Title: Persistence of Hippocampal and Striatal Multivoxel Patterns during Awake Rest after Motor Sequence Learning

Abbreviated title: Hippocampal reactivation after motor learning

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Abstract

Memory consolidation is thought to be mediated by the offline reactivation of brain regions recruited during initial learning. Evidence for hippocampal reactivation in humans comes from studies showing that hippocampal response patterns elicited during learning can persist into subsequent rest intervals. Such investigations have largely been limited to declarative memory, which is surprising given the critical role of the hippocampus in motor memory processes. The primary goal of this study was therefore to investigate whether motor learning induces persistence of hippocampal patterns into subsequent rest. Based on their critical roles in motor learning and memory consolidation processes, we also assessed persistence in the striatum and primary motor cortex (M1). Functional magnetic resonance imaging (fMRI) data were recorded during motor learning as well as pre- and post-learning resting periods from 55 young healthy adults (males and females). Patterns of brain responses were assessed with intra- and inter-regional multivoxel correlation structure (MVCS). Intra-regional multivoxel patterns during motor sequence learning within the hippocampus and the striatum – but not within M1 – were more similar to post-learning as compared to pre-learning resting epochs, indicating persistence of task-related patterns thought to reflect reactivation processes. Interestingly, the multivoxel pattern of hippocampal connectivity with the striatum (i.e., inter-regional MVCS) was strongly *dissimilar* between post-learning rest and task practice. Altogether, these results provide evidence for the persistence of learning-related response patterns within the hippocampus and striatum into rest following motor learning. They also suggest that striatal-hippocampal connectivity patterns elicited by task practice are reorganized in post-learning waking rest.

Significance statement

Memory consolidation, the process by which newly encoded and fragile memories become more robust, is thought to be supported by the reactivation of brain regions - including the hippocampus - during post-learning rest. While hippocampal reactivations have been demonstrated in humans in the declarative memory domain, it remains unknown whether such a process takes place after motor learning. Using functional MRI, we show that patterns of brain activity within both the hippocampus and striatum elicited during motor learning indeed persist into post-learning rest. In contrast, connectivity patterns between the striatum and the hippocampus do not persist but instead are reorganized during post-learning waking rest. These results thus provide novel insights into the reactivation of brain activity after motor sequence learning.

Introduction

Memory consolidation is thought to be supported by the replay of hippocampal activity during subsequent offline episodes, i.e. during post-encoding rest (O'Neill et al., 2010; Carr et al., 2011; Tambini and Davachi, 2019). Hippocampal replay, initially reported in the rodent literature, has recently been translated to human research with the development of multivariate pattern analyses of functional magnetic resonance imaging (fMRI) data (Tambini and Davachi, 2019). Using these approaches, several studies have shown that hippocampal brain patterns elicited by declarative learning can persist into subsequent rest episodes and that this spontaneous memory reactivation is related to better memory [e.g., (Tambini and Davachi, 2013; Gruber et al., 2016; Hermans et al., 2017)].

Hippocampal reactivations have predominantly been limited to examinations in the context of declarative memory, which is surprising given that the role of the hippocampus in procedural (motor) memory processes is now well established [see (Albouy et al., 2013a; King et al., 2017) for reviews]. Specifically, research has not only shown that the hippocampus is recruited during motor sequence learning [e.g., (Schendan et al., 2003; Albouy et al., 2008; Cousins et al., 2016)], but also that hippocampal activity and connectivity patterns elicited by task practice are linked to subsequent motor memory consolidation (Albouy et al., 2008, 2013b). In line with this, research using multivariate approaches has shown that task-related hippocampal patterns can discriminate newly learned as compared to consolidated motor sequences (Pinsard et al., 2019). Importantly, while there is recent evidence to suggest that the hippocampus is involved in micro-offline motor memory processes [i.e., fast consolidation occurring during short rest intervals between practice blocks; see (Jacobacci et al., 2020; Buch et al., 2021)], persistence of hippocampal patterns after motor learning has never been reported.

In the current study, we used multivariate analyses of task-related as well as pre- and post-learning resting state fMRI data to investigate whether motor sequence learning induced persistence of learning-related patterns into subsequent rest. Based on the aforementioned literature on the hippocampus as well as previous evidence demonstrating that: (i) the hippocampus can lead striatal replay (Lansink et al., 2009); (ii) task-related patterns in the primary motor cortex (M1) can be subsequently reactivated offline (Ramanathan et al., 2015; Eichenlaub et al., 2020); and, (iii) striatal and motor cortical regions are vital for the processes underlying motor learning and memory consolidation [e.g., (Doyon et al., 2009; Albouy et al., 2013a)], our analyses examined pattern persistence in 3 regions of interest: the hippocampus, striatum and M1. Moreover, given that the

interplay between the hippocampus and striatum serve a critical role in motor learning and memory consolidation processes (Albouy et al., 2008, 2013b), we also assessed the persistence of learning-related hippocampal-striatal connectivity patterns into subsequent rest. The overarching hypothesis was that that response patterns elicited by motor task practice would persist into immediate post-learning rest.

Material and Methods

Participants

Sixty-two young, right-handed (Oldfield, 1971), healthy volunteers were recruited by local advertisements to participate in this study which was approved by the Research ethics board of the Regroupement en Neuroimagerie du Québec (RNQ). Participants did not suffer from any known psychological, psychiatric [including anxiety (Beck et al., 1988) and depression (Beck et al., 1961)] or neurological disorders and reported normal sleep during the month preceding the experiment [Pittsburgh Sleep Quality Index, (Buysse et al., 1989)]. None of the subjects were taking medications at the time of testing. None received formal training on a musical instrument or as a typist. All participants were asked to follow a 4-day constant sleep schedule (according to their own rhythm ± 1 h) before the experiment. Compliance to the schedule was assessed using both sleep diaries and wrist actigraphy measures (Actiwatch AW2, Bio-Lynx scientific equipment Inc., Montreal, Canada).

Of the 62 participants recruited for the study, seven were discarded from analyses. One participant was excluded because his/her dataset was incomplete (i.e., no resting-state scans) and three participants were excluded for excessive head motion during one of the fMRI runs. Specifically, 2 participants had maximum linear translations that exceeded 3mm (~1 voxel) and an additional participant had more than 50% of the volumes removed as part of the scrubbing procedure (see details below), leaving minimal data for analyses. Three additional participants were excluded as they presented outlier similarity indices as part of the multivoxel imaging analyses described below (i.e., > 3 standard deviations away from the group average). Consequently, a total of 55 subjects were included in the analyses (mean age ± SD: 23.8 ± 3.4 years, 36 females).

Experimental design

The current research consisted of a within-subject experimental design, in which all participants were trained to perform a motor sequence learning task (see below) in an MRI scanner at approximately 11:30 am. Resting-state (RS) functional scans were acquired immediately prior and after the completion of the motor task (Figure 1A). Note that these 3 imaging runs analyzed for the current

research were the first 3 runs of a larger experimental protocol completed by the same participants [see (Albouy et al., 2015) for full protocol].

Motor sequence learning task

The sequential finger tapping task used in this research was coded in Cogent2000 (http://www.vislab.ucl.ac.uk/cogent.php) and implemented in MATLAB (MathWorks Inc., Sherbom, MA). Participants were instructed to tap a five-element finger sequence on an MR-compatible keyboard with their non-dominant (left) hand as fast and as accurately as possible (Figure 1B). The sequence to perform (i.e., 4 1 3 2 4, where 1 and 4 correspond to the index and the little fingers, respectively) was explicitly provided to the participants prior to training. Participants completed 14 consecutive practice blocks, each of which was separated by 15-second rest periods. Practice and rest blocks were indicated by green and red, respectively, fixation crosses on the center of the screen. During the rest blocks, participants were instructed to keep their fingers still and look at the red cross. The number and timing of each specific key press within each practice block was recorded (60 key presses, ideally corresponding to 12 correct sequences). This procedure effectively controlled the number of movements executed in each block. Yet, the duration of the practice blocks progressively decreased with learning, as subjects became faster at executing the 60 key presses. Performance on the motor sequence learning task was assessed with measures of speed (time to perform a correct sequence in s) and accuracy (number of correct sequences per block).

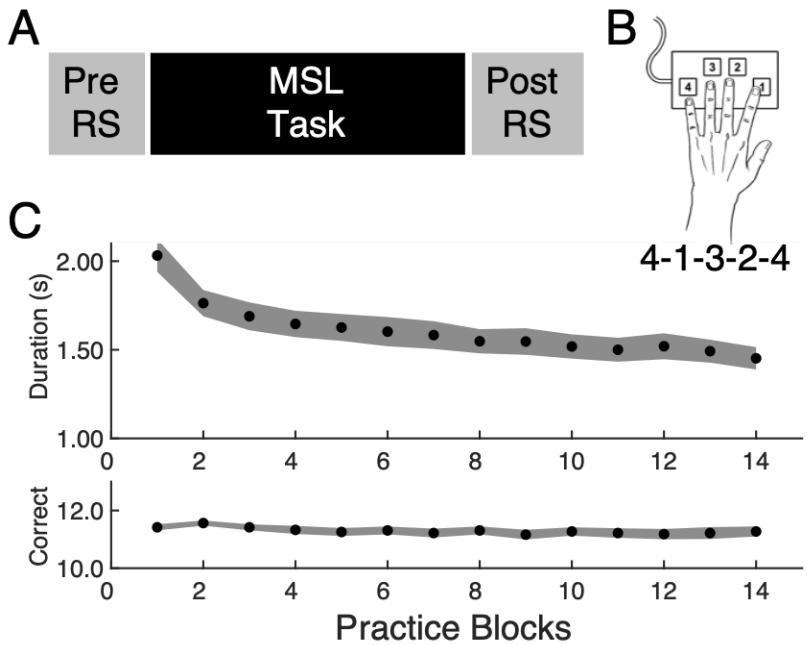

Figure 1: ***A.*** *Resting-state (RS) scans were acquired immediately prior to and after the execution a motor sequence learning (MSL) task. All three runs were completed inside the MRI scanner.* ***B.*** *The task consisted of an explicitly-known 5-element sequence (4-1-3-2-4,*

where 1 and 4 are the index and little fingers, respectively) completed with the left non-dominant hand. ***C.*** *Participants learned the novel sequence of movements, as evidenced by reductions in the duration to complete a correct sequence (in s) across blocks of practice (upper panel). Performance accuracy (number of correct sequences per block; maximum correct = 12) remained high and stable throughout training (lower panel). Dots represent means, shaded regions represent SEM. N = 55.*

fMRI data acquisition and processing

Acquisition

Functional MRI-series were acquired using a 3.0 T TIM TRIO scanner system (Siemens, Erlangen, Germany), equipped with a 32-channel head coil. Multislice T2*-weighted fMRI images were obtained, during both resting-state and task practice, with a gradient echo-planar sequence using axial slice orientation (TR = 2650 ms, TE = 30 ms, FA = 90°, 43 transverse slices, 3 mm slice thickness, 10% inter-slice gap, FoV = 220 × 220 mm2, matrix size = 64 × 64 × 43, voxel size = 3.4 × 3.4 × 3 mm^3). Slices covering the whole brain were acquired along the z-axis in an ascending direction. During each resting-state scan (6 min 40 s each), participants were instructed to keep their eyes open and fixate on a white cross in the middle of a black screen. They were asked to remain still and "not to think about anything in particular" during the resting state scan. A structural T1-weighted 3D MP-RAGE sequence (TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, FA = 9°, 176 slices, FoV = 256 × 256 mm^2, matrix size = 256 × 256 × 176, voxel size = 1 × 1 × 1 mm^3) was also acquired in all subjects. Head movements were minimized using cushions.

Analyses

Pre-processing

The fMRI data were preprocessed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/; Wellcome Centre for Human Neuroimaging, London, UK). The reoriented structural image was segmented into gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), bone, soft tissue, and background. Task-based and RS functional volumes of each participant were first slice-time corrected (reference: middle slice). Images were then realigned to the first image of each session and, in a second step, realigned to the mean functional image computed across all the individuals' fMRI runs using rigid body transformations. The mean functional image was co-registered to the high-resolution T1-weighted anatomical image using a rigid body transformation optimized to maximize the normalized mutual information between the two images. The resulting co-registration parameters were then applied to the realigned functional images. To optimize voxel pattern analyses, functional and anatomical data remained in subject-specific (i.e., native) space, and no spatial smoothing was applied to functional images (Tambini and Davachi, 2013).

ROI definition

Three ROIs were considered in the analyses (see Figure 2B) and consisted of the bilateral hippocampus, bilateral putamen and right M1 (i.e., contralateral to the hand practicing the task). The hippocampus and putamen ROIs were created in the native space of each individual using the FMRIB's Integrated Registration Segmentation Toolkit (FSL FIRST; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST). The bilateral hippocampus and putamen ROIs included an average of 309 (SD = 33; range of 258 – 385) and 309 (SD = 22; range of 274 – 362) voxels, respectively. The right M1 ROI was defined by masking the activation map from the group-level main effect of practice map (task > rest) derived from univariate analyses (threshold $p < 0.05$ whole brain FWE corrected) with the anatomically-defined right M1 from the Human Motor Area Template (Mayka et al., 2006). Details on the univariate analytic pipeline and corresponding group-level results are provided in (Albouy et al., 2015). As this functional map and anatomical template were provided in MNI space, the M1 ROI was mapped back to native space using the individual's inverse deformation field output from segmentation of the anatomical image. The right M1 ROI included an average of 254 voxels (SD = 23; range of 204 – 302).

Multi-voxel correlation structure (MVCS) analyses

The custom analysis pipeline was written in Matlab. Prior to running MVCS analyses, additional preprocessing of the time series was completed. Specifically, whole-brain signal was detrended and high-pass filtered (cutoff=1/128). The realignment parameters were used to compute framewise displacement (FD). If the FD of any volume *n* exceeded 0.5mm, volume *n* and *n*+1 were discarded from analyses. The percentage of volumes excluded from the pre RS, MSL task and post RS runs were 0.72 (SD=1.57), 5.96 (SD=7.99) and 1.60 (SD=3.20), respectively. At the ROI level, only voxels with >10% GM probability were included in the analyses. Regression analyses were performed on the fMRI time-series of the remaining voxels in each ROI to remove additional sources of noise. These nuisance regressors included the first three principal components of the signal extracted from the WM and CSF masks created during segmentation of the anatomical image, the 6-dimensional head motion realignment parameters, as well as the realignment parameters squared, their derivatives, and the squared of the derivatives (i.e., a total of 30 nuisance regressors). Last, the number of volumes included in the analyses were matched across runs within each individual. To do so, the run with the smallest number of volumes was identified, and for the other 2 runs, the corresponding number of volumes centered around the middle volume of that run were selected. The average number of volumes within each run included in the analyses was 146 (SD = 6; range 117-150).

To assess *intra-regional response patterns*, multi-voxel correlation structure (MVCS) matrices were computed for each ROI and each run of interest with similar procedures as in previous research (Tambini and Davachi, 2013; Hermans et al., 2017). Specifically, Pearson's correlations were computed between each of *n* BOLD-fMRI voxel time courses, yielding an *n* by *n* MVCS matrix per ROI, run and individual (see Figure 2A). Pearson's correlation coefficients were Fisher z-transformed to ensure normality. Each MVCS matrix is thought to reflect the response pattern of the ROI in the specific run (Tambini and Davachi, 2013).

To assess *inter-regional response patterns*, the pre-processed BOLD data from each ROI (i.e., putamen and hippocampus) in each fMRI run were first subjected to a principal component analysis (PCA). For each fMRI run, the time series of the first component for a given ROI (herein referred to as the seed) was extracted and correlated with the time courses of each voxel in a 2nd ROI (herein referred to as the target region). Figure 3A depicts a schematic with the putamen as the seed and the hippocampus as the target region for an exemplar run. The corresponding Fisher z-transformed correlation coefficients therefore constituted distinct inter-regional MVCS vectors for each run, conceptualized as multivoxel patterns of connectivity between the 2 ROIs (Tambini et al., 2017). For example, the schematic in Figure 3A represents the multivoxel pattern of hippocampal connectivity with the striatum.

Statistical Analyses

To ensure participants learned the novel motor sequence, behavioral measures of movement speed and accuracy were separately assessed with one-way repeated-measures ANOVAs, with block as the within subject factor. Greenhouse-Geisser corrections were applied if the sphericity assumption was violated.

To assess the influence of motor learning on multivoxel patterns within and between our ROIs, two separate, albeit related, analyses were conducted. First, similarity indices (SI) that reflect the similarity of the multivoxel patterns between two specific fMRI runs were computed as the r-to-z transformed correlation between the two MVCS matrices (for intra-regional patterns) or vectors (for inter-regional patterns) of interest (Tambini and Davachi, 2013; Tambini et al., 2017). Similarity indices (SIs) were computed between (1) pre RS and MSL task practice and (2) MSL task practice and post RS (SI in Figure 2A). The magnitude of the similarity indices between task and post RS reflects the degree of persistence of the learning-related patterns into the subsequent rest interval. To assess whether this persistence is significant, a comparison was made to the SI between the task and pre RS as a within-subject control. Specifically, paired t-tests were computed using Matlab to

compare SIs between pre RS / task and task / post RS. Cohen's d effect sizes corresponding to the paired t-tests are reported. Within each family of hypothesis tests (i.e., intra- and inter-regional MVCS analyses), results were considered significant if $p < 0.05$, with a false-discovery rate (FDR) correction for testing multiple ROIs (Benjamini and Hochberg, 1995).

Second, we adopted a partial correlation approach previously used in rodent literature to quantify replay of neuronal firing associated to previous experience (Kudrimoti et al., 1999; Lansink et al., 2008) and in human research to study persistent of task-related brain pattern into rest (Hermans et al., 2017). Specifically, we quantified the proportion of variance in the multivoxel pattern of the post RS epoch that was explained by the variance in the multivoxel pattern during task practice while controlling for pre-existing covariance reflected in the pre RS epoch. The explained variance (EV; Figure 2A) was computed with the following formula (Kudrimoti et al., 1999):

$$EV = r^2_{Task,Post|Pre} = \left(\frac{r_{Task,Post} - r_{Pre,Task}r_{Pre,Post}}{\sqrt{\left(1 - r^2_{Pre,Task}\right)\left(1 - r^2_{Pre,Post}\right)}}\right)^2$$

The magnitude of the EV then reflects the degree of persistence of the learning-related pattern into the subsequent rest interval. To assess whether this persistence is significant, a comparison was made to the "reverse" explained variance [REV; (Lansink et al., 2008); Figure 2A], in which the proportion of variance in the multivoxel pattern in the *pre* RS epoch is explained by the pattern during task practice while partialing out covariance reflected in the *post* RS epoch. The formula for REV is thus:

$$REV = r^2_{Pre,Task|Post} = \left(\frac{r_{Pre,Task} - r_{Task,Post}r_{Pre,Post}}{\sqrt{\left(1 - r^2_{Task,Post}\right)\left(1 - r^2_{Pre,Post}\right)}}\right)^2$$

As these explained variance metrics are not normally distributed, paired Wilcoxon signed ranked tests were computed using Matlab to compare EV and REV. Effect sizes corresponding to the Wilcoxon signed ranked tests are provided (r = Z-statistic / sqrt (N)). Similar to above, results were considered significant if $p < 0.05$, with a FDR correction for multiple comparisons (Benjamini and Hochberg, 1995).

Results

Behavior

Performance accuracy (number of correct sequences per block) remained high (11.3/12 correct sequences on average) and stable across training (no main effect of block; $F_{(13,702)} = 0.76$, Greenhouse-Geisser corrected p-value = 0.70; Figure 1C, lower panel). Conversely, performance

speed (time to perform a correct sequence in s) improved across blocks of practice, as evidenced by a significant effect of practice block ($F_{(13,702)} = 36.6$, Greenhouse-Geisser corrected p-value < 0.001; Figure 1C, upper panel). These results demonstrate the expected finding that participants were able to acquire the novel sequence of movements during the training session.

Intra-regional MVCS patterns

We first examined whether brain patterns *within* our ROIs (Figure 2B) showed evidence of persistence into post-learning rest. Results showed that the multivoxel response patterns within the hippocampus and putamen during MSL were significantly more similar to activity patterns in the post RS scan as compared to the pre RS scan (HC: (t(54) = 2.86, p(unc) = 0.006, p(FDR) = 0.018, Cohen's d = 0.39; Put: t(54) = 2.45, p(unc) = 0.018, p(FDR) = 0.026, Cohen's d = 0.33; Figure 2C). In contrast, the MSL task-related pattern of activity in M1 was equally similar to that in pre RS and post RS (t(54) = 1.37, p(unc) = 0.178, Cohen's d = 0.18; Figure 2C). These results indicate that task-related patterns within the hippocampus and striatum – but not in M1 – significantly persisted into rest after motor sequence learning.

An identical pattern of results was obtained using a partial correlation approach (Figure 2D). Specifically, in both the hippocampus (z(54) = 2.61, p(unc) = 0.009, p(FDR) = 0.022, r = 0.25) and the putamen (z(54) = 2.45, p(unc) = 0.014, p(FDR) = 0.022, r = 0.23), the variance of the *post* RS multivoxel pattern explained by the task multivoxel pattern (partialing out variance explained by the pattern in pre RS) was significantly greater than the opposite direction (i.e., REV; variance in pre RS multivoxel pattern explained by task multivoxel pattern controlling for post RS). There were no differences between EV and REV for M1 (z(54) = 1.37, p(unc) = 0.17, r = 0.13).

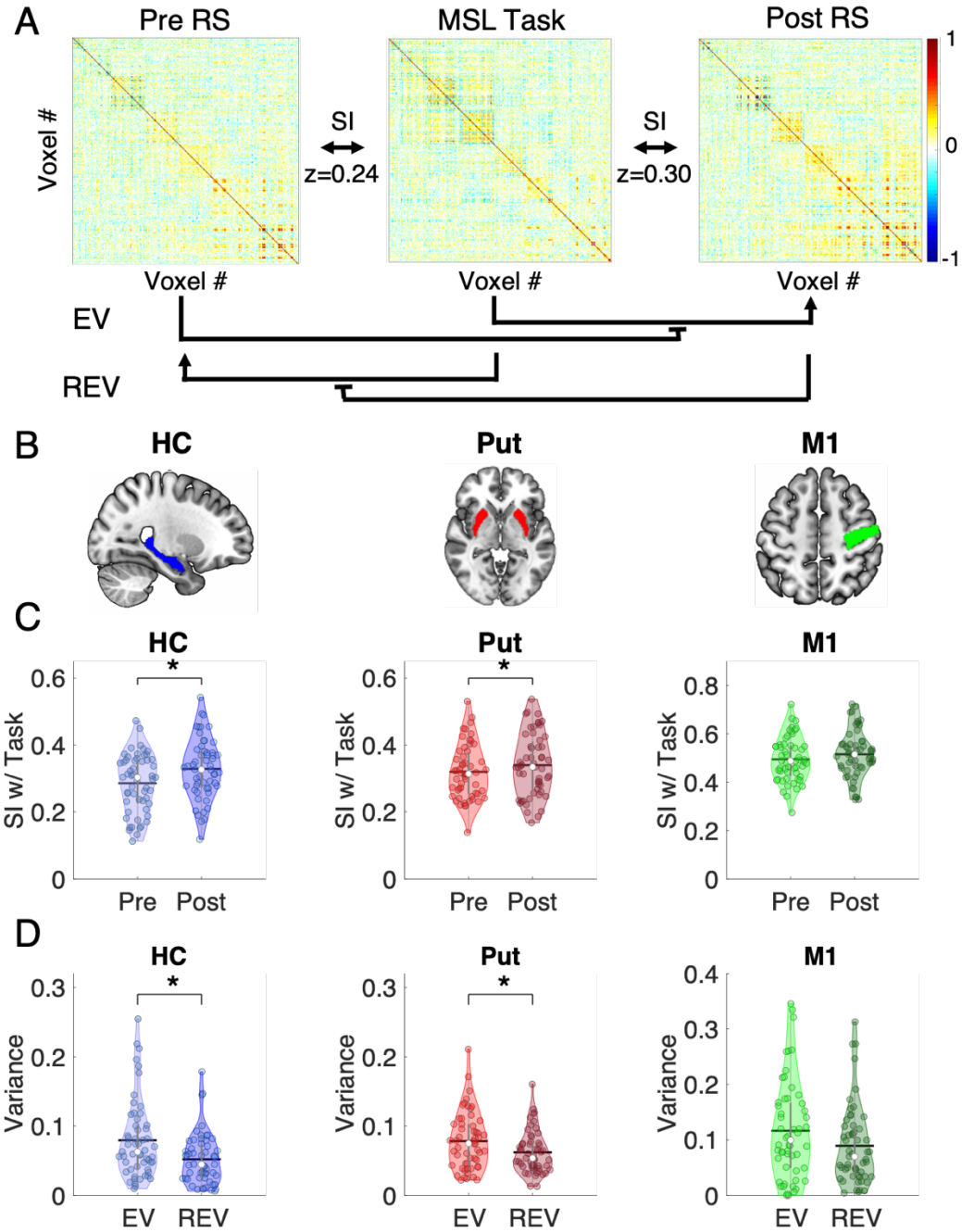

Figure 2. *(**A**) MVCS matrices are depicted for an exemplar ROI and participant. Each matrix shows the correlation between each of the n voxels of the ROI with all the other voxels of the ROI during the pre-learning resting state (Pre RS), motor sequence learning (MSL) task and post-learning resting state (Post RS) runs. The similarity index (SI) between two matrices (pre RS vs. MSL and MSL vs. post RS) is defined as the r-to-z transformed correlation between each pair of MVCS matrices. Pre RS/MSL and MSL/post RS SIs were compared to assess whether task-related patterns persisted significantly into post-learning rest (results presented in panel C). Additionally, a partial correlation approach was used to quantify the proportion of variance in the multivoxel pattern of the post RS epoch that was explained by the variance in the multivoxel pattern during task practice while controlling for pre-existing covariance reflected in the pre RS epoch (explained variance, EV). EV was statistically compared to the "reverse" explained variance (REV) in which the proportion of variance in the multivoxel pattern in the pre RS epoch is explained by the pattern during task practice while partialing out covariance reflected in the post RS epoch (results presented in panel D). (**B**) Depiction of the hippocampus (HC), putamen (Put) and primary motor cortex (M1) ROIs. (**C**) Violin plots*

*(Bechtold, 2016) depicting the Similarity Indices (SI; Fisher Z-transformed correlation coefficients) between Pre RS and MSL as well as between MSL and Post RS for the HC, Put and M1. (**D**) Violin plots depicting proportion of explained variance (EV) and reverse explained variance (REV) for the 3 ROIs. Results collectively indicate that task-related patterns in the hippocampus and putamen – but not in M1 – persist into post-learning rest intervals. Findings were consistent across the analyses comparing SI (panel C) as well as explained variance (panel D). For all violin plots, individual data points are shown as small colored circles jittered on the horizontal axis within the respective plot to increase visualization (N = 55); white circles and horizontal black lines depict group medians and means, respectively. * indicate p<0.05 after FDR correction for multiple comparisons.*

Inter-regional MVCS patterns

Given that (i) task-related activity patterns of both the hippocampus and the striatum showed persistence into subsequent rest and (ii) hippocampal-striatal interplays play a critical role in the motor learning and consolidation process (Albouy et al., 2008, 2013b; Cousins et al., 2016), we tested whether the task-related MVCS representing connectivity *between* the hippocampus and putamen showed reinstatement during post-learning rest. Results indicated that the multivoxel pattern of hippocampal connectivity with the putamen (i.e., putaminal seed and hippocampal target) was significantly altered by task practice. Specifically, the connectivity pattern during post-learning, as compared to pre-learning, rest was *less* similar to the task pattern ($t(54) = 3.28$, p(unc) = 0.002, p(FDR) = 0.004, Cohen's d = 0.44; Figure 3C, left panel). No significant difference was observed for the multivoxel pattern of putaminal connectivity with the hippocampus (i.e., hippocampal seed and putaminal target; $t(54) = 1.04$, p(unc) = 0.20, Cohen's d = 0.14; Figure 3C, right panel). These results collectively indicate that task-related multivoxel patterns of hippocampal and striatal connectivity do not persist during subsequent rest. However, the multivoxel pattern of hippocampal connectivity with the striatum during post-learning rest was strongly *dissimilar* to that observed during task practice, suggesting that striatal-hippocampal connectivity was reorganized in post-learning rest.

Similar results were observed using the partial correlation approach (Figure 3D). Specifically, EV was significantly lower than REV for the multivoxel pattern of hippocampal connectivity with the putamen ($z(54) = 3.22$, p(unc) = 0.001, p(FDR) = 0.003, r = 0.31), indicating the post RS pattern explained less variance in the task pattern than pre RS. There were no differences in EV and REV for the multivoxel pattern of putaminal connectivity with the hippocampus ($z(54) = 1.78$, p(unc) = 0.08, r = 0.17).

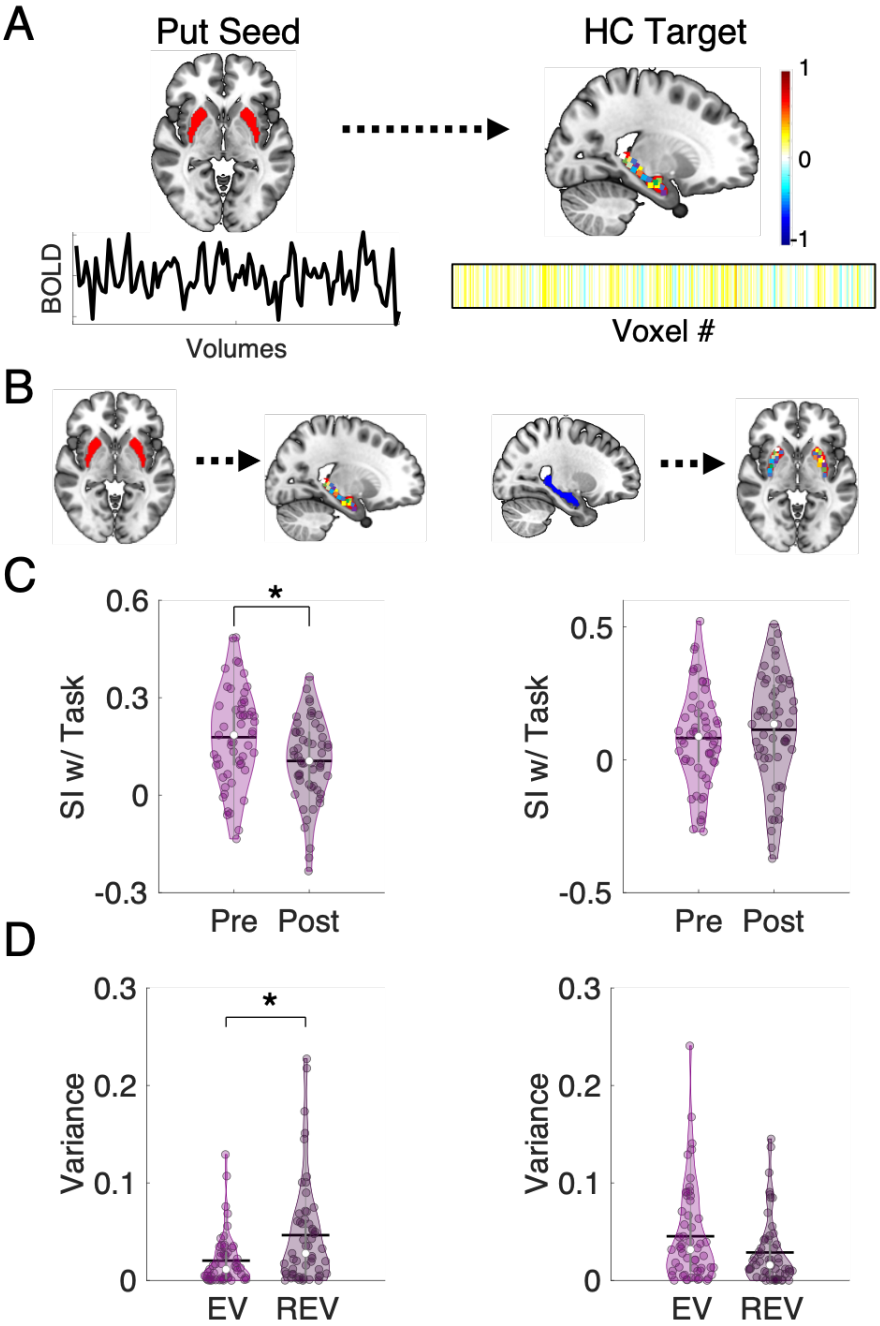

Figure 3*: (A)** *The BOLD time series within a seed region (putamen – Put - in this example) was correlated to the time series of each voxel of the target region (here the hippocampus - HC) separately for each of the three runs (only a single exemplar run depicted here). This resulted in distinct multivoxel connectivity pattern vectors containing the r-to-z transformed correlation for the n voxels of the target region.* ***(B) *Depiction of seed / target ROI pairs: left panel depicts Put as the seed and HC as the target (reflecting the multivoxel pattern of HC connectivity with the Put) whereas the right panel illustrates the HC seed and the Put target (reflecting the multivoxel pattern of Put connectivity with the HC).* ***(C)*** *Violin plots (Bechtold, 2016) depicting the Similarity Indices (SI; Fisher's Z-transformed correlation coefficients) for relevant pairs of fMRI runs (pre RS to MSL task and MSL task to post RS). The post-learning rest multivoxel pattern of HC connectivity with the Put was significantly less similar to the task connectivity pattern as compared to pre-learning rest (left panel). Conversely, the similarity between the RS and task-related multivoxel pattern of Put connectivity with the HC did not differ between the two RS runs (right panel).* ***(D)*** *Violin plots depicting explained variance (EV) and reverse explained variance (REV) for the 2 seed-target combinations. EV*

*was significantly lower than REV for the multivoxel pattern of HC connectivity with the Put (left panel), whereas no differences were observed for the pattern of Put connectivity with the HC (right panel). These results mirror those presented based on SI shown in panel C. For all violin plots, individual data points are shown as small colored circles jittered on the horizontal axis within the respective plot to increase visualization (n = 55); white circles and horizontal black lines depict group medians and means, respectively. * indicate p<0.05 after FDR correction for multiple comparisons.*

Discussion

In this study, we used multivariate analyses of motor task and resting state fMRI data to investigate whether brain patterns elicited by motor sequence learning persist into immediate post-learning waking rest. Results indicated that task-related patterns *within* the hippocampus and the striatum - but not in M1 - were reinstated during subsequent rest. In contrast, task-related connectivity patterns *between* the hippocampus and the striatum did not persist. Instead, the multivoxel pattern of hippocampal connectivity with the striatum was highly *dissimilar* between task practice and post-learning rest. Altogether, our results not only provide evidence for the persistence of motor learning-related patterns in the hippocampus and striatum into subsequent rest, but also demonstrate a reorganization of striato-hippocampal connectivity patterns in this offline epoch.

Motor-task-related hippocampal and striatal - but not M1 - patterns persist into post-learning wakeful rest

Persistence of task-related hippocampal patterns into post-learning rest has been consistently observed in humans in the declarative memory domain (Tambini and Davachi, 2013, 2019; Gruber et al., 2016; Hermans et al., 2017; Tambini et al., 2017; Schapiro et al., 2018; Schuck and Niv, 2019; Tambini and Esposito, 2020). It is argued that such pattern persistence reflects “reactivation” or “replay” of patterns that were previously expressed during learning (Tambini and Davachi, 2019). The current study reports task-related hippocampal pattern persistence into post-learning rest in the motor memory domain. These findings extend previous neuroimaging investigations highlighting the crucial role of the hippocampus in motor learning [e.g., (Schendan et al., 2003; Albouy et al., 2008)]. Based on evidence that the hippocampus supports abstract/spatial representations of motor sequences (Albouy et al., 2013a), we argue that such representations might be reactivated during the early consolidation window following learning. This is in line with observations from univariate MRI analyses showing increased hippocampal recruitment 30 minutes after motor learning (Albouy et al., 2008). However, these findings also challenge views according to which hippocampal-mediated motor memory processing is predominantly sleep-dependent [e.g., (Albouy et al., 2015; Cousins et

al., 2016)]. It remains unknown how the pattern persistence observed in the present study is related to consolidation processes; however, the present data nonetheless point towards hippocampal-mediated memory processing during wakefulness immediately following learning.

Interestingly, our findings are in line with recent examinations assessing hippocampal activity on the micro-offline time scale (i.e., during rest blocks interleaved with practice). Jacobacci et al. (2020) demonstrated that hippocampal activations during these micro-offline rest epochs - as revealed by univariate analyses - tended to be linked to the amplitude of micro-offline gains in performance and learning-related changes in hippocampal structure. Additionally, Buch and colleagues (2021) conducted multivariate pattern analyses of MEG data and their results demonstrated hippocampal replay during micro-offline epochs. Interestingly, they did not find clear evidence of replay or pattern persistence into post-learning rest (i.e., pre RS and post RS replay patterns were comparable). The reasons for these discrepant findings between Buch et al. and the current results are not immediately clear, but the two studies employed different imaging modalities (MEG vs. fMRI) with inherently different spatial and temporal resolutions and the corresponding analytic approaches were vastly different.

Recurrence of task patterns into subsequent sleep and wakeful rest has been reported in rodent research in the ventral striatum [after reward-based learning; (Lansink et al., 2008)] and putamen [after exploration of novel objects; (Ribeiro et al., 2004)]. While there is evidence from previous human research that striatal multivariate patterns are modulated during motor learning (Pinsard et al., 2019; Andersen et al., 2020; Berlot et al., 2020), the current research reports persistence of task-related striatal patterns into post-learning rest in humans. Interestingly, such reactivation or reverberation of striatal task patterns has been observed in rodents in conjunction with hippocampal replays (Ribeiro et al., 2004; Lansink et al., 2009). Even though there is no causal evidence that the hippocampus drives replay in the striatum, it has previously been argued that the hippocampus plays a network synchronization role by orchestrating memory trace consolidation in concert with other task-relevant areas such as the striatum (Rusu and Pennartz, 2020). Although our results cannot directly speak to the hippocampus as an orchestrator of such inter-regional communications (but see below for a discussion of the connectivity patterns), our data do demonstrate that both the hippocampus and striatum are reactivated during post-learning wakefulness and thus are consistent with the aforementioned rodent work (Ribeiro et al., 2004; Lansink et al., 2009).

The current results observed on M1 patterns stand in contrast with previous animal (Ramanathan et al., 2015) and human (Eichenlaub et al., 2020) research showing replay of task-related M1 activity

patterns during subsequent sleep. It could be argued that M1 reactivations preferentially occur during post-learning sleep as compared to wakefulness. However, this remains hypothetical, as earlier studies suggest that M1 does not play a prominent role in sleep-dependent motor memory consolidation [see (King et al., 2017) for a review]. Our results are, however, in line with recent evidence showing that, in contrast to premotor areas, activity patterns in M1 do not show motor sequence representations but rather exhibit patterns related to the execution of individual finger movements (Yokoi et al., 2018; Yokoi and Diedrichsen, 2019). It is thus tempting to speculate that M1 patterns do not code for the motor sequence memory trace *per se,* which might explain the lack of M1 pattern reactivation during post-learning rest reported in the current study.

Striatal-hippocampal connectivity patterns do not persist into - but are reorganized in - post-learning rest

In contrast to the present findings, reinstatement of task-related multivoxel *connectivity* patterns has previously been reported for other brain regions like the amygdala [i.e., from emotional to neutral memory encoding sessions; (Tambini et al., 2017)]. There is also evidence to suggest that post-acquisition functional connectivity between the hippocampus and the amygdala persist during a rest epoch following fear learning (Hermans et al., 2017). The present data indicate that instead of being reinstated, hippocampal-striatal interplay appears to be reorganized in the offline episode. The current results thus build on top of previous research speaking to the importance of hippocampal-striatal interplay for motor sequence learning. Such functional interactions during motor task practice have been described to be critical (i) for the development of a coherent motor memory trace during initial learning (Albouy et al., 2008, 2012); (ii) to trigger subsequent consolidation processes (Albouy et al., 2013b); and, (iii) to support optimal performance when motor memory traces are consolidated (Albouy et al., 2008; Cousins et al., 2016). Perhaps most interestingly, results from the current investigation are in line with evidence of reorganization of *task-related* hippocampal-striatal connectivity over the course of the consolidation process. Specifically, previous research has demonstrated that the hippocampal-striatal interplay proceeds from a competitive interaction during initial learning to a more cooperative relationship in a next-day retest (Albouy et al., 2008). The present data suggest that such reorganization – thought to be a signature of "longer-term" consolidation processes (Albouy et al., 2008) – might be initiated immediately after learning during wakeful rest.

Interestingly, the reorganization discussed above was only observed when assessing the multivoxel pattern of hippocampal connectivity with the putamen (not the multivoxel pattern of striatal

connectivity with the hippocampus). While the current analyses certainly do not afford any inferences about causality, it is tempting to speculate that hippocampal multivoxel pattern generated by motor sequences during learning and replayed during post-learning rest may lead striatal reactivation (Lansink et al., 2009). This possibility fits influential models proposing that the hippocampus plays the role of a central metronome pacing reactivation in other structures such as the striatum during the offline consolidation process (Rusu and Pennartz, 2020). Most interestingly, the current data also suggest that this temporal organization specifically arises during rest, after learning has ended, as connectivity patterns during post-learning rest were highly dissimilar to those observed during task practice. Based on evidence that hippocampal-striatal interplay is competitive during initial motor learning (Albouy et al., 2008, 2013b, 2015; Rieckmann et al., 2010), we propose that the dissimilarity of connectivity patterns observed during rest might reflect the co-reactivation occurring in concert between the hippocampus and the striatum. This interpretation, however, is certainly speculative.

Conclusions

Our results show that patterns of brain activity within the hippocampus and striatum – but not M1 - observed during motor learning persisted into post-learning rest. In contrast, connectivity patterns between the striatum and the hippocampus did not persist but instead were reorganized in the waking rest epoch following motor learning. Results thus provide novel insights into the reactivation of brain activity after motor sequence learning in early consolidation windows during wakefulness. Moreover, our findings add to the growing body of literature demonstrating that the hippocampus plays a vital role in learning and memory processes in the procedural (motor) memory domain.

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