual subject level and this contrast was subsequently tested at the second level using a
241 one-sample t-test.

242 *Psychophysiological interaction (PPI) analysis during cueing*

243 The previous analysis determined which of the regions increased in global inter-modular
244 network integration. To determine which regions in other modules of the brain were connected

245 with the 'hub' (the region found to display increased network integration during cueing), we
246 performed a follow-up seed-based functional connectivity analysis. This psychophysiological
247 interaction (PPI) analysis served to determine which brain regions were more connected with
248 the hub during the presentation of cue versus control sounds. Therefore, we extracted the
249 physiological signal time series from the seed region found in the network analysis (cluster-
250 forming threshold at $Z > 2.33$) to serve as a physiological regressor. This was entered into a
251 general linear model (GLM) along with the psychological variable (cue versus control sounds)
252 and the psychophysiological interaction regressor. Small-volume correction was used for the
253 bilateral parahippocampal gyri, as this region was activated by the object-location retrieval task
254 (van Dongen, Takashima, et al. 2012), and the thalamus and hippocampus, as these are smaller
255 structures hypothesized to be involved in memory reactivation. Here, anatomical masks
256 implemented in the IBASPM 71 Atlas of the WFU Pick Atlas Tool in SPM were used (Iturria-
257 Medina et al. 2007).
258
259 *Activity analysis during pre- and post-sleep test*
260 We next assessed whether network integration of visual cortex during cueing in SWS results in
261 increased involvement of cortical associative regions at retrieval. For this reason, we contrasted
262 the neural activations observed during the pre-sleep (Test 1) and post-sleep (Test 2) tests, and
263 related this contrast to the participation coefficient of the occipital cortex as observed in the
264 graph theory analysis (see Results section). The fMRI data for Test 1 and Test 2 were analyzed in
265 one GLM. Similar to van Dongen and colleagues (2012), this model included regressors for cued
266 and uncued associations, which were modeled as delta functions to be convolved with the

267 canonical hemodynamic response function (HRF), along with temporal derivatives provided by
268 SPM8. The design matrix included six regressors of no interest per test session to account for
269 head movement using the realignment parameters, as well as compartment regressors
270 accounting for non-specific signal fluctuations. Furthermore, a high-pass filter with a cutoff
271 period of 128s was implemented to remove low-frequency fluctuations from the time series.
272 Parameter images were generated on the first level (for each individual participant) based on a
273 contrast between all trials of Test 1 and all trials of Test 2, to assess changes in retrieval activity
274 from the pre- to the post-sleep test. We collapsed across cued and uncued associations
275 because network participation of the occipital cortex was not specifically related to only the
276 cued associations in the learned set (see 'Results'). These contrast images were entered into a
277 one-sample t-test on the second level, with the participation coefficient of the occipital cortex
278 during cue versus control sound presentation entered as a covariate. Small-volume correction
279 was used for the bilateral parahippocampal gyri, as this region was activated by the object-
280 location retrieval task and cue-sound presentation (van Dongen, Takashima, et al. 2012). Here,
281 an anatomical mask of the parahippocampal gyrus implemented in the IBASPM 71 Atlas of the
282 WFU Pick Atlas Tool in SPM was used (Iturria-Medina et al. 2007).
283

284 **Results**

285 *Network changes following cueing*

286 We probed changes in network dynamics in response to the presentation of cue sounds versus
287 control sounds. The presentation of cue sounds was hypothesized to induce reactivation of the
288 corresponding memory trace, which would presumably be reflected in increased integration of

289 areas that represent sensory features of the cued memory representation, e.g. occipital cortex.
290 Indeed, we found a specific increase in the participation coefficient of early visual (occipital)
291 cortex (peak MNI coordinates: *x,y,z* = [4, -70, 6], z_{21} = 3.12, cluster-forming threshold at Z>2.33,
292 corrected for the whole brain at $p < 0.05$ using Gaussian Random Field Theory), indicating that
293 occipital cortex displays an increased inter-modular connectivity when presented with cue
294 sounds during deep sleep. The region observed overlaps with regions that were activated in
295 response to the object-location task during Test 1 (see figure 2b). This overlap may be indicative
296 of replay of the original visuo-spatial neural memory trace in response to auditory cues
297 presented during sleep, in the absence of any external visual input.
298 To determine what brain regions this area in the occipital cortex preferentially connects to
299 when recruited in the network by the auditory cues, we performed a psychophysiological
300 connectivity analysis. The seed was defined as the region in cortex that displayed an increase in
301 participation coefficient during cueing (cluster-forming threshold at $z > 2.33$). The task contrast
302 was formed by the presentation of cue sounds versus control sounds. The occipital cortex was
303 found to be connected to the left hippocampus (peak MNI coordinates: *x,y,z* = [-32, -16, -18],
304 z_{21} = 3.36), right hippocampus (peak MNI coordinates: *x,y,z* = [22, -2, -22], z_{21} = 2.98), left
305 parahippocampal gyrus (peak MNI coordinates: *x,y,z* = [-26, -22, -26], z_{21} = 3.10), left thalamus
306 (peak MNI coordinates: *x,y,z* = [-4, -2, -0], z_{21} = 3.11), and right thalamus (peak MNI coordinates:
307 *x,y,z* = [2, -16, -12], z_{21} = 2.67), and a medial prefrontal region extending from premotor cortex
308 to anterior cingulate cortex and into dorsomedial prefrontal cortex (see figure 3, all cluster-
309 forming threshold at $z > 2.33$, cluster-size corrected for the whole brain, or reduced search
310 regions based on predefined anatomical areas for the bilateral hippocampus, parahippocampal

311 gyrus and thalamus, thresholded at $p < 0.05$ using Gaussian Random Field Theory). Thus, during
312 cueing, the occipital cortex was connected to several regions where memory replay has been
313 shown to occur (the hippocampus, medial prefrontal cortex), and that are involved in
314 oscillatory patterns of activity (spindles, ripples, slow-waves) that allow for systematic
315 interactions between brain regions (e.g., the hippocampus, medial prefrontal cortex and
316 thalamus).

317 *Network dynamics related to behavioral cueing effect*

318 As reported earlier (van Dongen, et al. 2012), memory performance decreased on average (Test
319 1 performance: error = 2.74 ± 0.12 cm; Test 2 performance: error = 3.12 ± 0.14: Δerror = 0.37 ±
320 0.08; $t_{21} = 4.48$, $p < 0.001$), both for cued (Δerror = -0.44 ± 0.11 cm, $t_{21}(3.86, P = 0.001)$), and
321 uncued associations (Δerror = −0.33 ± 0.11 cm, $t_{21}(2.56, P = 0.018)$). There was no effect of
322 cueing on participants' memory accuracy as tested after sleep ($F_{1,21} = 0.83$; $P = 0.374$). Thus,
323 there was forgetting across the sleep/rest session, and this forgetting appeared to be unspecific
324 across cued and uncued object-location associations.

325 However, there was substantial variability in the effect of cueing on retention. Therefore,
326 different neural responses to cueing might explain individual differences in the effect of cueing.
327 Particularly, increased network participation of the occipital cortex could be an index of how
328 much the visual-spatial memory trace became reactivated in a given subject when auditory
329 cues were presented. Indeed, the increased participation coefficient during cue sound
330 presentation was found to be associated (see figure 2) with reduced overnight forgetting of
331 visual-spatial object locations ($\tau_{21} = -0.311$, $p = 0.045$). This relationship was specifically present

for the visual-spatial locations that were associated with sound cues (τ_{21} = -0.338, p = 0.029), but not for other locations (τ_{21} = -0.211, p = 0.174), although the difference in these correlations did not reach significance (Williams-Hotelling test, t = 0.433, p = 0.670).

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

Overnight activity changes related to network integration during cueing

We then considered whether the increased network integration of the occipital cortex during sleep had subsequent effects on neural activity when retrieving object-locations during the post-sleep test. Here, we found that the participation coefficient was related to a positive increase in retrieval-related activity from Test 1 to Test 2 in both the posterior left parahippocampal gyrus (peak MNI coordinates: *x,y,z* = [-16, -32, -18], z_{21} = 4.14), and posterior right parahippocampal gyrus (peak MNI coordinates: *x,y,z* = [16, -32, -18], z_{21} = 3.26, cluster-forming threshold at Z>2.33, corrected for a reduced search volume based on anatomical masks of the left and right parahippocampal gyrus at a threshold of $p < 0.05$ using Gaussian Random Field Theory). Thus, increased network integration of the occipital cortex during cueing had a subsequent effect on the neural regions activated during the retrieval of object-locations at Test 2, thereby increasingly recruiting the bilateral parahippocampal gyrus.

Discussion

Here, we investigated the whole-brain neural network reorganization induced by cued reactivation of memory traces during deep sleep, and its behavioral and neural effects on the subsequent retrieval of those memories. For that purpose, we re-analyzed fMRI data reported in the study by van Dongen and colleagues (2012) using whole-brain connectivity measures. We demonstrate that when participants were in a state of slow-wave sleep (SWS), absent of visual input, and were cued with sounds associated with previously learned visuospatial information, the occipital cortex displayed increased network integration as measured with the participation coefficient. Furthermore, subjects who displayed an increase in cue-induced network

integration of the occipital cortex showed increased memory stabilization. This finding is indicative of a process in which the sound cue induces a global reprocessing of the learning episode, akin to memory replay, which is conducive to subsequent memory retention. Note that while the occipital cortex is not directly related to mnemonic processes, previous studies have shown that cue-triggered reactivation can lead to instantiations of previously experienced episodes in this region (Xu et al. 2012; Ekman et al. 2017). It is likely that this reinstitution is coordinated with other mnemonic regions. Indeed, various regions displayed increased connectivity with the occipital cortex during the presentation of cue sounds: memory and replay-related regions, such as the hippocampus, thalamus and medial prefrontal cortex; and higher-order associative cortices implicated in the encoding of the memory trace, such as the parahippocampal gyrus. These results are in line with a study by Ji & Wilson (2007) that shows coordinated replay between the hippocampus and a perceptual region that was involved in the initial processing of the stimulus (here: the occipital cortex). Cueing during SWS also showed an enduring neural effect at memory retrieval as measured during the post-sleep retrieval test. Specifically, subjects with greater network integration of the occipital cortex during SWS recruited the parahippocampal gyrus more at subsequent retrieval.

It should be noted that the network reorganization found during the presentation of cue sounds was not specifically related to memory stabilization of those object-location associations associated with the cues. The association of network participation with memory stabilization was numerically larger for cued, versus uncued material, but the interaction was not statistically significant. There could be various explanations for this unspecific effect. First, the MRI scanner is a suboptimal environment for auditory presentation during sleep. Therefore,

395 it is conceivable that the acoustic stimuli presented during deep sleep in a noisy environment
396 effectively modulated ongoing brain processes in some individuals, but not others, contributing
397 to large inter-individual differences and lower power of demonstrating this effect than in other
398 studies conducted outside of the MR-scanner environment (Rudoy et al. 2009). Indeed, we
399 observed a non-normal positively skewed distribution of increase in participation coefficient
400 during cueing. There was an overall effect on memory retention for participants who showed
401 increased participation coefficient during cueing – but there did not appear to be a cue-specific
402 memory benefit even when only considering this sub group. It is also possible that the study
403 was underpowered to observe an interaction effect, and that a larger sample would have
404 rendered a significant interaction. Furthermore, it is possible that the cueing had a non-specific
405 effect on all material acquired within the same temporal and spatial learning context (as also
406 suggested by the results reported in Oudiette et al., 2013). Indeed, all object-location
407 associations were acquired within the same learning context; the same scanning session and
408 temporal learning sequence, and most importantly, all objects were located on the same two-
409 dimensional spatial grid. Therefore, it is plausible that the various object-locations became
410 integrated into a unitary map, or spatial schema (Tse et al. 2007; van Buuren et al. 2014) at
411 initial learning and/or during replay (Durrant et al. 2015; Hennies et al. 2016). As such, object
412 locations could have been stored not only with reference to the two-dimensional grid, but also
413 directly or indirectly in spatial reference to the other objects. As such, a sound cue could have
414 reactivated certain object-location associations, but also objects located within the objects'
415 vicinity. In fact, it has been proposed that sleep-dependent memory replay is instrumental in
416 the integration of memories into a cognitive schema (Lewis and Durrant 2011). Thus, while

417 network integration of the occipital cortex during cueing is demonstrated to be positively
418 related to overall memory stabilization of a set of object-location associations, the fact that an
419 integrated spatial schema was cued may have contributed to non-specific reactivation of all
420 object-locations within this set.

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

439 correlations (Schabus et al., 2004; Holz et al., 2012). Moreover, intracranial recordings in
440 humans have found time-locked ripple events during sleep in both the hippocampus and
441 neocortex, the latter being related to memory consolidation (Axmacher et al., 2008).
442 Experimental disruption of hippocampal ripples in rats impaired consolidation of hippocampal-
443 dependent spatial memories (Girardeau et al., 2009). Therefore, during SWS, hierarchically
444 nested oscillations presumably serve to consolidate memories by tightly coupling the neocortex
445 (especially medial prefrontal cortex), thalamus and medial temporal lobes, which are the
446 regions that are displaying an increased interconnectivity during cueing here.

447 Thus, during SWS, there is increased cross-talk between the hippocampus, thalamus and
448 neocortex. If external auditory cueing would induce a reactivation of the visuo-spatial memory
449 traces, one would expect that cortical representation areas corresponding to the modality of
450 the memory trace would be recruited to participate in this cross-talk. Indeed, the occipital
451 cortex displayed an increase in global network integration during cueing, and when probing the
452 neural connectivity changes paired with this increase, we found that this region increases its
453 connectivity with the regions involved in active cross-talk during SWS, namely the
454 hippocampus, thalamus and medial prefrontal cortex. These regions resemble those that are
455 involved in the oscillatory patterns that orchestrate hippocampal-neocortical cross-talk (Mölle
456 et al. 2006; Staresina et al. 2015). At first sight, it might be surprising that the auditory cortex is
457 not recruited in response to auditory cueing. However, it should be noted that a contrast was
458 made between two conditions in which sounds were presented. The critical difference in the
459 contrast is that cue sounds were associated with specific visuo-spatial memories, but not
460 control sounds. It remains the case that one could still expect increased connectivity of the

461 auditory cortex with visual, spatial, and memory regions during cueing, the sounds being
462 associated with the object-locations. As psychophysiological interaction analyses were
463 informed by results of the graph theory analyses, we did not further probe connectivity from an
464 anatomically defined seed in the auditory cortex here. Despite this caveat, it remains to be
465 noted that cueing recruited the occipital cortex to engage with mnemonic regions that have
466 been shown to display increased cross-talk during SWS, and this promoted the stabilization of
467 the associated memory traces in neocortical storage sites.

468 Sleep supports memory retention in a selective manner, and information is prioritized based on
469 perceived future relevance at encoding (Wilhelm et al. 2011; van Dongen, Thielen, et al. 2012)
470 emotional salience (Hu et al. 2006; Wagner et al. 2006; Payne et al. 2007) and consistency
471 across episodes and with prior knowledge (Tamminen et al. 2013; Durrant et al. 2015). Possibly,
472 relevant information is selectively prioritized for active processing during sleep, whereas non-
473 prioritized memory traces are forgotten, perhaps aided by homeostatic regulation of synaptic
474 plasticity through global downscaling (Tononi and Cirelli 2003, 2006; Genzel et al. 2014). The
475 net result is an increase in signal-to-noise for the prioritized memory traces in the neocortex,
476 contributing to memory stabilization. Indeed, this appears to be the case in the
477 parahippocampal gyrus, where an increase in retrieval-related activation of the posterior
478 parahippocampal gyrus at post-sleep test versus pre-sleep test was related to an increase in
479 network participation of the occipital cortex during cueing. The parahippocampal gyrus is
480 known to be involved in representing the local visual environment (Epstein and Kanwisher
481 1998) and learning spatial layouts (Aguirre et al. 1996; Epstein et al. 1999). The increased
482 involvement of the parahippocampal gyrus during post-sleep retrieval is consistent with a role

483 of the parahippocampal gyrus in binding together low-level visual information from
484 downstream occipital cortex. Notably, the parahippocampal gyrus was activated already during
485 retrieval at the pre-sleep test, showed selective increased activation in response to cueing, and
486 displayed increased connectivity with the occipital cortex during cued reactivation, as originally
487 reported in van Dongen & colleagues (2012). The results reported here further elucidate the
488 mechanisms involved in targeted memory reactivation by taking a network perspective.

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

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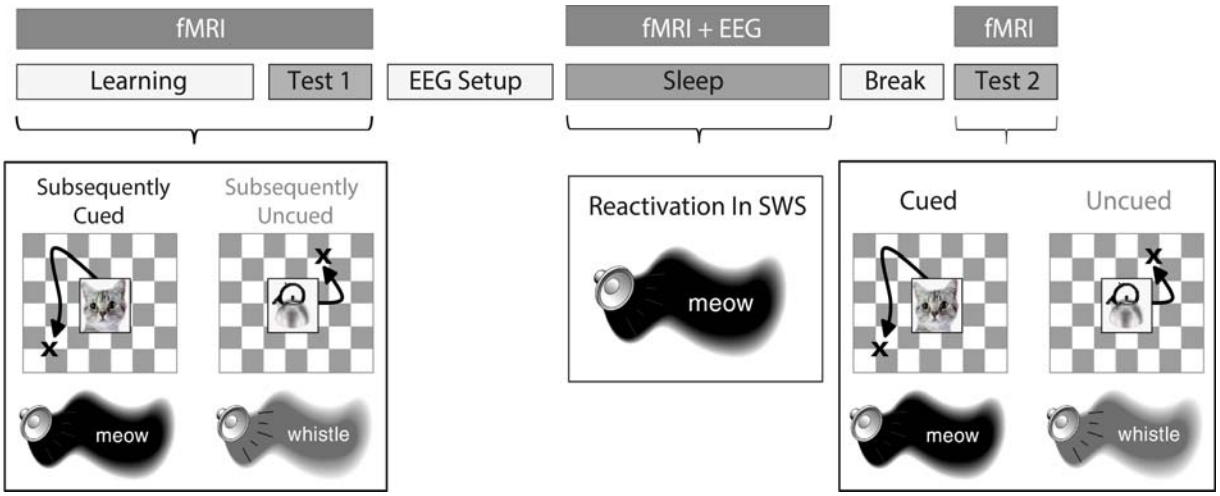
655

656 **Figures**

657 Figure 1



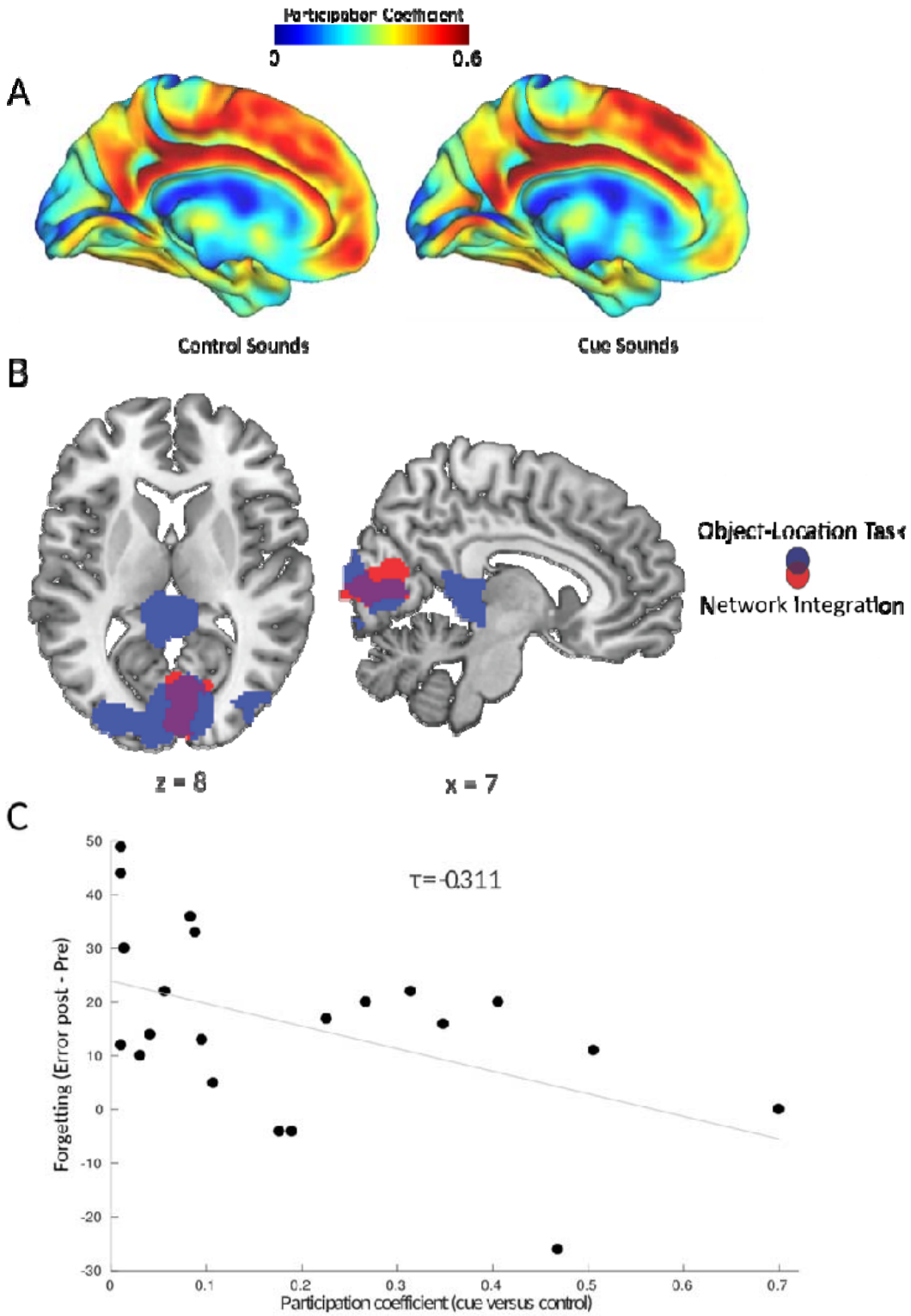
658

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

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

666

667 Figure 2



668

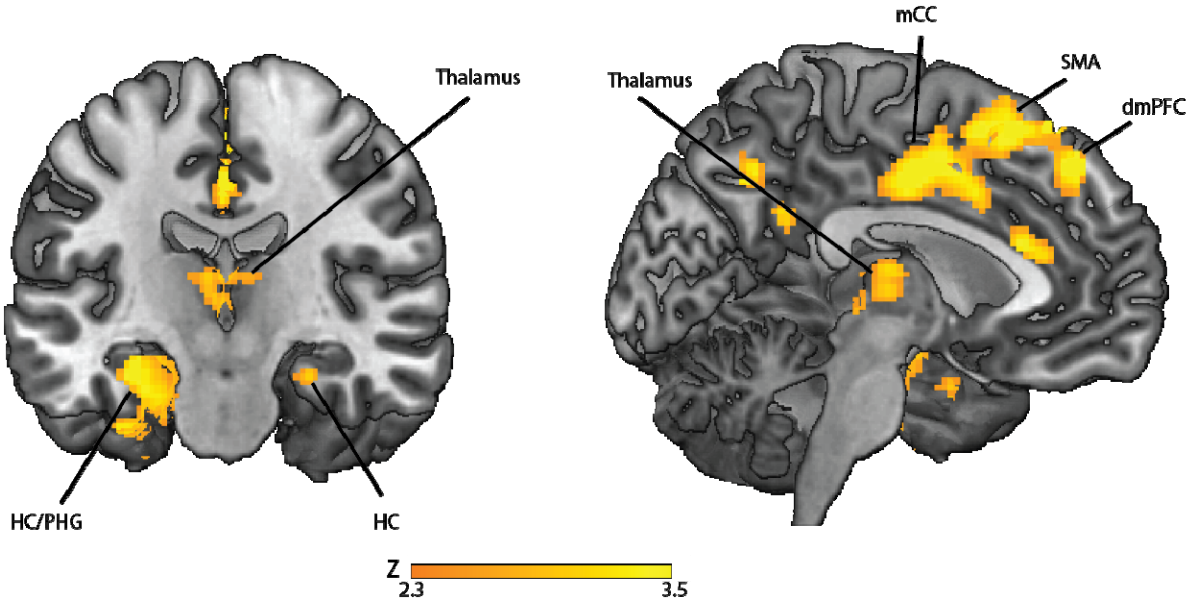
Replay-related changes in network integration predict behavioral memory stabilization.

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).

682 Figure 3



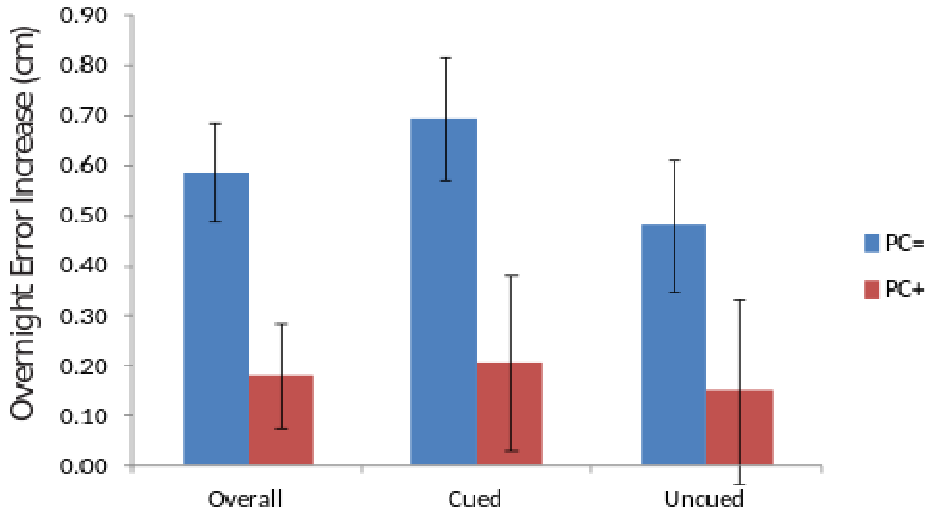
683

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

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

689

690 Figure 4

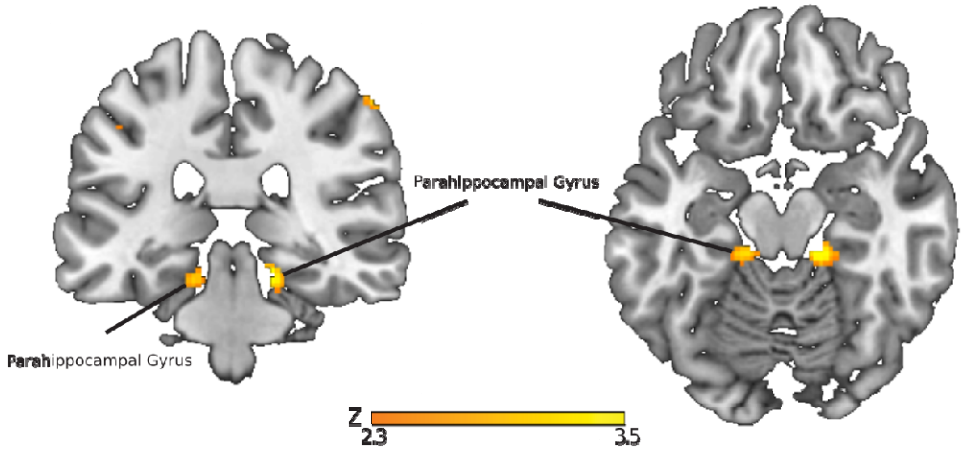


691
692 ***Overnight forgetting, split by network integration in occipital cortex***

693 *The bar graph depicts the change in error distance between the pre-sleep Test 1 and the post-sleep Test*
694 *2 for a group where there was an increase in network integration (as measured with the participation*
695 *coefficient) in occipital cortex (PC+) and a group where no such increase was found (PC=). The groups*
696 *were defined using a median split on the participation coefficient values acquired from the occipital*
697 *cortex in the cue sounds > control sounds comparison. The error distance is operationalized as the*
698 *distance between the location where the object is placed at test, and the actual object location as*
699 *presented during the learning phase. A higher overnight error increase indicates a reduction in accuracy*
700 *of placement across sleep, indicative of increased forgetting. The group where cueing induced increasing*
701 *network integration in the occipital cortex (PC+) displayed reduced forgetting overall, indicating that, in*
702 *this group, cueing stabilizes memories overnight.*

703

704 Figure 5



705

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

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

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

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

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