



Distinct connectivity profiles predict different in-time processes of motor skill learning

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ABSTRACT

Learning through intensive practice has been largely observed in motor, sensory and higher-order cognitive processing. Neuroimaging studies have shown that learning phases are associated with different patterns of functional and structural neural plasticity in spatially distributed brain systems. Yet, it is unknown whether distinct neural signatures before practice can foster different subsequent learning stages over time. Here, we employed a bimanual implicit sequence reaction time task (SRTT) to investigate whether the rates of early (one day after practice) and late (one month after practice) post-training motor skill learning were predicted by distinct patterns of pre-training resting state functional connectivity (rs-FC), recorded with functional MRI. We observed that both motor learning descriptors were positively correlated with the strength of rs-FC among pairs of regions within a SRTT-relevant network comprising cerebellar as well as cortical and subcortical motor areas. Crucially, we detected a double dissociation such that early post-training learning was significantly associated with the functional connections within cerebellar regions, whereas late post-training learning was significantly related to the functional connections between cortical and subcortical motor areas. These findings indicate that spontaneous brain activity prospectively carries out behaviorally relevant information to perform experience-dependent cognitive operations far distant in time.

1. Introduction

The ability to efficiently adapt our behavior to the environmental changes is pivotal in daily life. One way to face this challenge is through repeated practice leading to acquisition of new skills. Examples of training-induced learning have been largely described in motor (Dayan and Cohen, 2011) and sensory (Gilbert et al., 2001; Watanabe and Sasaki, 2015; Irvine, 2018) as well as higher-level domains such as language, e.g. learning foreign sounds (Ventura-Campos et al., 2013). Crucially, once new abilities are acquired, they can be retained for days or even months after training (Romano et al., 2010). While it is well known that different stages of experience-dependent learning are associated with patterns of functional and structural neural plasticity in spatially distributed brain systems (Dayan and Cohen, 2011; Lohse et al., 2014; Chang, 2014; Doshier and Lu, 2017; Doyon et al., 2018), it is less clear whether different processes over time of a novel skill learning can be predicted by distinct neural signatures before training. Several lines of evidence suggest that spontaneous brain activity as indexed by resting state functional connectivity (rs-FC)

(Fox and Raichle, 2007; Biswal et al., 1995) might represent a good candidate for a neural 'prior' for learning after practice (Harmelech and Malach, 2013). In the last fifteen years, a very large body of neuroimaging studies has shown that rs-FC can be used to identify large-scale resting state networks, whose topography strongly recapitulates the one observed during active behavior in different domains (visual, motor, attention, memory) (Power et al., 2011; Yeo et al., 2011; Gordon et al., 2014). Notably, some reports indicated that the degree of the strength of rs-FC within and between resting state networks can predict the extent of subsequent learning of visual perceptual (Baldassarre et al., 2012), motor (Mattar et al., 2018), auditory-motor (Herholz et al., 2016), language (Ventura-Campos et al., 2013; Chai et al., 2016) and arithmetic (Supekar et al. 2013) skills after intensive training, in the latter case up to eight weeks later. Furthermore, it has been reported that rs-FC can be modified by training in a behaviorally relevant fashion (Albert et al., 2009; Lewis et al., 2009; Tambini et al., 2010) suggesting that intrinsic activity contributes to memory formation and skill acquisition by maintaining previous information.

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Yet, it is still unknown whether different in-time processes of learning can be predicted by distinct patterns of rs-FC at rest. In other words, does the spontaneous brain activity prospectively carry out behaviorally relevant information required to perform experience-dependent operations at different time frames? To answer this question, here we employed resting state functional MRI combined with a short bimanual Serial Reaction Time Task (SRTT) (Nissen and Bullemer, 1987; Robertson, 2007) protocol including a one-month follow-up. The SRTT task requires the mapping between the position of a series of visual stimuli and the corresponding motor responses, thus providing the implicit learning of a motor sequence. Specifically, we investigated whether, once the sequence learning was accomplished, distinct profiles of pre-training rs-FC among SRTT-relevant areas were selectively predictive of the rates of skill maintenance at two different time-points: early and late post-training stages of learning. Neurophysiological and neuroimaging studies showed that SRTT task is mediated by several areas as cerebellum, primary motor cortex, basal ganglia, and thalamus, which play different functions during sequence learning over time (Doyon and Benali, 2005; Hardwick et al., 2013). Accordingly, we expect a selective association of different profiles of functional connectivity with early and late post-training learning of a newly acquired implicit motor sequence, as these two learning components might recruit different sets of neural circuits to carry out distinct in-time operations.

2. Material and methods

2.1. Participants

A cohort with an arbitrarily determined sample size of 18, right-handed subjects (mean age = 21.8; SD = 1.97; 9 F) was recruited in the study. Individuals with history of neurological, psychiatric or sleep disorders were excluded. Furthermore, musicians, video game players, and individuals with manual hobbies were not enrolled. The participants gave their written informed consent to participate to the study, which was approved by the Ethical Committee at the University “G. d’Annunzio” of Chieti-Pescara.

2.2. Behavioral procedures

Participants underwent a 3-session longitudinal protocol of bimanual serial reaction time task (SRTT) (Nissen and Bullemer, 1987; Robertson, 2007) (Fig. 1A), in which mapping between the position of visual stimuli and motor responses is required, providing the implicit learning of a motor sequence. At Session #1 (i.e. training day), participants performed a short (~20 min) training on a bimanual SRTT involving a series of sequential key presses. At each trial, an image of a response pad with four buttons arranged in a square shape was displayed with a white rectangle framing a single button. Subjects were asked to press the spatially corresponding button on the 4-key response pad as quickly and accurately as possible by using their left/right thumb/index fingers (Fig. 1B). Specifically, left and right hands corresponded to the left and right keys, respectively, as well as thumb and index fingers corresponded to the lower and upper keys, respectively. Each trial ended when the subject selected the appropriate response button, then the next trial started. Therefore, the duration of each trial varied trial-by-trial. Reaction times (RTs) were recorded and employed as primary measure of participants’ performance, whereas accuracy was not considered given the nature of the task. The SRTT training consisted of 30 repetitions of the learning (L) sequence preceded and followed by a random (R) sequence (Trapp et al., 2012), each one including 27 trials. The precise learning sequence combining the hand and finger at each trial is displayed in the Supplementary Data. At Session #2 and Session #3, respectively 1-day and 30-days after Session #1, participants performed the same SRTT protocol consisting of only two R and two L repetitions in the following order: R-L-L-R.

Furthermore, sleep quality during the night after training was monitored through a diary and sleep efficiency (SE) calculated as the ratio between total sleep time and time spent in bed. Average SE was 95,25% and all participants were above the cut-off (85%) (Lacks and Morin, 1992).

Finally, to keep the task implicit, two consent documents were collected, the first one on training session, in which participants were informed about the procedure (e.g. “you will see some stimuli on a computer screen. You have to respond by pressing some keyboard buttons”) and the second consent form at the end of the last retest session (1 month later), in which the experimental hypotheses were explained, and data use consent required. Moreover, in order to verify that the sequence did not become explicit, on the last experimental session participants were asked questions designed to probe for declarative knowledge (Howard et al., 2008; Romano et al., 2010) (see Supplementary Data). The revision of the questionnaires indicated that participants did not acquire declarative knowledge of the sequence structure during or after the task sessions.

2.2. fMRI procedure

Before training, participants underwent an fMRI session including resting state scans as well as scans of SRTT with only random sequences to identify task-relevant regions of interest (Fig. 1A). Acquisitions consisted in 6 resting state runs in which subjects were instructed to fixate a small cross under low-level illumination and to remain passive (free from pursuing focused thought), and 2 runs of a motor functional localizer to identify the regions selectively involved in bimanual visuomotor activity. Visual stimuli were generated using e-Prime 1.0 software, projected onto an LCD screen positioned at the back of the magnet bore and viewed through a mirror attached to the head coil. Presentation timing was triggered by the acquisition of fMRI frames. In the motor localizer, three types of blocks were adopted: Motor Response (MR), Passive View (PV), and Fixation (F). Each run lasted approximately 7 min and started with 10 s of fixation, then the first block was presented starting by the MR. MR and PV blocks consisted of 18 trials and were displayed 8 times. The task blocks were alternated with F blocks of variable duration (10, 12 or 14 s with equal probability) (e.g. MR – F – PV – F – MR – F and so on). For the fMRI session, we employed a complete random version of the above-described SRTT. Images of the MRI-compatible four-key response pad were displayed on the computer screen. On each trial, one of the four keys were randomly surrounded by a white frame. The frame lasted 600 ms, followed by an interstimulus interval of 200 ms and then another trial appeared. Subjects were required to press the corresponding button with their thumb and index fingers of both hands. In the PV blocks, trial presentation time was the same of MR blocks, but participants were presented four white frames surrounding each of the keys, to avoid any activation due to movement intention and detect just activation evoked by passive view.

In all scanning sessions, data were collected using a 3T Philips Achieva Scanner. Anatomical images were acquired using a sagittal magnetization-prepared rapid acquisition gradient echo T1-weighted sequence (MPRAGE) with time of repetition (TR) = 8.3 ms; echo time (TE) = 3.9 ms; flip angle (FA) = 8°; time for inversion = 1100 ms; voxel size = 1 × 1 × 1 mm. Functional images were acquired with a gradient echo planar imaging sequence to measure the BOLD contrast (TR = 1815 ms; TE = 30 ms; FA = 90; slice thickness = 3.59 mm) in the axial plane (matrix = 64 × 64, field of view = 230 mm, 3.59 mm in-plane resolution). Thirty-three slices were acquired in ascending interleaved order for whole-brain coverage. Resting state runs included 125 frames (volumes) and localizer runs 249 frames.

2.3. Behavioral descriptors of learning

In order to investigate the time course of the learning sequence during the training session, the averaged psychophysical performance was

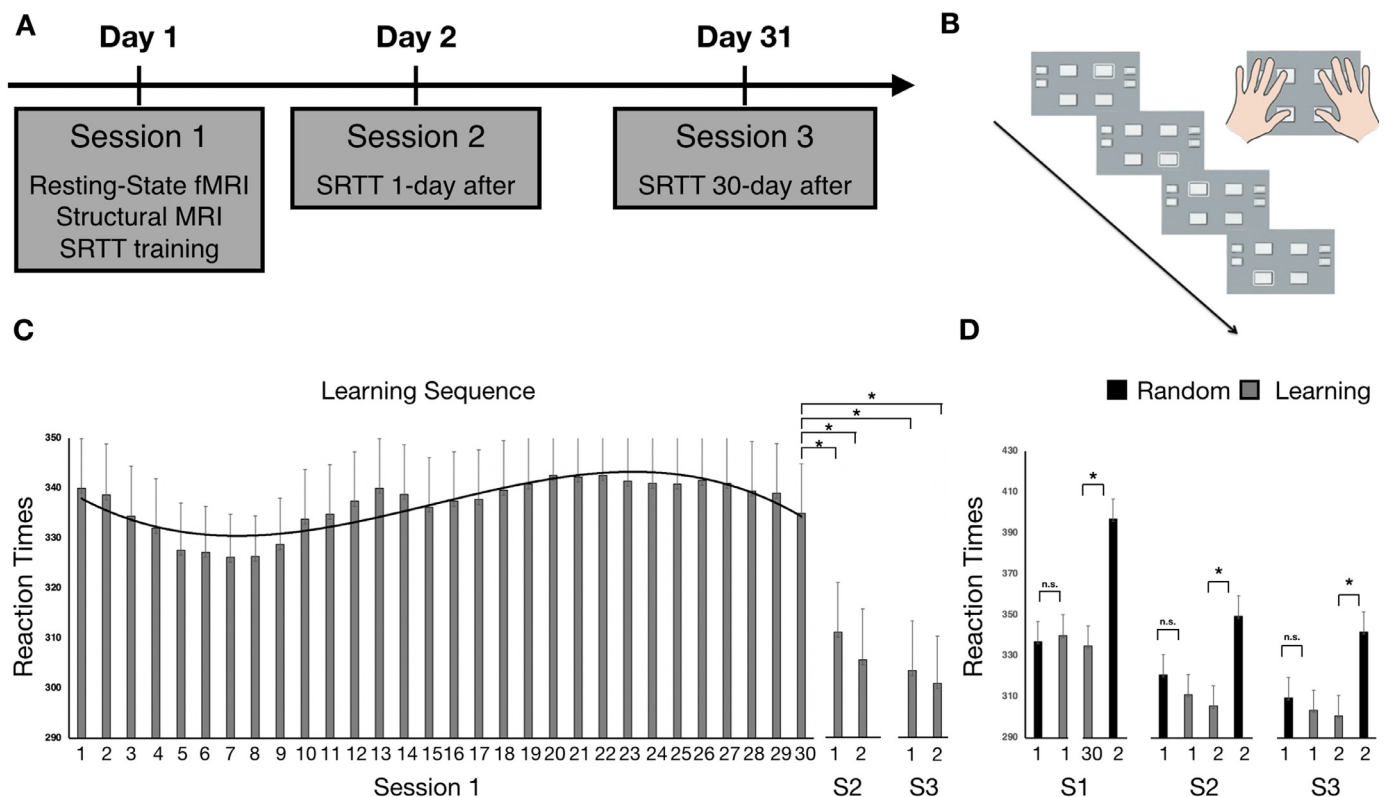


Fig. 1. Experimental protocol and behavioral results.

(A) Timeline of the experimental protocol. (B) Example of four trials of the bimanual serial reaction time task (SRTT) (Nissen and Bullemer, 1987; Robertson, 2007). (C) (D) Behavioral results. (C) Global learning. X-axis: number of learning blocks on Session 1 (block 1-30) and Session 2 (S2, block 1-2) and Session 3 (S3, block 1-2). Y-axis: reaction times (RTs) averaged across subjects. Of note, the RTs on training sessions underwent a minimal smoothing (5-block window), in order to compute the psychophysical fitting curve (solid line) $f(x) = p_1 \cdot x^3 + p_2 \cdot x^2 + p_3 \cdot x + p_4$, with coefficients (with 95% confidence bounds): $p_1 = -0.006307 (-0.008227, -0.004387)$; $p_2 = 0.2883 (0.1978, 0.3787)$; $p_3 = -3.189 (-4.406, -1.971)$; $p_4 = 340.8 (336.4, 345.3)$; * $p < 0.05$ 2-tail t-test. (D) Sequence-specific learning. The bar graph displays the mean RTs of the first and last random (black) and learning (gray) sequence block on each Session (S1, S2, S3); * $p < 0.05$ 2-tail t-test, n.s. = not significant 2-tail t-test.

first smoothed by employing a 5-block moving average and then fit by means of a 3-degree polynomial curve ($y = -0.0063 \cdot x^3 + 0.29 \cdot x^2 - 3.2 \cdot x + 3.4e+02$, goodness of fit, R-square=0.7716) by using `cftool` toolbox in MatLab (Fig. 1B). Next, to assess the global learning within training session and between sessions, we carried out two sets of analyses: a one-way ANOVA on the RTs of the 30 blocks learning as well as paired t-tests on the RTs between the last learning block of the training session and the two learning blocks on session 2 and 3, respectively.

The overarching goal of the study was to investigate whether distinct patterns of pre-training functional connectivity predict different motor learning components at two different time points, namely 24 hours and 1 month after training. To this aim, we computed the difference in RT between the last learning (L) sequence vs. the last random (R) sequence, i.e., sequence-specific learning, on session 1 (i.e., training) as well as on session 2 (day after) and session 3 (1 month after), respectively early and late post-training learning. The sequence-specific learning was statistically assessed by means of paired t-tests within each session.

2.4. fMRI analysis

2.4.1. Preprocessing

All functional MRI data (resting state and localizer scans) were pre-processed using SPM12 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London). For each subject, the functional images were first spatially corrected for head movements using a least-squares approach and six-parameter rigid-body spatial transformations (Friston et al., 1995). The realigned functional images were then corrected for differences in the timing between slices, using the middle

slice acquired in time as the reference. The high-resolution anatomical image and the functional images were co-registered and then stereotactically normalised to the Montreal Neurological Institute (MNI) brain template used in SPM12 (Mazziotta et al., 2001). Finally, the functional images were spatially smoothed with a three-dimensional Gaussian filter (8-mm full-width at half maximum) to accommodate anatomical variations between subjects (Friston et al., 1995).

2.4.2. Additional preprocessing for resting state Functional Connectivity

All the resting state analyses were performed with Conn Toolbox (version 13.1) (<http://web.mit.edu/swg/software/> or <http://www.nitrc.org/projects/conn>) for SPM (Whitfield-Gabrieli and Nieto-Castanon, 2012). Since the spontaneous, coherent, and low frequent fluctuations of the BOLD-signal are used for the resting state analysis, the BOLD time series for each participant were extracted and band-pass-filtered (0.008–0.08 Hz). As non-neural low frequent (<0.1 Hz) signals such as heart rate or respiration are able to modulate the BOLD-signal, they may likewise influence the resting state maps. Hence, a further important step is to correct for these physiological confounding signals. The Conn Toolbox accomplishes this by means of the CompCor approach. This method extracts principal components (5 each) from WM and CSF time series. WM and CSF voxels are identified via a segmentation of the anatomical images. These components were added as confounds in the denoising step of the Conn toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012; Restom et al., 2006). Six parameters obtained by rigid body head motion correction (three rotation and three translation parameters) were used as temporal covariates to reduce the impact of motion. Hereafter we report

the means (and SD) of 3 translation and 3 rotation parameters of head movements, translation (mm): $x=0.0162$ (0.0819), $y=-0.1149$ (0.0884), $z=-0.0581$ (0.0989); and rotation (degrees): $x=-0.0004$ (0.0033), $y=-0.00001$ (0.0013), $z=0.0002$ (0.0016). Furthermore, the CONN denoising pipeline included also the scrubbing of head motion-induced artifacts (Power et al., 2012) in order to avoid any potential confounding on the BOLD signal.

2.4.3. Functional localizer

The images were subsequently analyzed using a random-effects approach. At the first stage, the time series of the functional MR images obtained from each participant were analyzed separately. The effects of the experimental paradigm were estimated on a voxel-by-voxel basis, according to the general linear model extended to allow the analysis of fMRI data as a time series (Worsley and Friston, 1995). The experimental blocks (MR and PV) were modeled as box-car functions, convolved with a synthetic hemodynamic response function. Subject-specific contrast images (MR>PV) were then entered at the second-level group analysis into a random-effects group analysis using one-sample t-test. The resulting statistical parametric map of the t statistic was thresholded with $p = 0.05$ (FWE).

2.4.4. Task-relevant regions of interest (ROIs)

The task-relevant network was defined from the second-level analysis on task-evoked activity of the functional localizer. A set of regions of interest (ROIs) was generated around the activation peaks derived from the contrast between Motor Response (MR) and Passive View (PV) blocks of the visuomotor localizer task (MR>PV). The threshold for the activation map was set to whole brain cluster level of $p = 0.05$ (FWE) with an extent threshold of $k = 10$. A set of ROIs was built using the Matlab-based software BrainShow (in-house software implemented in Matlab, part of the BIL Matlab toolbox of the Neuroimaging Lab, Santa Lucia Foundation in Rome, Italy), by means of a peak-finding procedure that extracts activation peaks from the statistical map and creates ROIs by including supra-threshold voxels within a maximum distance from the peak (Tosoni et al., 2015). Such procedure retained portions within 8 mm from the activation peaks selected from each cluster (Table 1).

2.4.5. Resting state Functional Connectivity

After the preprocessing steps which are computed at single runs separately, the six single runs were aggregated (by skipping the first 4 volumes) and underwent the first-level connectivity analysis in which the single runs were aggregated. Specifically, for each subject, and for each ROI, the BOLD time courses of the six resting state runs were extracted and concatenated, then they were treated as a single one to compute the functional connectivity values. Then, the connectivity matrix was built by computing the pairwise temporal correlation (Pearson correlation coefficient r) across all pairs of ROIs. Finally, the r -score matrix was then converted by Fisher Z-transform into a normalized Z-score matrix.

2.5. Connectivity-behavior analysis

We then investigated whether the strength of pre-training functional connectivity (rs-FC) of task-relevant network was selectively associated with early and late post-training sequence-specific learning. Hence, we correlated across subjects the rs-FC matrices with early and late post-training learning metrics, regressing out the baseline performance as indexed by the first-ever random sequence on the training session. Furthermore, to control that connectivity-behavior patterns were not driven by the partial correlation approach, we also carried out a total correlation analysis without regressing the baseline (see Supplementary Data).

To evaluate the statistical significance of these correlations, we used a permutation test. Specifically, for all node pairs 1000 random permutations of FC values across subjects were generated and the corresponding FC-behavior partial correlations were computed. Then, we selected the 99th percentile of statistic values of the connectivity-behavior partial

Table 1

SRTT-relevant network.

The table lists the set of SRTT-relevant regions of interest (ROIs) employed for computing the pre-training resting state functional connectivity. The coordinates are in the Montreal Neurological Institute (MNI) space used in SPM12 (Mazziotta et al., 2001).

Area	Hemisphere	x	y	z
Cerebellum VIII	Right	30	-55	-55
Cerebellum VIII	Right	12	-76	-52
Cerebellum VIII	Right	21	-64	-55
Cerebellum Crus I	Left	-6	-76	-25
Cerebellum VI	Left	-24	-55	-28
Cerebellum VIIb	Left	-12	-76	-46
Cerebellum VIIa	Left	-15	-64	-52
Cerebellum Vermis VI	Medial	0	-67	-13
Cerebellum Vermis VI	Right	6	-73	-19
Cerebellum VI	Right	30	-52	-31
Putamen	Left	-24	11	2
Caudate	Left	-15	-4	14
Thalamus	Left	-15	-16	14
Putamen	Right	24	5	2
Caudate	Right	15	-7	14
Thalamus	Right	15	-16	8
Dorsal Pre-Central Gyrus	Left	-39	-10	53
Middle Cingulate Cortex	Left	-3	5	47
Ventral Pre-Central Gyrus	Left	-57	8	29
Ventral Post-Central Gyrus	Left	-51	-19	20
Supplementary Motor Area	Right	6	-4	65
Dorsal Pre-Central Gyrus	Right	39	-10	56
Dorsal Post-Central Gyrus	Right	42	-25	47
Middle Cingulate Cortex	Right	3	8	41
Ventral Pre-Central Gyrus	Right	54	14	29

correlations over all ROI pairs to build the null distribution of correlation with the specific learning index. The critical threshold was computed as the $c+1$ highest value of the permutation distribution where $c = \alpha \times N = 0.05 \times 1000$ rounded down. This computation retained the top 1% of the statistic values over all ROI pairs. The partial correlations with behavior obtained from unpermuted data at any ROI pair exceeding the critical threshold were considered statistically significant at level $\alpha = 0.05$.

Next, in order to statistically assess whether the patterns of rs-FC associated with the two behavioral descriptors were dissimilar, we computed the Mantel's test (Mantel and Haenszel, 1959) on the functional connections exhibiting a significant correlation at least with one learning component. Furthermore, we employed an unsupervised hierarchical clustering procedure, implemented in MATLAB (The MathWorks Inc., Natick, MA), to determine whether the ROI pairs identified in the previous analysis could be divided into different groups according to their partial correlations with the behavioral descriptors. A hierarchical cluster tree was created from the Euclidean distances between ROI pairs, which were characterized by the partial correlations with early and late post-training learning. The threshold for the pruning of the cluster tree, i.e., grouping in n -clusters, was calculated searching for a maximum value of the linear combination of the Dunn and Silhouette indices (Spadone et al., 2012; Sinibaldi et al., 2018; Baldassarre et al., 2019). The former indicates the quotient between the shortest distance between elements of distinct clusters and the maximum distance between points belonging to the same cluster; the latter denotes how a given point is similar to the other points belonging to the same cluster, in relation to points in different clusters. Finally, in a further analysis we compared the strength of significant connectivity-behavior partial correlations by means of Steiger's z-transform test (Steiger, 1980), implemented on the web version of the software "cocorr" of the R package (comparingcorrelations.org/) (Diedenhofen and Musch, 2015) (see Supplementary Data).

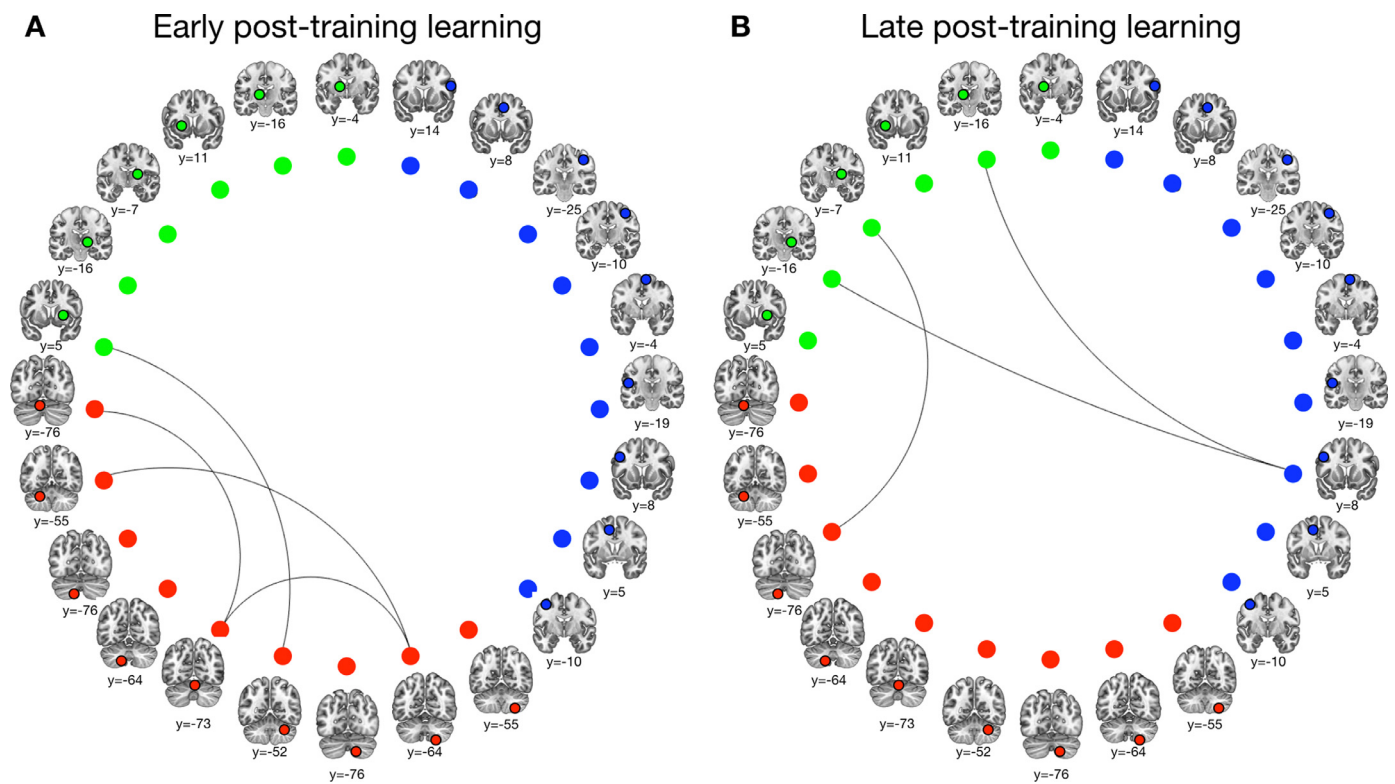


Fig. 2. Double dissociation between early/late post-training learning and functional connectivity profiles.

In each circular plot the arches connect two regions of interests (ROIs) whose resting state functional connectivity (rs-FC) was positively correlated (higher rs-FC = better performance) with early (A) and late (B) post-training sequence-specific learning (Pearson r values corrected after permutation test). Red, blue and green circles indicate cerebellar, cortical and subcortical ROIs, respectively.

3. Results

3.1. Global learning and early/late post-training sequence-specific learning

The curve fitting analysis on the RTs of the 30 learning blocks of the training session indicated a multi-phase sinusoid-like pattern showing a substantial decrease of RTs in the first 10 blocks, with a subsequent rebound (up to block ~20) and finally, a descent trend in the last 5 blocks (Fig. 1C). While the one-way ANOVA did not indicate a significant main effect of repetition of learning sequences ($F_{(29)}=0.41$; $p = 0.9$) within the training session, in both Sessions 2 and 3, all learning sequences were significantly faster than the last learning sequence on Session 1 (two-tail t -test: $t_{(17)} = 2.47$, $p = 0.024$ for block 1 and $t_{(17)} = 2.74$, $p = 0.013$ for block 2 of Session 2; $t_{(17)} = 3.81$, $p = 0.0014$ for block 1 and $t_{(17)} = 3.69$, $p = 0.0018$ for block 2 of Session 3) (Fig. 1C). These results indicate the presence of global learning 24 hours after training and that such effect is maintained after one month.

Next, we investigated the in-time components of sequence-specific learning, namely early and late post-training learning, which were defined as the difference in RTs between the last learning sequence vs. last random sequence at Session 2 (24 hours after training) and Session 3 (1 month after training), respectively (see Material and methods). Paired t -tests showed a significant increase in RTs for random as compared to learning sequence at each session (Fig. 1C, 2-tail t -tests: $t_{(17)} = 6.91$, $p = 2.5 \times 10^{-6}$; $t_{(17)} = 9.18$, $p = 5.3 \times 10^{-8}$; $t_{(17)} = 5.98$, $p = 1.4 \times 10^{-5}$, for Session #1, #2, and #3, respectively) (Fig. 1D). Notably, the comparison between the first random block vs. the first sequence block on Session 1 revealed no statistical differences in reaction times ($t_{(17)} = 0.5$, $p = 0.6$) (Fig. 1D). The same analysis on Sessions 2 and 3 indicated that the first random and learning sequences in both Sessions 2 and 3 did not differ ($t_{(17)} = 1.35$, $p = 0.19$; $t_{(17)} = 1.46$, $p = 0.16$) (Fig. 1D). Overall, these findings indicated that sequence-specific learning took place at

the end of the training session as well as that it was detected at the end of Sessions 2 and 3, respectively 24 h and one month after training. Of note, the two descriptors of early and late post-training sequence-specific learning exhibited a negative correlation ($r = -0.58$, $p = 0.012$), indicating that subjects with larger learning at 24 h after training exhibited less pronounced late learning at one month and vice versa.

3.3. Double dissociation between pre-training rs-FC of different circuits and post-training learning components: cerebellar-related rs-FC for early learning and cortical-subcortical rs-FC for late learning

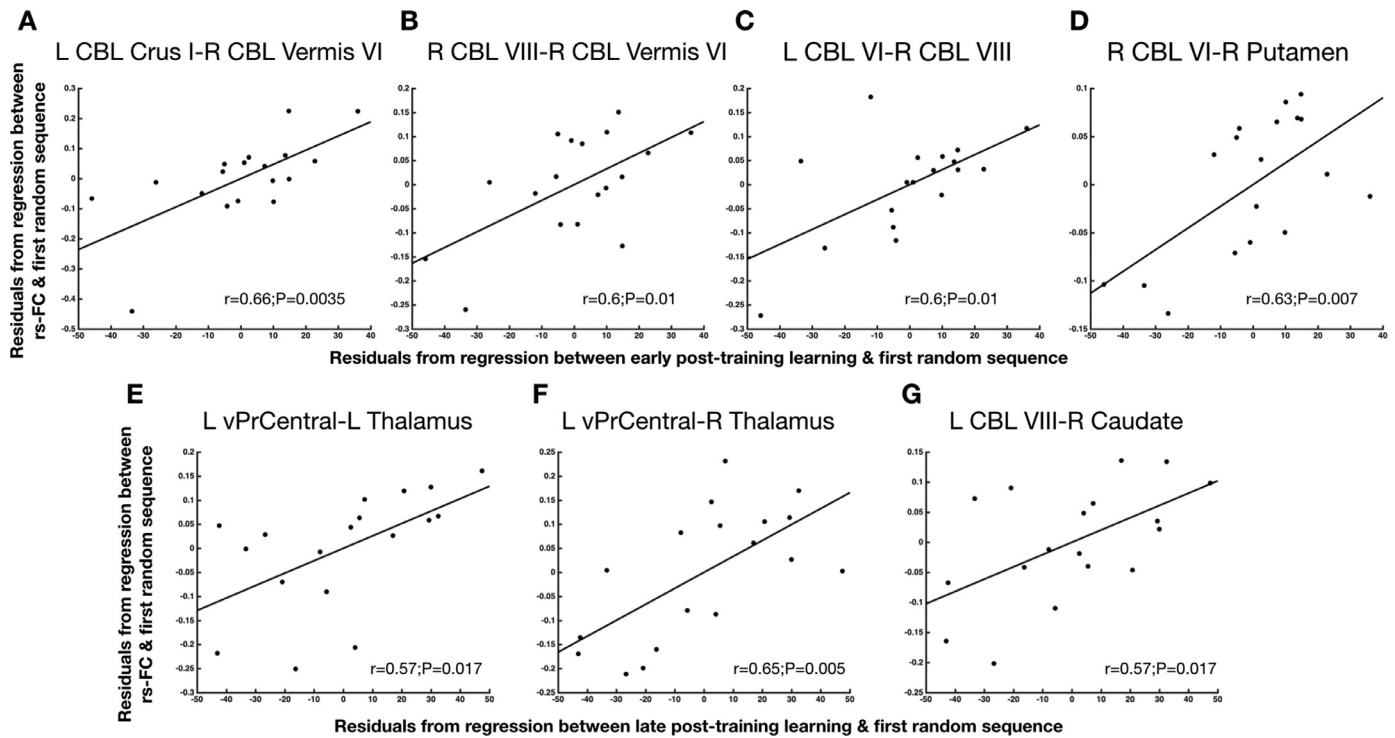
The overall goal of the current study was to investigate whether different profiles of pre-training resting state functional connectivity (rs-FC) (Fox and Raichle, 2007; Biswal et al., 1995) among SRTT-relevant brain areas were selectively associated with sequence-specific early and late post-training learning. To this aim, at Session #1 before the SRTT training, participants underwent an fMRI session including resting state scans (open eyes while fixating a small cross) as well as scans of a functional localizer for SRTT-relevant regions, in which they performed the task on solely random sequences (Fig. 1A) (see Material and methods). It was detected a bilateral SRTT network comprising cerebellar cortex (Crus, VI, VII, VIII), cortical motor areas such as dorsal/ventral pre- and post-central gyrus as well as subcortical structures including caudate, thalamus, and putamen. Next, for each subject we computed the pairwise rs-FC between the functionally defined regions of interest (ROIs) within the SRTT network (Table 1). Finally, in order to identify specific functional connections associated with early and late post-training learning, we computed the partial correlation between pre-training rs-FC and each descriptor across subjects, regressing out the baseline performance as indexed by the first-ever random sequence on the training session. We observed that early post-training learning was positively correlated with the strength of functional connections within cerebellar regions as well as between one cerebellar and one sub-cortical area ($p < 0.05$ after correction by permutation test) (see circular plot at

Table 2

Significant functional connections associated with early post-training learning.

The table lists the pairs of ROIs showing a significant partial correlation ($p < 0.05$ corrected after permutation test) with early post-training learning. Early and late learning columns report the r -Pearson and associated p -value for the partial correlations of the corresponding behavioral descriptors (bold indicates $p < 0.05$ corrected after permutation test).

ROI	ROI	Early learning	Late learning
Left Cerebellum Crux I	Right Cerebellum Vermis VI	$r = 0.66; P = 0.003$	$r = -0.33; P = 0.2$
Left Cerebellum VI	Right Cerebellum VIII	$r = 0.6; P = 0.01$	$r = -0.002; P = 0.99$
Right Cerebellum Vermis VI	Right Cerebellum VIII	$r = 0.6; P = 0.01$	$r = -0.34; P = 0.17$
Right Cerebellum VI	Right Putamen	$r = 0.63; P = 0.007$	$r = -0.21; P = 0.4$

**Fig. 3.** Early/late post-training learning and functional connectivity partial correlations.

The figure displays the scatterplots of the partial correlations between learning descriptors and resting state functional connectivity, regressing out the initial performance for each of the node pairs whose rs-FC was significantly associated with the Early (A-D) or Late (E-G) post-training Learning. L=left; R=right; CBL=cerebellum; vPrCentral= ventral pre-central gyrus.

Fig. 2A and Table 2 for the r - and p -values of the partial correlations). The positive sign of the partial correlations means that individuals with stronger pre-training rs-FC exhibited larger early post-training learning, that is higher increase of RT in random vs. learning sequence at Session #2 (1-day after training) regardless of the performance at the very beginning of training (see Fig. 3A–D for the scatterplots). Specifically, early post-training learning was associated with the functional connectivity among the following pairs of regions: right cerebellum vermis VI-left cerebellum crux I; right cerebellum vermis VI- right cerebellum VIII; right cerebellum VIII-left cerebellum VI; right cerebellum VI-right putamen. By contrast, late learning was positively related to the strength of functional connections between cortical and subcortical regions as well as between one subcortical and one cerebellar structure ($p < 0.05$ after correction by permutation test) (see circular plot at Fig. 2B and Table 3 for the r - and p -values of the partial correlations). Once again, the positive partial correlation indicates that subjects exhibiting higher pre-training rs-FC showed more pronounced late learning at Session #3 (~30 days after training), independently of the initial performance at the training session (see Fig. 3E–G for the scatterplots). In greater detail, late learning was associated with the functional connectivity between left ventral pre-central gyrus and bilateral thalami as well as between

left cerebellum VIIb and right caudate. Overall, two different components of the newly acquired motor sequence learning seem to be associated with distinct patterns of pre-training rs-FC as there were no connections associated with both time points. Taken together, these results suggest that early post-training learning exhibits a stronger association with cerebellar-related functional connections (Fig. 2A), whereas late learning shows a stronger association to cortical-subcortical functional connections (Fig. 2B). Furthermore, to control for a potential effect of the partial correlation, we carried out a total correlation analysis (i.e., without regressing out the baseline performance), which yielded a similar pattern of connectivity-behavior associations for early and late post-training learning components (see Supplementary Data).

In order to statistically assess whether the patterns of rs-FC associated with the two behavioral descriptors were different, we computed the Mantel's test for dissimilarity (Mantel and Haenszel, 1959) on the functional connections exhibiting a significant correlation at least with one learning component. Results indicated that the two sets of connectivity-behavior partial correlations were not similar ($Z_M = -0.96$; $p = 0.98$), suggesting that the sets of functional connections associated with the two learning measures are distinct (see scatter plot at Fig. 4A). To corroborate this hypothesis, we employed an unsupervised hierar-

Table 3

Significant functional connections associated with late post-training learning.

The table lists the pairs of ROIs showing a significant partial correlation ($p < 0.05$ corrected after permutation test) with late post-training learning. Early and late learning columns report the r -Pearson and associated p -value for the partial correlations of the corresponding behavioral descriptors (bold indicates $p < 0.05$ corrected after permutation test).

ROI	ROI	Early learning	Late learning
Left ventral Pre-Central Gyrus	Right Thalamus	$r = -0.28$; $P = 0.26$	$r = 0.65$; $P = 0.005$
Left ventral Pre-Central Gyrus	Left Thalamus	$r = -0.39$; $P = 0.11$	$r = 0.57$; $P = 0.017$
Left Cerebellum VIIIb	Right Caudate	$r = -0.14$; $P = 0.58$	$r = 0.57$; $P = 0.017$

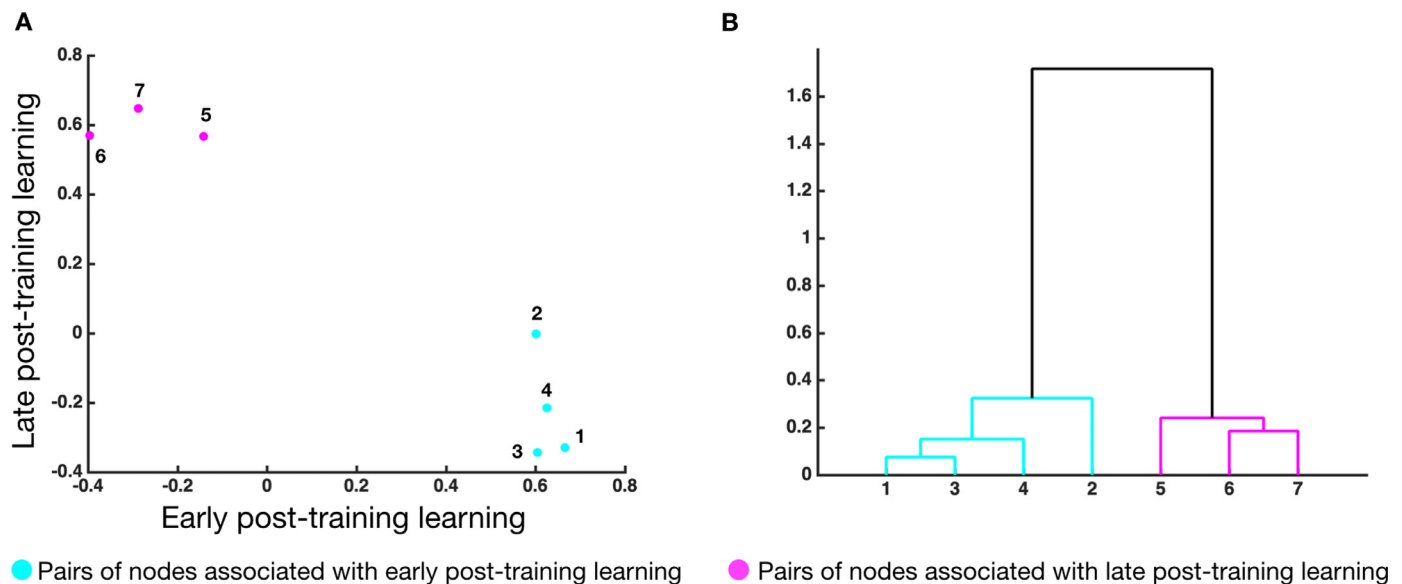


Fig. 4. Different clusters for early/late post-training learning and functional connectivity.

(A) Each dot in the scatterplot displays a pair of nodes showing a significant correlation with sequence-specific early (cyan) or late (magenta) post-training learning. The x- and y-axes indicate the magnitude of the connectivity-behavior partial correlations (r Pearson) for each learning descriptor. (B) Dendrogram derived from applying a clustering algorithm to the pairs of nodes exhibiting a significant association with at least one learning descriptor. Cyan and magenta colors failing in the same cluster. Of note, connectivity-behavior correlations associated with early and late post-training learning were grouped in two distinct clusters (cyan and magenta, respectively)

chical clustering procedure to determine whether the 7 ROI pairs identified in the previous analysis could be divided into different groups according to their partial correlations with behavior (see Methods). This analysis suggested an optimal number of clusters of two, which separated the connectivity-behavior partial correlations of the two learning descriptors (Fig. 4B). Overall, these findings clearly show a double dissociation regarding the differential engagement of cerebellar and cortical-subcortical functional connections for early and late post-training learning, respectively.

4. Discussion

In the current study we showed that distinct profiles of pre-training resting state functional connectivity (FC) among SRTT-relevant areas were selectively predictive of the degree of early (one day after training) and late (one month later) post-training learning of the newly acquired bimanual motor sequence. To be noted, the term prediction is employed to refer to “in-sample correlation” as variables measured at one time point (i.e., before training) were correlated with other variables measured at subsequent time points (i.e., one day and one month later), avoiding generalization to “out-of-sample predictions” (Gabrieli et al., 2015).

The psychophysical analyses on the learning sequence blocks of the training session revealed a multi-phase sinusoid-like pattern of the RTs time course. A possible factor to explain such pattern can be represented by the fatigue since subjects performed overall 810 trials. This factor

might also additively affect the increase of RTs in the last random sequence. However, it is unlikely that the effect of fatigue applies to Session 2 and 3, since solely two repetition blocks were performed.

Despite the lack of RTs reduction within these sessions, all learning sequences were significantly faster than the last learning sequence on Session 1. Therefore, these results indicate the presence of global learning 24 h after training and that such effect is maintained after one month, suggesting that the learning sequence has been encoded and consolidated.

The comparison between the last learning and random sequences within the training session showed a significant increase of RTs i.e., sequence-specific learning. Notably, such effect was detected 24 h and one month after training and it was stable across sessions. On the contrary, the first random and learning sequences did not differ in the training session, indicating that subjects exhibited equivalent performance in the two conditions before training. The same pattern was observed in Session 2 and 3 possibly due to the presence of partial transfer effects from learning to random sequences, as reported in previous studies (Mussegens and Ullen, 2015; Sanchez et al., 2015; Song et al., 2015). Instead, the repetition of two learning sequences might, then, re-activate the implicit knowledge of the sequential elements, which in turn would lead to a deterioration in performance on the subsequent, last random sequence, as observed.

To summarize, we detected global learning across sessions as well as sequence-specific learning effects both 24-hours and one month after training. These findings are consistent with previous works show-

ing that retention of acquired motor skills is observed in both humans (Hikosaka et al., 2002b; Romano et al., 2010) and monkeys (Hikosaka et al., 2002b) for a long period up to one year after training.

Albeit recent neuroimaging studies have shown that the resting state functional connectivity before training was correlated with the extent of subsequent forms of motor learning such as variants of SRTT (Mattar et al., 2018; Sugata et al., 2020) to our knowledge the present work is the first one providing evidence that distinct sets of functional connections are able to disentangle motor learning components requiring different in-time operations.

Overall, current findings indicate that individuals with stronger cerebellar pre-training rs-FC exhibited better sequence-specific early post-training learning the day after practice, independently of their baseline performance. In other words, the functional connections of cerebellum play a relevant role for promoting such stage of learning, whereas they are not involved in the processes of late learning when the motor sequence has been consolidated. Hence, coordinated endeavor between cerebellar regions is selectively crucial at the first stage of learning, but not (or less) for the late learning of the acquired sequence movements, which are selectively indexed by synchronized patterns of activity in cortical and subcortical motor structures. This pattern of behaviorally relevant pre-training rs-FC might reflect distinct sets of in-time functions carried out by cerebellum, cortical and subcortical motor areas at different stages of implicit motor learning (Hardwick et al., 2013). While the cerebellum plays a role in error prediction and correction by forwarding information to sub-cortical and cortical motor networks (Hikosaka et al., 2002a; Tseng et al., 2007; Shadmehr and Krakauer, 2008; Krakauer and Mazzoni, 2011; Galea et al., 2011; Penhune and Steele, 2012), the cortical motor network is strongly devoted to develop and maintain the muscle coordination in order to execute faster and more accurate finger movements (Krakauer and Mazzoni, 2011; Shmuelof and Krakauer, 2011; Penhune and Steele, 2012). Furthermore, the basal ganglia and thalamus are engaged in probabilistic computation involved in movement selection and its related reward (Hikosaka et al., 2002a; Krakauer and Mazzoni, 2011; Penhune and Steele, 2012), and in generation of movements and ongoing supervision of action, respectively.

Albeit all set of above-described operations are required for early post-training learning, the greater engagement of cerebellar connectivity suggests that the error monitoring exerted by cerebellum results fundamental for strengthening the sequence learning performance. Accordingly, task-based fMRI (Jueptner and Weiller, 1998; Toni et al., 1998) and lesion-behavior (Nixon and Passingham, 2000) studies indicate the importance of the cerebellum early on to learn a new motor sequence. This interpretation is supported by two observations regarding right lobule VIII, a cerebellar area exhibiting several functional associated with early post-training learning. First, a task-based meta-analysis of imaging studies reported that such region is recruited during motor tasks (Stoodley and Schmahmann, 2009). Second, the right lobule VIII at rest is functionally connected with the somato-motor and cingulo-opercular (Dosenbach et al., 2007) networks (Buckner et al., 2011; Sang et al., 2012; Shinn et al., 2015), which are devoted to motor control/planning and error monitoring, respectively.

One other not-mutually exclusive interpretation is that the association between cerebellum rs-FC and early post-training learning is related to the higher attention demand on this stage of learning. As a matter of fact, lobule VIII exhibits long-range functional connectivity with the so-called dorsal attention network (Buckner et al., 2011; Sang et al., 2012; Shinn et al., 2015), a set of fronto-parietal regions which are increasingly activated during attentionally demanding tasks (Corbetta and Shulman, 2002). Consistently, patients with aberration to lobules VI-VIII might present deficits of spatial attention (Townsend et al., 1999). Similarly, crus I exhibits significant functional connections with higher-order systems such as default mode and fronto-parietal control networks (San et al., 2012), which have been shown to be engaged in filtering out non-relevant information (Shulman et al., 2003) and to initiate and ad-

just control on each trial (Dosenbach et al., 2006) during task execution, respectively. This hypothesis well fits with findings coming from previous works on the visual domain showing higher activation of dorsal attention and de-activation of default mode networks early on perceptual learning when the task is more difficult, therefore requiring stronger attentive effort (Sigman et al., 2005; Lewis et al., 2009; Baldassarre et al., 2016a).

A second pattern of behaviorally relevant connectivity was also detected: albeit associated with both stages of SRTT performance learning, coordinated activity among cortical and subcortical motor regions appeared more relevant for the long-term outcome. This finding fits well with the putative function of primary motor cortex in maintaining the representation of coordination among muscles which are required to execute precise and fast movements (Krakauer and Mazzoni, 2011; Shmuelof and Krakauer, 2011; Penhune and Steele, 2012). Moreover, the involvement of thalamic-related functional connectivity in predicting late post-training learning suggests its preferential role in the processes required when a learned sequence is consolidated, i.e. retention/recall of learned moments. Different observations from both human and animal studies argue in favor of this interpretation: first, a focal damage to the thalamus in monkeys leads to the inability to re-learn a movement (Canavan et al., 1989); second, thalamic activations are stronger when subjects have an explicit awareness of the presence of a learned sequence (Hardwick et al., 2013); third, thalamus tends to be deactivated at the very early phase of SRTT training (Rauch et al., 1998). Taken together, current findings indicate that synchronized spontaneous activity between cortical and subcortical thalamic structures would be crucial for better recall of stored motor representations as well as for finest implicit selection of the learned movements sequence at one month after training.

Furthermore, we observed that the functional connections linking the putamen and caudate with the cerebellar areas VI and VIII were associated with early and late post-training descriptors of learning, respectively. At first glance, these findings might appear in contrast with previous neuroimaging reports, as the caudate is more involved in the early acquisition phase, whereas the putamen is recruited as learning progresses (Albouy et al., 2008; Jueptner et al., 1997; Lehericy et al., 2005; Steele et al., 2010) (Toni et al., 1998). However, it should be noted that such studies investigated the task-driven regional activity at different learning stages, whereas our findings rely on the pre-training resting state, i.e., in absence of task, functional connectivity between such striatal structures and cerebellar areas. The observed connectivity-behavior dissociation might be related to the opposite profiles of the baseline (i.e., not weighted by behavior) resting state functional connectivity derived from the caudate and putamen (Di Martino et al., 2008). Specifically, the caudate exhibits functional connections with higher-level areas belonging to domain general networks such as default mode, fronto-parietal and dorsal attention as well as negative associations with supplementary and primary motor areas. By contrast, the putamen shows preferential functional connectivity with primary and secondary motor areas as well negative correlation with default mode regions (Martino et al., 2008). Future studies, by employing directionality approach e.g., effective connectivity and granger causality, should be carried out in order to clarify the inhibitory/facilitatory role of caudate and putamen on the functional connectivity between cerebellar and higher-order domain-general areas at different phases of motor sequence learning.

Overall, current findings seem to be consistent with the neurobiological model of motor sequence learning proposed by Doyon's group (Doyon and Benali, 2005; Doyon et al., 2018), which posits that the long-term representation of the well-learned motor sequence is mediated by a network comprising cortico-subcortical structures, such as striatum and motor cortices, whereas there would be a decrease of cerebellar-related interactions.

Different yet not mutually exclusive interpretations have been proposed about the function of the spontaneous brain activity as indexed by resting state functional connectivity. A largely accepted hypothe-

sis is that functional connections at rest might reflect the previous history of co-activated areas over time (Fox and Raichle, 2007; Deco and Corbetta, 2011; Buckner and Vincent, 2007), thus intrinsic activity would contribute to memory formation and skill acquisition by maintaining previous information (Albert et al., 2009; Lewis et al., 2009; Tambini et al., 2010). One more prominent hypothesis is that correlated patterns of spontaneous fluctuations would represent a prior of cognitive bias (Deco and Corbetta, 2011; Harmelech and Malach, 2013) as the strength of rs-FC within and across brain networks correlates with off-line task performance in healthy individuals as well as with behavioral deficits in clinical populations (Baldassarre et al., 2016b; Sheffield and Barch, 2016). Current findings suggest that two putative functions of spontaneous brain activity, i.e., memory of previous experience through brain co-activations and prediction of subsequent behavior, might be closely intertwined. Indeed, we observed that separated sets of pre-training functional connections predict distinct in-time learning processes, which are likely the outcome of different previous experiences and the result of patterns of brain co-activations. Accordingly, the association between early post-training learning and cerebellar-based connections might be the result of past co-activations of such structures for computing operations (e.g., error monitoring) required at this specific learning stage. On the same line, the connections involving cortical and subcortical structures might reflect their history co-activation for carrying out the processes tailored for the recollection in time of acquired motor skills.

In summary, we report a double dissociation linking distinct temporal components of implicit motor skill learning to two pre-training functional connectivity profiles comprising task-relevant brain areas. The findings of an association between intrinsic brain activity and subsequent motor memories at different time points have both theoretical and clinical implications. Theoretically, they indicate that functional brain architecture acts as prior biasing learning abilities by carrying out behaviorally relevant features presumably based on past experience. Clinically, they might provide insights for targeting specific regions at different time-points in protocols aiming at improving motor functions by combining motor training and non-invasive brain stimulation in different neurological populations, such as stroke, brain tumors and Parkinson's disease.

5. Limitations

The current study has several limitations. First, the relatively small sample size ($n=18$) might potentially have an effect on the behavior-connectivity partial correlations. Further studies with larger cohorts might be useful. Second, although the reaction time represents the primary measure of SRT and error rate is usually very low (1–2%) (Cohen and Robertson, 2010; Robertson, 2007; Sami, Robertson and Mial, 2014), the absence of accuracy recording does not allow to differentiate slow response from a first incorrect response eventually followed by correct response. Third, in order to identify the SRTT- regions, we employed the random, rather than learning, sequences. This could raise the possibility of missing other regions such as the hippocampus/medial temporal lobe (Rieckmann et al., 2010; Stillman et al., 2013) or circuits such as the executive frontal network (Sami et al., 2014), which play a role in different stages of motor skill acquisition. Fourth, further investigations are needed to disentangle the role of structural connectivity into the association between distinct patterns of functional connectivity and different processes of motor learning. Finally, future studies might investigate whether current findings can be generalized to other domains such as associative conditioning, perceptual learning, semantic associations.

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Declaration of Competing Interest

The authors declare no competing financial interests.

Data for reference

The dataset and codes used in this study are available to other researchers on request to the corresponding authors A.B. a.baldassarre@unich.it and G.C. giorgia.committeri@unich.it.

Supplementary materials

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