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Abstract

People retain more new verbal episodic information for at least 7 days if they rest for a few minutes after learning than if they attend to new information. It is hypothesised that rest allows for superior consolidation of new memories. In rodents, rest periods promote hippocampal replay of a recently travelled route, and this replay is thought to be critical for memory consolidation and subsequent spatial navigation. If rest boosts human memory by promoting hippocampal replay/consolidation, then the beneficial effect of rest should extend to complex (hippocampal) memory tasks, for example tasks probing associations and sequences. We investigated this question via a virtual reality route memory task. Healthy young participants learned two routes to a 100% criterion. One route was followed by a 10-minute rest and the other by a 10-minute spot the difference game. For each learned route, participants performed four delayed spatial memory tests probing: (i) associative (landmark-direction) memory, (ii) cognitive map formation, (iii) temporal (landmark) order memory, and (iv) route memory. Tests were repeated after 7 days to determine any long-term effects. No effect of rest was detected in the route memory or cognitive map tests, most likely due to ceiling and floor effects, respectively. Rest did, however, boost retention in the associative memory and temporal order memory tests, and this boost remained for at least 7 days. We therefore demonstrate that the benefit of rest extends to (spatial) associative and temporal order memory in humans. We hypothesise that rest allows superior consolidation/hippocampal replay of novel information pertaining to a recently learned route, thus boosting new memories over the long term.

INTRODUCTION

People retain more new verbal episodic information (e.g., word lists and stories) if they rest for a few minutes after learning than if they attend to new information (Cowan et al., 2004; Della Sala et al., 2005; Dewar et al., 2007, 2012; Craig et al., 2014). This rest-related benefit in memory retention, which is not dependent on explicit rehearsal (Dewar et al., 2014) and unlikely to be due to reduced retrieval interference, is long-lasting, remaining for at least 7 days (Dewar et al., 2012, 2014; Alber et al., 2014).

It is hypothesised that new memories are consolidated, i.e. strengthened, better during rest than during periods of sensory stimulation (Wixted, 2004; Dewar et al., 2009, 2012; Mednick et al., 2011), perhaps due to minimal encoding of novel information (Wixted, 2004; Mednick et al., 2011; Dewar et al., 2012). Research suggests that consolidation is associated closely with hippocampal replay, i.e. the automatic reactivation of recent experiences (Foster and Wilson, 2006; Tambini et al., 2010; Carr et al., 2011).

Hippocampal replay has been studied predominantly in rodents using spatial memory paradigms. During movement, hippocampal place cells fire when the animal holds a particular location in space (Jackson et al., 2006; Davidson et al., 2009; Karlsson and Frank, 2009). Importantly, during pauses in movement, i.e. rest and sleep, hippocampal place cells re-fire in the same forward, and reverse, direction as during the initial experience (Foster and Wilson, 2006; Carr et al., 2011). Replay is hypothesised to be a critical mechanism for successful memory and spatial navigation functions, including (i) the integration and consolidation of (spatial) associative information (Mahut and Moss, 1982; Degonda et al., 2005; Ramadan et al., 2009), (ii) the integration of (spatial) experiences in to a temporal order (Foster and Wilson, 2006; Derdikman and Moser, 2010; Gupta et al., 2010), and (iii) the formation of a cognitive map (Tse et al., 2007; Gupta et al., 2010). Indeed, disruption of replay impairs spatial memory in rodents (Girardeau et al., 2009; Ego-Stengel and Wilson,

2011). Moreover, replay magnitude is associated positively with subsequent (spatial) memory in rodents (Foster and Wilson, 2006; Carr et al., 2011) and with subsequent visual associative memory in humans (Tambini et al., 2010). Importantly, the predominance of replay during periods of relative immobility, i.e. rest and sleep (Carr et al., 2011; Foster & Wilson, 2006) hints at links between rest-related increases in (i) replay and (ii) memory retention.

To date the effect of rest on memory in humans has been examined only via simple memoranda, i.e. lists of unrelated words and short stories. If, as hypothesised, rest boosts memory by promoting hippocampal replay/consolidation of recent experiences, then the beneficial effect of rest in humans should extend to complex hippocampal tasks, for which replay has been demonstrated, i.e. associative memory and sequences. In the study reported here we examined this hypothesis via a route memory task. This task tapped into a number of such hippocampal functions and resembled the task used in rodent replay research, thus bridging the divide between human and rodent work. Participants learned two routes, each followed by one of two 10-minute delays: rest or a spot the difference game. Participants performed four spatial memory tests at two time points, 10-20 minutes and 7 days after route learning. We predicted that rest would boost the hippocampal replay/consolidation of new information from a recently experienced route, and would thus improve retention across our test measures.

METHODS

Ethics Statement

This research was approved by the University of Edinburgh's Psychology Research Ethics Committee (Ref: 446-1112). All participants provided their informed consent in writing prior to taking part in our research.

Participants

Thirty-six healthy young adults (8 males, 28 females; mean age = 20.78 years, SD = 2.15) were recruited. All participants had normal or corrected-to-normal vision.

Design

The study took place over 2 sessions, Session 1 and Session 2, which were separated by 7 days. Figure 1 illustrates an overview of the procedure. In Session 1 participants learned two routes through different virtual environments, each followed by one of two delay conditions. One route was followed by 10-minutes of wakeful rest, and the second by a 10-minute spot the difference game. The order of learned routes and delay conditions was counterbalanced across participants using 4 rotations (A1-B2, B1-A2, A2-B1 and B2-A1, where A and B = learned routes, and 1 and 2 = delay conditions). Figure 1 shows the example order: Route A → wakeful resting → Route B → spot the difference game. After completion of the second delay condition, retention of spatial information pertaining to the learned routes was probed via 4 surprise memory tests (10-20-min recall) that comprised: (i) an associative (landmark-direction) memory test, (ii) a cognitive map test, (iii) a temporal order memory test, and (iv) a route memory test. The four memory tests were repeated after 7 days (Session 2; 7-day recall), in the same order as during 10-20-min recall. We applied a within-subjects design with within-subjects factors delay condition (wakeful rest vs. spot the difference) and test time (10-20 minutes vs. 7 days).

<<INSERT FIGURE 1 ABOUT HERE>>

Materials. Virtual environments were created using the virtual reality environment builder "Abashera Maze Editor" (copyright Magnus Norman Software). A simple grid-shaped, virtual environment was created for participants to roam within during the familiarisation phase. Virtual environments used during the learning phase, and route memory test during test phases were more complex in their design. As illustrated in Figure 2, the two environments were mirror images of one another, and thus were matched in terms of their environmental size and layout. The virtual environments used during learning and test differed aesthetically in that two different textures were used in each environment, one on the floor and one on the walls. There was no overlap between environments in the textures used.

Each environment contained a single, long, indirect route from a start point to a goal location. Routes contained eight decision points, i.e. junctions, with a different landmark positioned at each decision point, eight in total. There was no overlap in landmarks between virtual environments. Abstract landmarks (as illustrated in Figure 2 and 3) were used so as to minimise the potential use of verbal codes. All environments contained a white fog that ensured that distant landmarks, environmental features and route direction were obscured from view. The virtual environments were presented via a 17-inch laptop computer screen.

<<INSERT FIGURE 2 ABOUT HERE>>

Procedure

Familiarisation. Participants were first instructed on how to control movement through a simple virtual environment. Movement was possible in two dimensions, forwards/backwards movement and yaw movement (i.e. right/left rotations). Forward and

reverse movement was controlled via the ↑/↓ keyboard arrow keys. Yaw movement was controlled via the ←/→ keyboard arrow keys. Participants were free to roam within the simple virtual environment and were instructed to inform the experimenter when they were familiar and comfortable with the control of movement, after which they proceeded to the learning phase.

Learning. Participants learned two routes to a 100% criterion. Route learning was broken into learning cycles; each learning cycle comprised of one learning trial followed by one probe trial. In a learning trial, controlling their movement via the arrow keys, participants were required to follow a series of large, red guidance arrows. Guidance arrows were presented on the floor of the virtual environment and indicated a pre-set route between the start point and goal location (see Figure 2). After reaching the goal location, participants performed a probe trial. In a probe trial, again controlling their movement via the arrow keys, participants were required to recall and navigate the route presented during the prior learning trial. No guidance arrows were presented during probe trials. If they made an error during a probe trial, i.e. a deviation from the correct route by making an incorrect turn at a decision point (junction), the participant was immediately instructed that they had made an error, and the probe trial ceased. The participant was not provided with the correct direction to turn at the location of the error. Instead they started a new learning cycle, in which they were again presented the route during a learning trial, followed by a further probe trial. The participant exited the learning phase and entered the delay phase as soon as they were able to successfully recall the route without making a single error. The order in which the two routes were learned was counterbalanced between-participants. While there was no time limit on each learning/probe trial, or on the learning phase as a whole, a maximum of 10 learning cycles were allowed.

Delay Phase. During the delay phase, participants underwent one of two 10-minute delay conditions in which they either: (i) rested wakefully, or (ii) performed a spot the difference game. Participants were given no prior indication as to what a delay condition would entail, until instructions were provided at the beginning of a delay condition.

In the 10-minute *wakeful rest* delay condition, participants were asked to sit quietly in a dimly-lit testing room and relax while the experimenter left the room to "set up the next section of the experiment" (Dewar et al., 2012; Craig et al., 2014). Care was taken to ensure that the laboratory was devoid of any visual and/or audible sensory cues.

In the 10-minute *spot the difference* delay condition, participants were asked to perform a spot the difference game for the duration of the 10-minute delay (Dewar et al., 2012). Participants performed 20 spot the difference trials in silence; each trial was 30 seconds in duration. A trial comprised of the presentation of a pair of real-world photos on a 17-inch laptop computer screen. Photo pairs were identical other than for two discrete differences. Participants were instructed to search for differences between photos in a pair, and to point out any discovered differences to the experimenter, using their index finger.

Test Phase. Following the completion of the second delay condition, participants underwent four surprise delayed (10-20 min) recall tests for the *first* learned route in the order of: (i) associative (landmark-direction) memory test, (ii) cognitive map test, (iii) temporal (landmark) order memory test, (iv) route memory test. The same tests were then repeated, in the same order, for the *second* learned route. The route memory test was positioned last to ensure that participants were not exposed to the environment and route again prior to performing the other three memory tests. Memory for both routes was probed at the end of Session 1 (after 10-20 min) rather than after each delay condition separately to ensure that memory tests for the latter learned route would come as a surprise. Thus, the likelihood of

conscious rehearsal of the routes and/or environmental features should be minimal (Dewar et al., 2012).

The *associative memory test* was applied to examine memory for landmark-direction associations pertaining to the learned routes (e.g. triangle-right turn). Participants were sequentially presented the eight landmarks from the learned route, via a 17-inch laptop computer screen. Landmarks were presented in a random order that was consistent across all participants. No contextual information from the environment was shown (see Figure 3a). For each presented landmark, participants were asked to verbally state whether they had turned left, right, or had gone straight on.

The *cognitive map test* was applied to assess the accuracy of an internally generated mental map of the experienced environments (Tolman, 1948). Participants performed eight trials. In each trial, two of the eight landmarks from the learned route were presented, i.e. a landmark A-B pair. Landmark pairs were presented via a 17-inch laptop screen on a white background, i.e. no contextual information from the environment was shown (see Figure 3b). The order of landmark pair presentation was random but consistent across participants. For each landmark A-B pair, participants were asked to imagine that they were stood at landmark A within the environment. They were then asked to verbally state the direction of landmark B relative to their position at landmark A, assuming their starting position in the environment was facing North. Participants were asked to provide responses based on an eight-point compass, e.g. North, North East, East, South East etc.

The *temporal order memory test* was applied to examine memory for a sequence of spatial events, i.e. the order of landmarks in a travelled route. Participants were provided with scale cut-outs of the eight landmarks from the route. Landmarks were presented without any contextual information from the environment (see Figure 3c). Participants were required to

place the cut-outs of landmarks in the order in which they had been positioned during route learning, from the start point to the goal location.

The *route memory test* was applied to examine memory for the learned route within its original context, i.e. this test assessed participants' ability to retrace their steps along the learned route. Participants were placed at the start point of the earlier learned route and asked to recall the route taken between the start point and goal location (see Figure 3d). As during learning, participants controlled movement using the four keyboard arrow keys. No guidance arrows were presented during the route memory test. If participants made an error, i.e. if they deviated from the correct route, they were immediately instructed that they had made an error, were returned to the start point, and were asked to try to recall the route again. Participants exited the route memory test when they could recall the route without error. While there was no time limit on each test trial, or the route memory test on the whole, a maximum of 10 attempts was allowed.

<<INSERT FIGURE 3 ABOUT HERE>>

Upon completion of the four delayed memory tests for *both* learned routes, participants completed a detailed post-experimental questionnaire. Participants were required to provide ratings of difficulty for: (i) route learning, and (ii) each of the delayed recall tests. Ratings of difficulty were on a scale of 1 (very easy) to 5 (very difficult). Participants were also asked to provide information regarding: (i) whether they had expected to perform delayed recall tests for information associated with the learned routes, and (ii) whether they had spontaneously/intentionally thought about the virtual environments, learned routes and/or landmarks, during one or both delay conditions. If intentional thoughts were reported

participants were asked a follow-up question of whether any information was actively rehearsed during one or both delay conditions. Ratings of past experience using computers, playing video games and using virtual reality software were also collected (Rodgers et al., 2012).

After 7 days, participants returned to the lab for Session 2, in which they performed the same four memory tests, in the same order as during the 10-20-min recall test of Session 1. A different post-experimental questionnaire was then completed to establish whether participants expected to again perform recall tests relating to the learned routes, and whether they had spontaneously/intentionally thought about any information pertaining to the routes learned 7 days earlier between Sessions.

Scoring

Associative Memory Test. The total number of correct responses, i.e. the number of correctly stated directions travelled at decision points (e.g. right turn), was extracted. Raw scores were then transformed into a percentage of correct responses by dividing the number of correct responses by the total number of landmarks, i.e. (number of correct responses / 8) * 100.

Cognitive Map Test. The total number of correct responses, i.e. the number of correct direction judgments (e.g. North East), was extracted. Raw scores were then transformed into a percentage of correct responses by dividing the number of correct responses by the total number of landmarks, i.e. (number of correct responses / 8) * 100.

The accuracy of responses was also calculated. Based on an eight-point compass, the number of points of error was calculated for each trial. Error scores comprised of: 0 = correct

response, 1 = 45 degrees incorrect, 2 = 90 degrees incorrect, 3 = 135 degrees incorrect and 4 = 180 degrees incorrect. For example, assuming the correct direction was West, if the participant responded with "South", an error score of 2, i.e. 90 degrees of error, would be allocated. A mean error score was calculated for each participant (i.e. sum of errors / 8).

Temporal Order Memory Test. The accuracy of responses was calculated by extracting for each landmark the number of positions of error, i.e. the deviation between correct landmark position and recalled landmark position. For example, if landmark 6 was incorrectly placed in position 3, this would be an error score of 3, i.e. 3 positions from the correct response. Alternatively, if landmark 2 was incorrectly placed in position 7, this would be an error score of 5, i.e. 5 positions from the correct response. Error scores could range between 0 (correct response) and 7. A mean accuracy error score was calculated for each participant, (i.e. sum of accuracy errors / 8).

We also calculated a Spearman's rank coefficient for the recalled order of route landmarks. All Spearman's rank coefficients were transformed using Fisher r-to-z transformations for analysis.

Route Memory Test. The total number of errors made, i.e. the number of deviations from the correct route, was extracted.

Statistical Analyses

The alpha level was set to .05 for all analyses. For each of the four memory tests we carried out repeated measures ANOVAs with factors delay condition (wakeful rest vs. spot the difference) and test time (10-20 min vs. 7 days).

RESULTS

Route Learning

Participants made an average of 0.69 errors (SEM = 0.12) in route learning prior to the wakeful rest delay condition and an average of 0.75 errors (SEM = 0.13) in route learning prior to the spot the difference delay condition. No significant main effect of delay condition was observed in the number of errors made during route learning ($F(1,35)$ = 0.327, p = .571). Thus, as expected, there was no difference between delay conditions in participants' ability to learn a route that was presented prior to the onset of a delay condition.

Associative Memory Test

Mean percentage correct scores are shown in Figure 4. Performance was significantly higher in the wakeful rest delay condition than in the spot the difference delay condition ($F(1,35)$ = 12.597, p = .001). Performance decreased significantly over 7 days ($F(1,35)$ = 6.807, p = .013). However, superior performance in the wakeful rest delay condition was sustained after 7 days, with no additional benefit after 7 days, i.e. no significant interaction between delay condition and time ($F(1,35)$ = 0.005, p = .943).

<<INSERT FIGURE 4 ABOUT HERE>>

Cognitive Map Test

    Total Correct Responses. The total number of correct responses was only marginally above chance (1 / 8 = 0.125) in both delay conditions during both test times. In the wakeful rest delay condition, the mean percentage correct response score was 0.28 (SEM = 0.03) after 10-20 minutes and 0.22 (SEM = 0.03) after 7 days. In the spot the difference delay condition, the mean percentage correct response score was 0.21 (SEM = 0.02) after 10-20 minutes and

0.21 (*SEM* = 0.03) after 7 days. There was no significant main effect of delay condition ($F(1,35)$ = 3.204, p = .082), time ($F(1,35)$ = 1.712, p = .199), or a significant interaction between delay condition and time ($F(1,35)$ = 2.356, p = .063).

Accuracy of Responses. In the wakeful rest delay condition, the mean error score (max = 4) was 2.53 (*SEM* = 0.08) after 10-20 minutes and 2.30 (*SEM* = 0.08) after 7 days. In the spot the difference delay condition, the mean error score was 2.45 (*SEM* = 0.13) after 10-20 minutes and 2.31 (*SEM* = 0.07) after 7 days. There was no significant main effect of delay condition ($F(1,35)$ = 0.111, p = .741). Error scores increased significantly over 7 days ($F(1,35)$ = 5.551, p = .024), however there was no significant interaction between delay condition and time ($F(1,35)$ = 0.292, p = .592). Taken together, the low number of correct responses and the substantial directional errors show that the cognitive map task was very difficult for participants, leading to floor effects in both conditions.

Temporal Order Memory Test

Accuracy of Responses. Mean error scores (max = 7) are shown in Figure 5. Error scores were significantly lower in the wakeful rest delay condition than in the spot the difference delay condition ($F(1,35)$ = 16.605, p < .001). Error scores increased significantly over 7 days in both delay conditions ($F(1,35)$ = 6.060, p = .010). However, the lower error score in the wakeful rest delay condition relative to the spot the difference delay condition was sustained after 7 days, with no additional benefit after 7 days, i.e. there was no interaction between delay condition and time ($F(1,35)$ = 0.365, p = .550).

<<INSERT FIGURE 5 ABOUT HERE>>

Spearman's Rank Analysis. The mean recalled positions of landmarks vs. the correct positions of landmarks during learning are shown in Figure 6. The following analysis examined the strength of the relationship between *correct* order of landmarks and *recalled* order of landmarks. Comparison of *z* scores revealed superior performance in the wakeful rest delay condition than in the spot the difference delay condition ($F(1,35) = 15.030$, $p < .001$), i.e. wakeful rest boosted memory for the order in which landmarks were presented during route learning. Performance decreased significantly in both delay conditions over 7 days ($F(1,35) = 4.783$, $p = .036$). However, the superior performance in wakeful rest delay condition, relative to the spot the difference delay condition, was sustained after 7 days, with no additional benefit after 7 days, i.e. no significant interaction between delay condition and time ($F(1,35) = 0.823$, $p = .370$).

<<INSERT FIGURE 6 ABOUT HERE>>

Route Memory Test

Participants made very few errors in the route memory test, irrespective of delay condition. In the wakeful rest delay condition the mean number of errors made was 0.67 (*SEM* = 0.14) in the 10-20-min recall test and 1.92 (*SEM* = 0.14) in the 7-day recall test. In the spot the difference delay condition the mean number of errors made was 0.89 (*SEM* = 0.14) in the 10-20-min recall test and 2.01 (*SEM* = 0.15) in the 7-day recall test. There was no significant main effect of delay condition in the number of errors made ($F(1,35) = 2.046$, $p = .161$). The

number of errors increased significantly over 7 days ($F(1,35) = 88.927$, $p < .001$). However, there was no interaction between delay condition and time ($F(1,35) = 0.179$, $p = .674$).

Our procedure meant that participants performed the associative memory, cognitive map and temporal order memory tests prior to the route memory test. It is possible that performing these three tests prior to the route memory test led to the cuing of weaker memory traces and subsequent masking of a rest-related benefit in route recall. To investigate this, we tested 12 further participants (5 males, 7 females; mean age = 20.08 years, SD = 1.83) who performed only the route memory test during 10-20-min recall.

As in the main experiment, participants made very few errors in the route memory test. In the wakeful rest delay condition the mean number of errors made was 0.83 (SEM = 0.21). In the spot the difference delay condition the mean number of errors made was 0.91 (SEM = 0.26). There was no significant main effect of delay condition in the number of errors made ($F(1,11) = 0.167$, $p = .658$). Importantly, the number of errors made did not differ significantly between participants who received only the route memory test and those who received the three other tests prior to the route memory test, neither in the wakeful rest delay condition ($t(46) = -0.815$, $p = .419$); nor in the spot the difference delay condition ($t(46) = -0.265$, $p = .792$). Thus, there is no strong evidence to suggest that a rest-related improvement in the route memory test could have been masked by the preceding associative memory, cognitive map and temporal order memory tests in the main experiment.

Between-route Differences

Across all learning and test measures there was no significant main effect of route, or significant interactions between route and delay condition, or route and time of recall (all p > .05). Thus, there were no significant differences in the ability to learn Route A and Route B,

nor the ability to retain information from Route A and Route B. This suggests that the routes/environments employed in this study were well matched.

Effect of Past Experience

When including self-ratings of past-experience (using computers, playing video games and using virtual reality software) (Rodgers et al., 2012) as a covariate, no significant main effect of past experience was observed in: (i) the number of errors made during route learning ($F(1,34) = 1.934$, $p = .173$), (ii) the associative memory test ($F(1,34) = 0.069$, $p = .795$), (iii) the cognitive map test (total correct: $F(1,34) = 2.708$, $p = .108$; accuracy: $F(1,34) = 1.347$, $p = .254$), (iv) the temporal order memory test (accuracy: $F(1,34) = 1.742$, $p = .196$; spearman's rank $F(1,21) = 1.398$, $p = .242$), or (v) the route memory test ($F(1,34) = 0.162$, $p = .690$). Moreover, no significant interactions were observed between past experience and time (all $p > .065$), or between past experience, time and delay condition (all $p > .261$).

Post-experimental Reports

Twelve participants (33.33 %) reported expecting to be tested on information from the learned routes during the 10-20-min recall test of Session 1. No results changed when these participants were removed from the analyses. Seventeen participants (47.22 %) reported having (intentional or spontaneous) thoughts about the learned routes and environments during one or both of the delay conditions. When these 17 participants were removed from our analyses, significant main effects of time in the associative memory test and the temporal order memory test (Spearman's rank analysis) were no longer observed, i.e. performance in these measures was not significantly poorer after 7 days than after 10-20 minutes in either delay condition. No other change in results was observed. Of the 17 participants who reported spontaneous/intentional thoughts related to the learned routes, 5 participants (13.89 % of all participants) reported consciously rehearsing information from one or both learned routes

during one or both of the delay conditions. When these 5 participants were removed from our analyses, no change in results was observed.

Twenty-three participants (63.89 %) reported expecting to be tested on information from the learned routes during the 7-day recall test of Session 2. Twenty-two participants (61.11 %) reported having (intentional or spontaneous) thoughts about the learned routes and environments in the 7 days between Session 1 and Session 2. When these 22 participants were removed from our analyses, significant main effects of time in the cognitive map test (accuracy of responses) and temporal order memory test (Spearman's rank analysis) were no longer observed, i.e. performance in these measures was not significantly poorer after 7 days than after 10-20 minutes in either delay condition.

Of the 22 participants who reported spontaneous/intentional thoughts related to the learned routes, 4 participants (11.11 % of all participants) reported consciously rehearsing information from one or both learned routes during one or both of the delay conditions. When these 4 participants were removed from our analyses, no change in results was observed.

Thirteen participants (36.11 %) reported using a memory strategy, comprising of: (i) verbally encoding and recalling the route as a sequence of turns (5 participants; 13.89 % of all participants), (ii) visualising travelling along the route during one or both delay conditions (3 participants; 8.30 % of all participants), (iii) verbally encoding landmark-direction associations e.g. "blue triangle – left turn" (3 participants; 8.30 % of all participants), (iv) using non-specific verbal encoding (1 participant; 2.78 % of all participants), and (v) encoding the route by connecting landmarks as part of a "story" (1 participant; 2.78 % of all participants). When these 13 participants were removed from our analyses, no change in results was observed.

Participants provided ratings of difficulty for route learning and the four memory test measures performed during Session 1 and Session 2. Participants rated difficulty on a scale of 1 (very easy) to 5 (very difficult). Table 1 shows mean ratings of difficulty collapsed across delay conditions.

<<INSERT TABLE 1 ABOUT HERE>>

As in previous research (Dewar et al., 2012; Craig et al., 2014), the majority of participants (N = 30) reported mind-wandering during the wakeful rest delay condition, incidentally recalling the past and thinking about the future.

Effect of Route Position and Position of the Wakeful Rest Delay Condition

Given that our test measures for both learned routes were probed in a single recall test session at the end of Session 1, the delay intervals between learning and delayed (10-20 min) testing varied between the route presented first (~ 20 minutes) and the route presented second (~ 10 minutes). We therefore examined whether delay interval length affected performances in our test measures. No main effect of delay interval length was observed in any of our delayed test measures (all $p > .237$).

DISCUSSION

Our results demonstrate that rest boosts the retention of at least some types of complex hippocampal-dependent memories over the longer term. Specifically, a few minutes of rest, relative to a spot the difference game, improved the retention of associative (landmark-

direction) and temporal order (landmark 1, landmark 2, landmark 3…) information. In both cases, this memory boost persisted for at least 7 days, which supports the hypothesis that rest promotes the consolidation of novel information into stable long-term memory traces, a process thought to be critically dependent on offline hippocampal replay.

The associative memory test measured the retention of landmark-direction associations, i.e. the direction travelled at each decision point (junction) during route learning. The rest-related boost in this test could in principle result from increased retention of verbal information, as found in previous work using verbal material (Dewar et al., 2007, 2012; Craig et al., 2014). To reduce the likelihood of verbal coding of landmark-direction associations we used abstract landmarks. Despite this manipulation, a minority of participants reported verbally encoding information from the learned routes. However, no change in results was observed when these participants were removed from our analyses. It therefore appears unlikely that a rest-related boost in verbal memory could be masquerading as a rest-related boost in landmark-direction memory.

Our associative memory and route memory tests share some common features. For example, in both tests, sound knowledge of landmark-direction associations was required. However, a rest-related boost was observed in the associative memory test, but not the route memory test. It is possible that the lack of visuospatial contextual information in the associative memory test increased test difficulty as fewer visuospatial cues were present than in the route memory test, which was performed in the same context as during route learning. The contextual information in the route memory test may have enabled features such as distance judgement between decision points and the cueing of memories for the upcoming section of the route (Janzen, 2006), thus lowering difficulty. This is unlikely to have occurred in the associative memory test, where no contextual information was presented and landmarks were presented in a randomised order. Indeed, research demonstrates the positive

influence of ambient visual information on haptic spatial memory for real-world scenes (Pasqualotto et al., 2013). Our results indicate a similar effect in spatial memory tests for information pertaining to a newly learned route.

The temporal order memory test measured the retention of a sequence of spatial events. A rest-related boost in memory was even clearer in this test since responses were more accurate when route learning was followed by rest than by the spot the difference game. As argued above, it is unlikely that the rest-related boost in memory can be accounted for by a boost in the retention of verbal codes. Moreover, even after excluding the minority of participants who used verbal codes, the rest-related boost in temporal order memory remained, thus suggesting that rest boosted temporal order memory directly, rather than via verbal memory.

Could processes other than consolidation account for the rest-related boost observed in associative and temporal order memory?

Given the design of our paradigm it is unlikely that the rest-related memory enhancement observed here could be accounted for by reduced retroactive interference at retrieval following the rest delay, as compared to following the spot-the-difference delay. Retrieval interference would have been minimal in both conditions given that the spot-the-difference material differed somewhat from the virtual environment material. Nonetheless, performance was superior following the rest delay than following the spot-the-difference delay.

Our paradigm also rules out the hypothesis that rest had a mere passive and transient effect on the new memory traces. This hypothesis of a 'passive' effect of rest, originating from the sleep/memory field (Ellenbogen et al., 2006), posits that the benefit of rest lasts only until people are exposed to interfering material (Dewar et al., 2012; Mednick, Cai, Shuman, Anagnostaras, & Wixted, 2011; Ellenbogen, Payne, & Stickgold, 2006). Our finding of a

lasting rest-related boost in retention following 7 filled days is incompatible with this passive effect hypothesis.

In contrast, this is not the case for the hippocampal replay/consolidation account, which posits that new memories strengthen over time, thus becoming less susceptible to the detrimental effects of subsequent interfering new information (Dudai, 2004).

There is, however, the possibility that our rest period boosted memory consolidation by allowing participants to intentionally think about/rehearse information pertaining to learned routes. Several participants reported having intentional or spontaneous thoughts pertaining to the learned routes during one or both of the delays. However, the rest-related memory boost did not subside after the removal of these participants (Dewar et al., 2012; Craig et al., 2014). Thus, it is unlikely that the rest-related memory boost observed here can be accounted for merely by such intentional thoughts. A more plausible explanation is that rest can boost memory consolidation automatically (Dewar et al., 2012). This view is in keeping with research demonstrating (i) a long-lived verbal memory boost via rest even when memoranda are unrehearsable (Dewar et al., 2014), and (ii) increased retention of a newly learned route through a virtual environment via sleep (Peigneux et al., 2004; Ferrara et al., 2006, 2008; Wamsley et al., 2010), a behavioral state during which intentional rehearsal is highly unlikely.

This automatic consolidation hypothesis resonates with the hippocampal replay work in rodents, which presumably do not rehearse recently travelled routes intentionally. This work shows that when rodents travel a route through a new spatial environment, hippocampal place cells fire sequentially, continuously encoding the currently held location within space (Jackson et al., 2006; Davidson et al., 2009; Karlsson and Frank, 2009), even when navigating through a virtual environment (Erkstrom et al., 2003; Harvey et al., 2009; Dombeck et al., 2010). Importantly, during brief pauses in movement, i.e. rest, these same

place cells re-fire in the same forward, and reverse direction as during initial travel (Foster and Wilson, 2006; Karlsson and Frank, 2009; Carr et al., 2011), and this re-firing is thought to reflect automatic, offline hippocampal replay of the recently travelled route. This replay is hypothesised to have a critical function in the consolidation of spatial-associative information (Degonda et al., 2005; Girardeau et al., 2009; Ramadan et al., 2009; Ego-Stengel and Wilson, 2011), and the integration of spatial events into a temporal sequence (Foster and Wilson, 2006; Derdikman and Moser, 2010; Fouquet et al., 2010; Gupta et al., 2010; Bellassen et al., 2012).

The rest period in our human study could have been conducive to hippocampal replay/consolidation due to the minimal encoding of new information. It is hypothesised that novel encoding interrupts the consolidation of recently learned material (Craig et al. 2014; Mednick et al., 2011; Dewar et al., 2012). In our spot the difference delay, participants likely encoded much novel picture information. This novel encoding of picture information could have interrupted the consolidation, i.e. hippocampal replay, of the recently learned route. In contrast, the reduced sensory stimulation during the rest delay probably resulted in only minimal novel encoding. This could have provided superior conditions for hippocampal replay/consolidation, resulting in a boost in retention.

We acknowledge that our spatial associative and temporal order tasks were not exclusively spatial, and thus that the rest-related boost in these tasks may be accounted for by hippocampal replay/consolidation of relational memory more generally (e.g. a sequence of events, associative memory) rather than spatial memory specifically (Eichenbaum and Cohen, 2014). Indeed, the hippocampus is associated with a range of relational memory functions (c.f. Konkel and Cohen, 2009; Eichenbaum and Cohen, 2014), including memory for spatial relations (the locations of objects), temporal/sequential relations (the order of objects), and associative relations (the objects that appeared in the same trial) (Konkel et al., 2008; Watson

et al., 2013), and human fMRI work has revealed hippocampal replay of non-spatial visual associative information (Tambini et al., 2010).

It is of note that the benefit of rest remained over the long-term despite all participants having 7 filled days between recall tests. This supports the notion that the few minutes that immediately follow learning form a critical period for the hippocampal replay/consolidation of new memories, determining their retention over the longer term (Dewar et al., 2012; Alber et al., 2014).

In summary, we demonstrate that wakeful rest boosts the long-term retention of new (spatial) associative and temporal memories. We hypothesise that rest allows superior consolidation/hippocampal replay of novel information pertaining to a recently learned route, thus boosting new memories over the long term. Future work should examine the effects of rest in exclusively spatial memory measures.

Acknowledgments

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FIGURE LEGENDS

Figure 1. Experimental Procedure. The study took place over 2 sessions, Session 1 and Session 2, which were separated by 7 days. In Session 1 participants learned two routes, each followed by one of two 10-minute delay conditions (wakeful rest vs. spot the difference game). The order of the two learned routes and two delay conditions was counterbalanced across participants. The figure shows the example order: Route A → wakeful resting → Route B → spot the difference game. After completion of the second delay condition, retention of spatial information pertaining to the two learned routes was examined via four surprise memory tests (10-20-min recall). Participants returned after 7 days (Session 2) and repeated the same memory tests (7-day recall). See Figure 3 for further information on the different memory tests.

Figure 2. Virtual Environments. *Top:* Schematic layout of Environment A (left) and Environment B (right). *Bottom:* Screen captures of Environment A (left) and Environment B (right). The figure demonstrates the symmetrical design of the environments and the long, indirect routes that were learned. The position of the start point ("S") and goal location ("G") are shown alongside the position of all landmarks ("L"). The screen captures illustrate the textures and example of landmarks used in the two environments. The position that the landmarks shown in the screen captures held within each environment are indicated by a darker "L".

Figure 3. Graphical Representations of the Four Recall Tests. *Top*: (3a) associative memory test, (3b) cognitive map test. *Bottom:* (3c) Temporal order memory test, (3d) route memory test. The four memory tests were first performed for Route A, in the order shown in the figure (top left – bottom right). In the associative memory test (3a) participants were asked to verbally state the direction turned at each landmark. In the cognitive map test (3b) participants were asked to provide a judgment of the direction of a target landmark. In the temporal memory test (3c) participants were asked to place cut outs of landmarks in the order in which they had travelled past them during earlier route learning, from the start point to the goal location. In the route memory test (3d), controlling their movement, participants were asked to recall the earlier learned route. The tests were then repeated, in the same order, for the second learned route. All tests were repeated during Session 2, 7 days later.

Figure 4. Associative Memory Test. Mean percentage of correct responses in the associative memory test as a function of delay condition (wakeful resting vs. spot the difference) and time of recall (10-20 min vs. 7 day). Error bars display the standard error of the mean (SEM).

Figure 5. Temporal Order Memory Test: Accuracy of Responses. Mean error of responses (max = 7) in the temporal order memory test as a function of delay condition (wakeful resting vs. spot the difference) and time of recall (10-20 min vs. 7 day). Error bars display the standard error of the mean (SEM).

Figure 6. Temporal Order Memory Test: Spearman's Rank Analysis. Mean recalled positions of landmarks (*y axis*) during 10-20-min recall (*left*), and 7-day recall (*right*) for the wakeful rest and spot the difference delay conditions, plotted against the order in which landmarks were presented in during route learning (*x axis*).

Table 1. Post-experimental Ratings of Route Learning and Test Difficulty. Mean ratings (+SEM) of difficulty for route learning and each of the test measures, collected during detailed post-experimental questioning after completing 10-20-min recall (Session 1) and 7-day recall (Session 2) tests. Participants rated these on a scale from 1 (very easy) to 5 (very difficult).
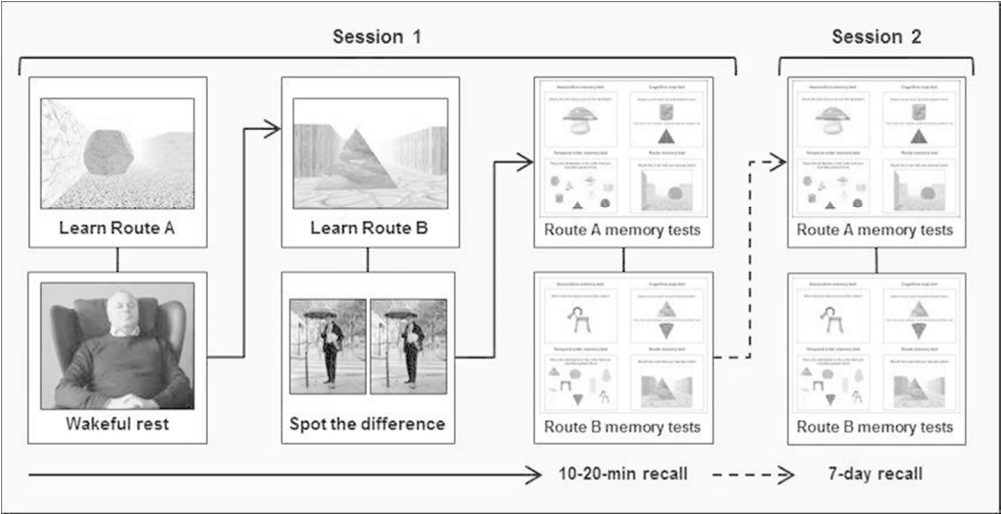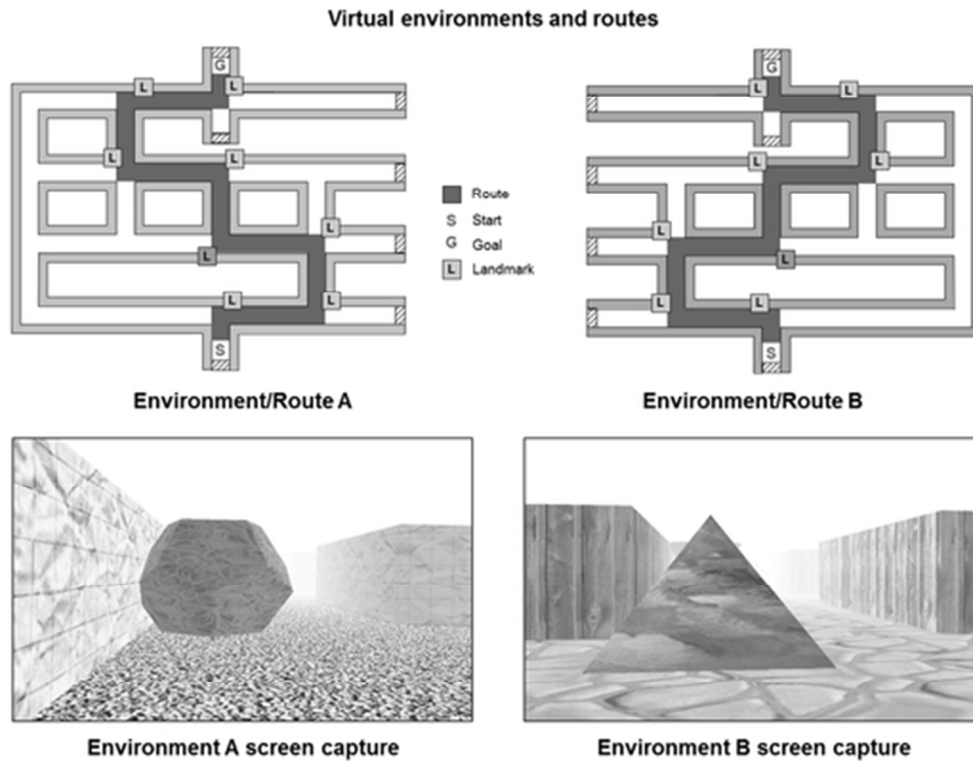
Figure 1. Experimental Procedure. The study took place over 2 sessions, Session 1 and Session 2, which were separated by 7 days. In Session 1 participants learned two routes, each followed by one of two 10-minute delay conditions (wakeful rest vs. spot the difference game). The order of the two learned routes and two delay conditions was counterbalanced across participants. The figure shows the example order: Route A ◊ wakeful resting ◊ Route B ◊ spot the difference game. After completion of the second delay condition, retention of spatial information pertaining to the two learned routes was examined via four surprise memory tests (10-20-min recall). Participants returned after 7 days (Session 2) and repeated the same memory tests (7-day recall). See Figure 3 for further information on the different memory tests.
178x91mm (122 x 122 DPI)

Virtual environments and routes

Environment/Route A

Environment/Route B

Route
S   Start
G   Goal
L   Landmark

Environment A screen capture

Environment B screen capture

Virtual Environments. Top: Schematic layout of Environment A (left) and Environment B (right). Bottom: Screen captures of Environment A (left) and Environment B (right). The figure demonstrates the symmetrical design of the environments and the long, indirect routes that were learned. The position of the start point ("S") and goal location ("G") are shown alongside the position of all landmarks ("L"). The screen captures illustrate the textures and example of landmarks used in the two environments. The position that the landmarks shown in the screen captures held within each environment are indicated by a darker "L".
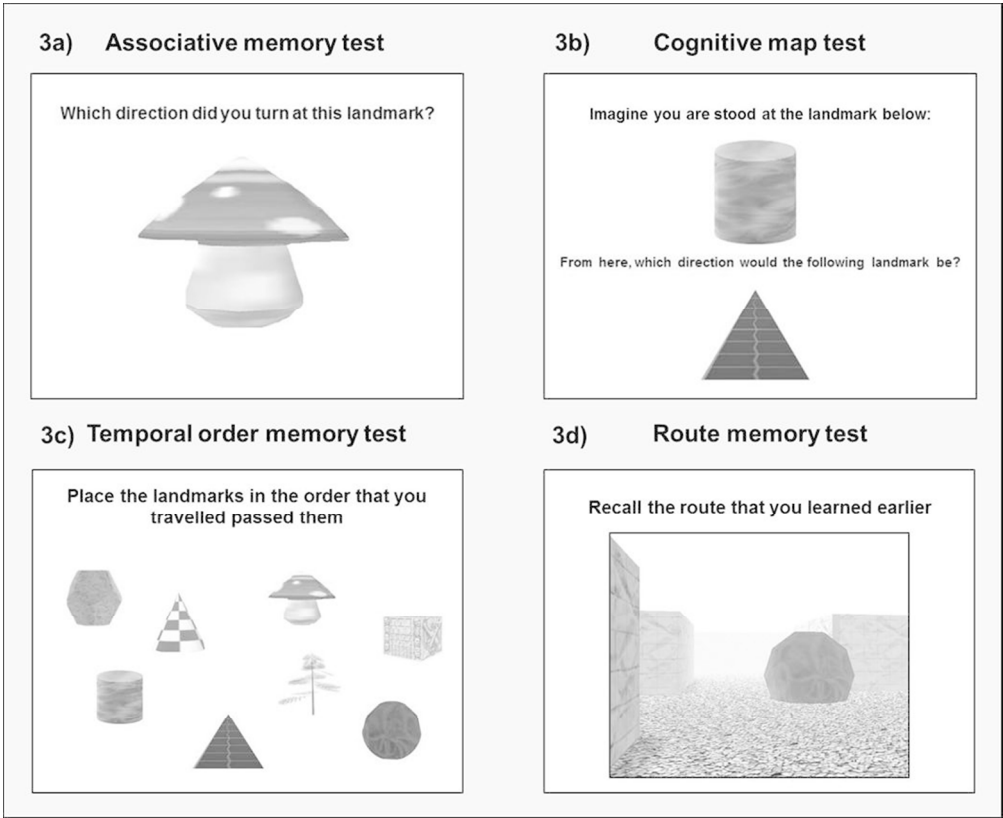44x34mm (300 x 300 DPI)

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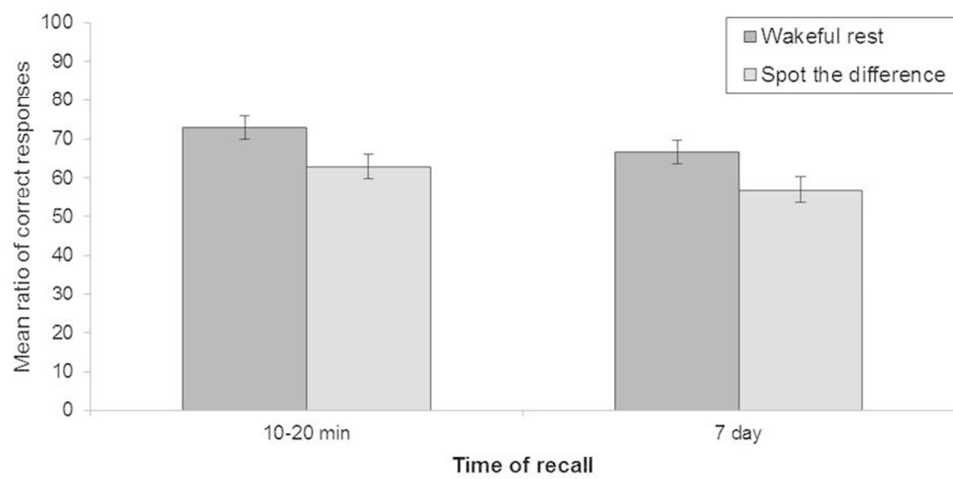178x144mm (150 x 150 DPI)

Figure 4. Associative Memory Test. Mean percentage of correct responses in the associative memory test as a function of delay condition (wakeful resting vs. spot the difference) and time of recall (10-20 min vs. 7 day). Error bars display the standard error of the mean (SEM).
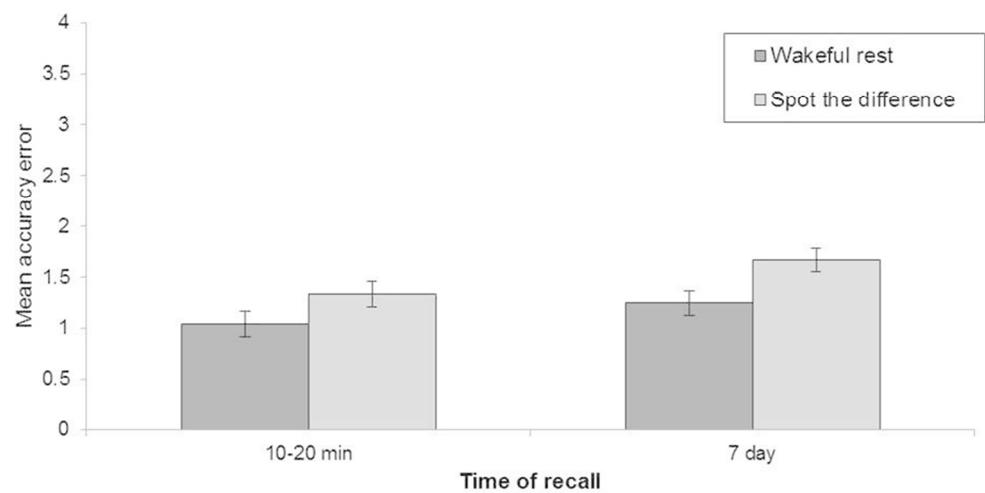178x88mm (135 x 135 DPI)

Figure 5. Temporal Order Memory Test: Accuracy of Responses. Mean error of responses (max = 7) in the temporal order memory test as a function of delay condition (wakeful resting vs. spot the difference) and time of recall (10-20 min vs. 7 day). Error bars display the standard error of the mean (SEM).
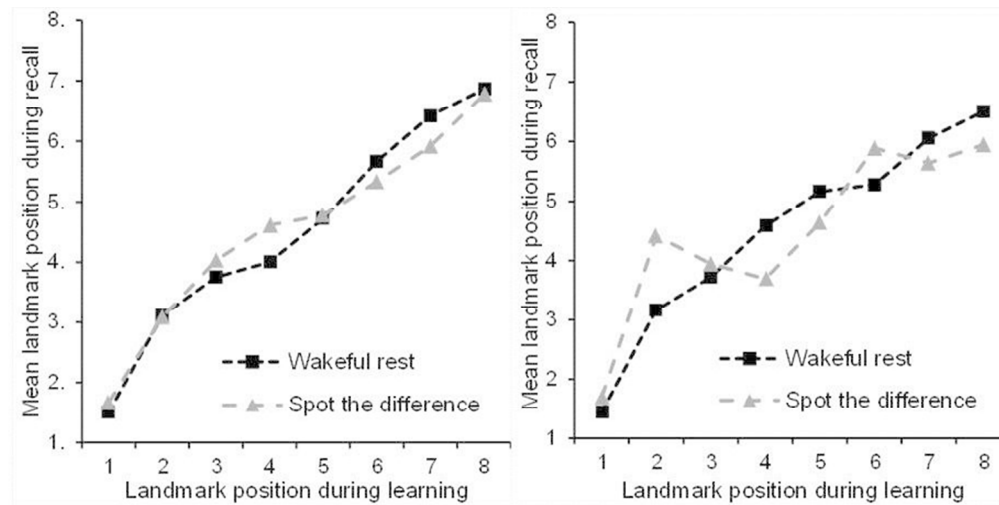178x88mm (135 x 135 DPI)

Figure 6. Temporal Order Memory Test: Spearman's Rank Analysis. Mean recalled positions of landmarks (y axis) during 10-20-min recall (left), and 7-day recall (right) for the wakeful rest and spot the difference delay conditions, plotted against the order in which landmarks were presented in during route learning (x axis).
177x89mm (130 x 130 DPI)

Table 1. Post-experimental Ratings of Route Learning and Test Difficulty. Mean ratings (+SEM) of difficulty for route learning and each of the test measures, collected during detailed post-experimental questioning after completing 10-20-min recall (Session 1) and 7-day recall (Session 2) tests. Participants rated these on a scale from 1 (very easy) to 5 (very difficult).

Time of recall test	Route learning	Associative memory test	Cognitive map test	Temporal order memory test	Route memory test
10-20 min	2.56 (0.14)	3.65 (0.12)	4.60 (0.11)	3.93 (0.12)	2.50 (0.15)
7 day	-	3.88 (0.13)	4.51 (0.11)	3.79 (0.10)	2.49 (0.15)