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Original Article

ORIGINAL ARTICLE

Stress Modulates the Balance between Hippocampal and Motor Networks during Motor Memory Processing

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Abstract

The functional interaction between hippocampo- and striato-cortical regions during motor sequence learning is essential to trigger optimal memory consolidation. Based on previous evidence from other memory domains that stress alters the balance between these systems, we investigated whether exposure to stress prior to motor learning modulates motor memory processes. Seventy-two healthy young individuals were exposed to a stressful or nonstressful control intervention prior to training on a motor sequence learning task in a magnetic resonance imaging (MRI) scanner. Consolidation was assessed with an MRI retest after a sleep episode. Behavioral results indicate that stress prior to learning did not influence motor performance. At the neural level, stress induced both a larger recruitment of sensorimotor regions and a greater disengagement of hippocampo-cortical networks during training. Brain-behavior regression analyses showed that while this stress-induced shift from (hippocampo-)fronto-parietal to motor networks was beneficial for initial performance, it was detrimental for consolidation. Our results provide the first experimental evidence that stress modulates the neural networks recruited during motor memory processing and therefore effectively unify concepts and mechanisms from diverse memory fields. Critically, our findings suggest that intersubject variability in brain responses to stress determines the impact of stress on motor learning and subsequent consolidation.

Key words: hippocampus, memory consolidation, motor sequence learning, stress

Introduction

The cerebral processes underlying motor learning and memory consolidation, defined as the process by which newly learned motor skills are transformed into more robust forms (Robertson et al. 2004), are well described. Various models of motor sequence learning (MSL) indicate that the encoding of a new motor skill relies on "cortico-cerebellar, cortico-striatal, and cortico-hippocampal" circuits, whose recruitment follows specific dynamics during the learning process (see Doyon et al. 2009; Penhune and Steele 2012; Albouy, King, et al. 2013a; for various models). While activity in cortico-hippocampal networks progressively decreases during initial encoding, recruitment of circuits including sensorimotor cortical, striatal, and cerebellar areas increases as a function of practice. Interestingly, functional responses in striato- and hippocampo-cortical systems have been particularly linked to the consolidation process (for a review see Albouy, King, et al. 2013a). Specifically, interindividual approaches indicate that the

level of activity in the hippocampus as well as the strength of its competitive interaction (i.e., connectivity) with the striatum during memory acquisition forecasts gains in performance observed after sleep (Albouy et al. 2008; Albouy, Sterpenich, et al. 2013b). Critically, while these past studies effectively linked the neural signatures of encoding to consolidation, it remains unclear whether modulating striatal and hippocampal systems during learning would influence motor memory processes.

Based on extensive evidence that experimental induction of stress can alter the balance between hippocampal and striatal recruitment during declarative and perceptual learning (Schwabe and Wolf 2012; Schwabe et al. 2013; Vogel et al. 2015; Wirz, Reuter, et al. 2017a; for reviews see Vogel et al. 2016; Schwabe 2017), stress was used in the present study as a potential modulator of the neural signatures supporting motor memory consolidation. While it is widely accepted that stress modulates hippocampal function (for reviews see Kim and Diamond 2002; Joëls et al. 2007), contradictory patterns of results have been reported in the literature with respect to both the direction of the effects (e.g., increase vs. decrease in brain activity, Quaedflieg and Schwabe 2017) and the link to memory performance (Schwabe 2017). Importantly, a more consistent effect of stress on brain function has been described for nonmotor learning tasks in which the hippocampus and striatum compete during initial learning (see Wirz et al. 2018 for a review of the different tasks). Indeed, neuroimaging studies have shown that stress prior to probabilistic classification learning consistently elicits a shift towards the use of striatum-based "rigid" strategies at the expense of more "flexible" hippocampus-dependent strategies (Schwabe and Wolf 2012; Schwabe et al. 2013; Wirz, Wacker, et al. 2017b). Crucially, in this previous research, the stress-induced behavioral shift was paralleled by reduced hippocampal activity during task performance. Given the competitive interaction between striatal and hippocampal systems during classification learning (Poldrack et al. 2001; Poldrack and Packard 2003), it was recently suggested that stress-induced reduction in hippocampal recruitment may allow the striatum to dominate learning under stress (Schwabe 2017). Thus, based on the evidence that stress interferes with neural processing in the hippocampus and can bias the balance between hippocampal and striatal recruitment, i.e., two main actors ensuring optimal motor sequence memory consolidation (Albouy, King, et al. 2013a), the goal of the present study was to investigate whether stress can be used to modulate motor memory consolidation processes.

To the best of our knowledge, the effect of stress on motor sequence memory consolidation has only been investigated at the behavioral level in a recent study from our group. Our results showed that stress, induced prior to learning, did not alter motor memory consolidation at the group level. However, interindividual approaches revealed that the glucocorticoid response to stress was negatively related to subsequent memory consolidation processes (Dolfen et al. 2019). The present study is directly based on these previous observations and the first to investigate the effect of experimentally induced stress on the neural correlates of motor memory in general and hippocampal-mediated motor memory consolidation in particular. To do so, participants were exposed to the Socially Evaluated Cold Pressor test (SECPT; Schwabe et al. 2008; Schwabe and Schachinger 2018) or to a nonstressful control intervention prior to performing a motor sequence learning task in a magnetic resonance imaging (MRI) scanner. Consolidation was assessed with an MRI retest taking place after a 6-h delay. Based on evidence that sleep facilitates motor sequence memory consolidation processes and the neural signatures targeted in the present study support this sleep-dependent enhancement (King et al. 2017), a 90-min nap opportunity monitored with polysomnography was introduced in this interval. Our overarching hypothesis was that exposure to acute stress prior to initial memory acquisition will challenge the recruitment of the hippocampus and favor striato-cortical networks over hippocampal circuits during initial motor learning. Based on 1) brain-behavior regression approaches showing that responses in these brain networks during learning are related to motor memory consolidation (Albouy et al. 2008; Albouy, Sterpenich, et al. 2013b) and 2) evidence of intersubject variability in the effect of stress on motor memory consolidation (Dolfen et al. 2019), we used regression analyses to investigate the link between stress-induced modulation of brain function and motor behavior. We hypothesized that the predicted stress-induced modulation of hippocampo- and striato-cortical systems will forecast a disruption of the subsequent sleep-related consolidation process.

Material and Methods

Participants

Eighty healthy, young (mean age: 22.2, range: 18–31, 48 females), right-handed (Edinburgh Handedness questionnaire; Oldfield 1971) adults provided written informed consent to participate in this research. They did not report any current or previous neurological or psychiatric diseases and were free of medications. Based on standardized questionnaires, participants exhibited no indication of fear of pain (Pain Catastrophizing Scale; Sullivan et al. 1995), extreme stress (Perceived Stress Scale; Cohen et al. 1983), excessive daytime sleepiness (Epworth Sleepiness Scale; Johns 1991), anxiety (Beck Anxiety Inventory; Beck et al. 1988) or depression (Beck Depression Inventory; Beck et al. 1961). Participants were not extreme morning or evening chronotypes (Circadian Rhythm questionnaire; Horne and Ostberg 1976) or shift workers. All participants reported normal sleep quality and quantity during the month prior and during the study, as evaluated with the Pittsburgh Sleep Quality Index (Buysse et al. 1989) and the St Mary's Hospital questionnaire (Ellis et al. 1981), respectively. The study was approved by the Medical Ethics Committee of the University Hospital Leuven, Belgium (B322201525025).

Experimental Procedure

The experimental procedure is depicted in Figure 1. One week prior to the experimental session, all participants were invited to the sleep lab for a 90-min habituation nap (start ~1 pm), which was monitored using standard polysomnography (PSG; see details below). Participants were instructed to respect a regular sleep/wake schedule (according to their own schedule ±1 h) starting 3 days before the experimental session. Compliance to this schedule was assessed using sleep diaries and wrist actigraphy (ActiGraph wGT3X-BT). Alcohol, nicotine, and caffeine (and other vigilance-altering substances) consumption was not permitted the day before as well as the day of the experimental session.

On the day of the experimental session, participants spent 9 consecutive hours in the lab (from ~8 am to 5 pm). Participants were instructed to wake a minimum of 1 h before the start of

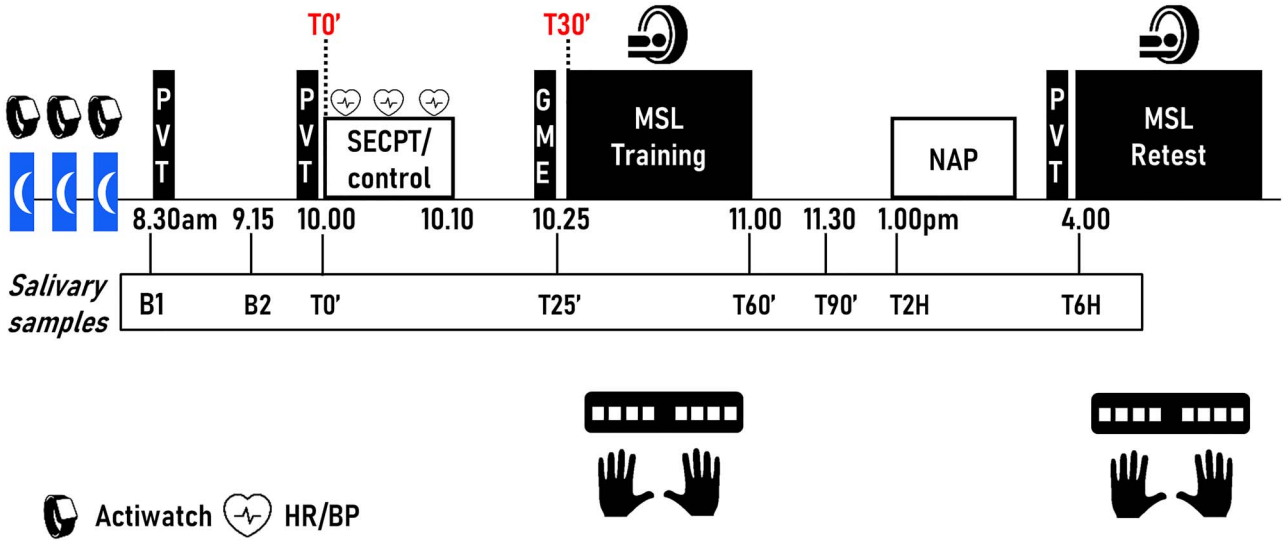

Figure 1. Experimental design. Participants followed a constant sleep/wake schedule for 3 nights prior to the experimental session. They were trained on a motor sequence learning (MSL) task (bimanual finger-tapping task) at 2 different occasions in the scanner, referred to as MSL training and retest. The MSL task was performed in a self-initiated manner and required participants to learn an 8-element sequence (4-7-3-8-6-2-5-1, using 8 fingers, no thumbs) through repeated practice. Prior to MSL training, subjects were exposed to the stress (SECPT) or control intervention (T0′). MSL training started 30-min postintervention (T30′) and participants were retested 6 h after initial training (MSL retest). Immediately before the MSL training, the effect of stress on general motor execution (GME) was assessed using a random serial reaction time task. Between sessions, all participants had a 90-min nap opportunity monitored with polysomnography. Salivary samples were collected at baseline (T0–90′ (B1) and T0–45′ (B2)), immediately before the stress/control intervention (T0′), before (T25′) as well as immediately after (T60′) the MSL training, 90 min after the intervention (T90′), before the nap (T2H) and before the MSL retest (T6H). Heart rate (HR) and blood pressure (BP) were taken before, during and after feet immersion. PVT, Psychomotor Vigilance Testing; SECPT, Socially Evaluated Cold Pressor Task.

the experimental session to account for the cortisol awakening response (Fries et al. 2009). They were also instructed to refrain from brushing their teeth, eating, and drinking (apart from water) for 1 h before the experimental session to ensure adequate saliva sampling for cortisol assessment (see below). Participants were assigned to one of two groups according to whether they were exposed to a control or a stress intervention (SECPT). The intervention was administered in a testing room in the vicinity of the MRI scanner on average 30 min (range: 29–34) before the training on the MSL task (a self-initiated bimanual finger tapping task, see next Section Motor Sequence Learning Task and Behavioral Measures) that took place in the fMRI scanner (at ~10 am). This timing was chosen because SECPT-induced secretion of cortisol is known to reach peak levels 25 min after the onset of the intervention (Schwabe et al. 2008; Schwabe and Schachinger 2018). After the fMRI session, at approximately 12 pm, participants were offered a standardized lunch followed by a 90-min nap opportunity (start nap around 1 pm) that was recorded using PSG in the sleep lab. Approximately, 1.5 h after the end of this 90-min nap interval (around 4 pm), participants were scanned again while they completed the MSL retest. Note that this protocol is part of a larger design that included a supplemental MRI session prior to the intervention (from 8.30 to 9.15 am) that is not reported in the present paper (see Supplementary Fig. 1 for the complete design).

The physiological response to stress was assessed using blood pressure and heart rate measures immediately before, during and immediately after the intervention as well as with salivary cortisol samples that were collected throughout the experimental day (see below for details). A random serial reaction time task was administered before the MSL training (15 min after the intervention) in order to assess the effect of the intervention on general motor execution. At arrival, immediately before the control/stress intervention as well as before the MSL retest, vigilance was measured subjectively using the Stanford Sleepiness questionnaire (Maclean et al. 1992) and objectively using a Psychomotor Vigilance Task (PVT) (Dinges and Powell 1985). Methods and results with respect to the assessment of general motor performance, sleep prior to and vigilance during the experimental session are reported in the Supplementary Material. Importantly, results from these measures indicate that stress and control groups did not differ with respect to sleep prior to the study, general motor execution as well as subjective and objective measures of vigilance at the time of testing.

Motor Sequence Learning Task and Behavioral Measures

Participants were scanned at two different occasions while they performed a bimanual finger-tapping task implemented in Matlab Psychophysics Toolbox version 3, referred to as MSL training and retest. The task required participants to tap an 8-element finger sequence on a specialized keyboard, using both hands (8 fingers, no thumbs; see Fig. 1), as rapidly and accurately as possible. The sequence to perform (4-7-3-8-6-2-5-1, where 1 and 8 correspond to the little fingers of the left and right hands, respectively) was presented on the screen during task practice. Each session started with a brief pretraining phase during which participants performed the sequence repeatedly and slowly until three consecutive correct sequences were completed. Both the MSL training and retest sessions consisted of 20 practice blocks. In addition, the MSL training session was followed by an immediate post-test (after a 2-min break) of 4 practice blocks in order to minimize the confounding effect of fatigue on end-training performance (Pan and Rickard 2015). The task was performed in a self-initiated manner; i.e., the start

of each practice block was indicated by a green fixation cross displayed in the middle of the screen with the sequence of numbers shown slightly above and participants were instructed to continuously tap the sequence until a stop signal (red cross) was given. Each practice block included 48 keypresses (ideally corresponding to 6 correct sequences) after which the cross automatically turned red, indicating a rest block (duration 15 s). During rest blocks, a sequence of eight asterisks (∗-∗-∗-∗-∗-∗-∗-∗) replaced the sequence of numbers and participants were instructed to keep their fingers still and look at the red fixation cross. Motor performance was measured in terms of speed (mean inter-response interval between two consecutive correct keypresses in s) and accuracy (% of correct two-element chunks).

Stress Induction Method

In the stress condition, participants were exposed to a modified version of the socially evaluated cold pressor test (SECPT) (Schwabe et al. 2008; Larra et al. 2015; Schwabe and Schachinger 2018) as described in Dolfen et al. (2019). The task required participants to immerse their feet (up to and including the ankles) in ice water (0–2 °C) while being videotaped for pretended analysis of facial expression and monitored by an unsociable and nonreinforcing experimenter. While feet were immersed, participants were asked not to talk or move, to keep their eyes focused on the camera and to keep their feet in the water until the experimenter gave the instruction to withdraw (after 3 min). The duration of the cold water stimulation was not provided to the participants in order to increase the unpredictability of the intervention. In contrast to the stress condition, participants in the control condition submerged their feet up to and including the ankles for 3 min in warm water (35–37 °C). They were neither monitored by an unsociable experimenter nor videotaped.

To measure the effectiveness of the stress induction by the SECPT, subjective, and physiological responses were repeatedly measured during the experiment. Participants were asked to rate their subjective feeling of stress, pain, and unpleasantness on a visual analogue scale from 0 ("Not at all") to 100 ("Very much") immediately following the control/stress manipulation. Heart rate and blood pressure (systolic and diastolic) were assessed using an automatic upper arm blood pressure monitor (BP6000, Braun) before (pre), during and immediately following (post) feet submersion. Finally, for each participant, a total of eight salivary cortisol samples were collected using Salivette collection device (Sarstedt Salivette) to assess the endocrine stress response. The start of the intervention is referred to as T0′ (see Fig. 1). Salivary samples were collected 90 min (8.30 am, T0–90′, Baseline 1, B1) and 45 min (9.30 am, T0–45′, Baseline 2, B2) before the intervention, immediately before the stress/-control intervention (10 am, T0′), immediately before (T25′), immediately after (T60′) and 30 min after (T90′) MSL training, before the nap (1 pm, T2H) and before the MSL Retest (4 pm, T6H). All samples were taken while participants lied supine in the scanner with the exception of sample T0′ and T2H, which were taken seated. After collection, the samples were stored at −20 °C until analyzed using immunoassay (analyses performed by Dresden Labservice GmbH, Germany).

Polysomnographic Data Acquisition

Both habituation and experimental naps were monitored with a digital sleep recorder (V-Amp, Brain Products, Gilching, Germany; bandwidth: DC to Nyquist frequency) and were digitized at a sampling rate of 1000 Hz. Standard electroencephalographic (EEG) recordings were made from Fz, C3, Cz, C4, Pz, Oz, A1, and A2, with A2 used as the recording reference and A1 as a supplemental individual EEG channel according to the international 10–20 system (note that Fz, Pz, and Oz were omitted during habituation). An electrode placed on the middle of the forehead was used as the recording ground. Bipolar horizontal eye movements (electrooculogram: EOG) were recorded from electrodes placed on the outer canthus of both eyes. Bipolar submental electromyogram (EMG) recordings were made from the chin. Electrical noise was filtered using a 50-Hz notch. Polysomnographic data of the experimental naps were visually scored in 30-s windows by a registered polysomnographic technologist according to AASM criteria (AASM Manual for the Scoring of Sleep and Associated Events version 2.5, 2018). To easily visualize the relevant features of sleep and wakefulness, EEG was re-referenced to an average of A1 and A2 displayed from 0.3 to 30 Hz, EOG between 0.3 and 30 Hz and EMG above 10 Hz using software filters.

Note that the nap episode in this study was included based on previous research demonstrating that sleep facilitates motor sequence memory consolidation processes (King et al. 2017). Sleep characteristics are reported in Supplementary Table 2. Importantly, results showed that participants experienced on average 66.22 min of sleep (with a minimum sleep duration of 16 min) and that the stress and control groups did not statistically differ in any of the sleep measures.

Statistical Analyses

Statistical analyses were performed using SPSS Statistics 24 (IBM). For all analyses, the probability level was set at $P < 0.05$. In case of violation of the sphericity assumption, Greenhouse–Geisser corrections were applied. Results of planned pairwise comparisons were corrected using Bonferroni correction for multiple comparisons.

Magnetic Resonance Imaging

Data Acquisition

Both functional and anatomical images were acquired with a Phillips Achieva 3.0 T MRI System and a 32-channel head coil. During the MSL sessions, BOLD signal was acquired with a T2∗ gradient echo-planar sequence using axial slice orientation that covers the whole brain (TR = 2000 ms, TE = 30 ms, FA = 90°, 54 transverse slices, 3-mm slice thickness, 0.2-mm interslice gap, FoV = 210 × 210 mm^2, matrix size = 84 × 82 × 54 slices, voxel size = 2.5 × 2.56 × 2.5 mm^3). A structural T1-weighted 3D MP-RAGE sequence (TR = 9.5 ms, TE = 4.6 ms, TI = 858.1 ms, FA = 9°, 160 slices, FoV = 250 × 250 mm^2, matrix size = 256 × 256 × 160, voxel size = 0.98 × 0.98 × 1.20 mm^3) was also obtained for each participant.

Preprocessing

Functional images were preprocessed and analyzed using SPM12 (Welcome Department of Imaging Neuroscience) implemented in MATLAB. Preprocessing included the realignment of the functional time series using rigid body transformations, iteratively optimized to minimize the residual sum of squares between each functional image and the first image of each session separately in a first step and the across-session mean functional image in a second step. The mean functional image was coregistered to the structural T1-image using a rigid body

transformation optimized to maximize the normalized mutual information between the two images. Coregistration parameters were then applied to the realigned BOLD time series. Spatial normalization to an average subject-based template created using DARTEL in SPM12 (registered to the MNI space) was performed on both functional and anatomical images. Finally, spatial smoothing was applied to the functional images (Gaussian kernel, 8-mm full-width at half-maximum [FWHM]).

Statistical Analyses

The analysis of fMRI data was conducted in 2 serial steps accounting for fixed and random effects, respectively. Changes in brain responses were estimated using a general linear model including the responses to motor sequence practice and their linear modulation by performance speed (mean inter-response interval between two correct consecutive keypresses by block) during MSL training and retest sessions. Performance speed, rather than accuracy, was chosen as a parametric modulator because accuracy was not modulated by task practice (see Supplementary Material Section 2.5). The 15-s rest blocks occurring between each block of motor practice served as the baseline condition modeled implicitly in the block design. These regressors consisted of box cars convolved with the canonical hemodynamic response function. Movement errors (i.e., incorrect key presses) as well as key presses during rest were modeled as events of no interest. Movement parameters (derived from realignment of the functional volumes) as well as the average time series extracted from the cerebrospinal fluid and white matter segments were entered as regressors of no interest. High-pass filtering with a cut-off period of 128 s served to remove low-frequency drifts from the time series and an autoregressive (order 1) plus white noise model and a restricted maximum likelihood (ReML) algorithm was used to estimate serial correlations in fMRI signal. Subsequently, linear contrasts were generated that assessed the main effect of practice and its linear modulation by performance speed within each session as well as between sessions. The modulation contrasts identified regions wherein brain responses decrease or increase in proportion to performance speed. These linear contrasts generated statistical parametric maps (SPM[T]). The contrast images were further spatially smoothed (Gaussian kernel 6 mm FWHM) and entered in second-level analysis accounting for intersubject variance.

The second-level analyses were performed using full factorial ANOVAs. Three groups were considered as participants in the stress group were further split into two subgroups according to whether they showed a stress-induced cortisol response or not (i.e., stress cortisol responders (SCR) and stress cortisol non-responders (SCNR), respectively, see Participants Results Section). Furthermore, we investigated the relationship between interindividual differences in brain responses on the contrasts described above (i.e., main of effect of practice and modulation during training as well as between-session changes in brain responses) and motor behavior within and between groups. To do so, separate regression models including the individual's performance as covariate of interest were performed at the second level. Two separate regression models tested the relationship between brain activity and 1) "the level of performance reached at the end of training" using the individual's performance averaged across the 4 blocks of the immediate post-training test and 2) "the offline consolidation process" using the individual's between-session changes in performance computed as the percent change from the end of training (average 4 blocks of the immediate post-training test) to the beginning of the retest (average first 4 blocks).

Finally, psychophysiological interaction (PPI) analyses were performed using, as seed region, the hippocampal area that showed a differential pattern of dynamical activity between groups (see results). To do so, for each individual, the first eigenvariate was extracted on the training session data using singular value decomposition of the time series across the voxels included in a 6-mm radius sphere centered on the peak of the activation reported at the group level (right hippocampus: 28–36 −2 mm). New linear models were generated at the individual level, using three regressors representing: 1) the practice of task modulated by performance speed, 2) activity in the reference area extracted as described above and 3) the interaction of interest between the first (psychological) and the second (physiological) regressors. To build this regressor, the underlying neuronal activity was first estimated by a parametric empirical Bayes formulation, combined with the psychological factor and subsequently convolved with the hemodynamic response function (Gitelman et al. 2003). The design matrix also included movement parameters as well as average time series extracted from the cerebrospinal fluid and white matter segments as regressors of no interest. A significant PPI indicated a change in the regression coefficients (i.e., a change in the strength of the functional interaction) between any reported brain area and the reference region, related to changes in performance speed during MSL training. Similar to the activation-based analyses, individual summary statistic images obtained at the first-level (fixed effects) analysis were spatially smoothed (6-mm FWHM Gaussian kernel) and entered in a second-level (random-effects) analysis using full factorial design ANOVAs including three groups. Regression analyses between connectivity maps and performance at the immediate post-training test and offline changes in performance were also performed using similar procedures as described above.

The resulting set of voxel values for each analysis described above (activity and connectivity) constituted maps of the *t* statistic (SPM[T]), thresholded at $P < 0.005$, uncorrected for multiple comparisons. Statistical inferences were performed at a threshold of $P < 0.05$ after family-wise error (FWE) correction for multiple comparisons over small spherical volumes (small volume correction (SVC) approach; Poldrack 2007; Poldrack et al. 2008). Spheres (10-mm radius) were centered on coordinates from literature in regions of interest (see Supplementary Material). All results reported and discussed in the main text survived SVC. An additional correction for multiple volumes of interest was performed using Holm-Bonferroni correction procedures within each contrast ($P < 0.05$) (Holm 1979). Results surviving this additional Holm-Bonferroni correction are simply indicated with an asterisk in the tables.

Results

Participants

Sample size estimation in the current study was based on our previous work showing a significant correlation between offline gains in performance and cortisol response to stress (Dolfen et al. 2019). As earlier studies have shown that some individuals do not show any cortisol response to the SECPT intervention (i.e., cortisol nonresponders, Schwabe and Schachinger 2018), individual cortisol data were analyzed during collection. In line

Table 1 Subjective and autonomic (heart rate, systolic, and diastolic blood pressure) responses to the intervention

Subjective ratings	Pain	Stress	Unpleasantness
Control	1.39 ± 4.79	1.26 ± 4.34	1.1 ± 4.33
SCR	68.15 ± 21.07	55.47 ± 24.9	88.87 ± 12.13
Control vs. SCR[a]	$P < 0.001$	$P < 0.001$	$P < 0.001$
Autonomic responses	**Pre**	**During**	**Post**
SBP (mmHg)			
Control	122.29 ± 10.27	120.82 ± 9.33	120.43 ± 9.87
SCR	130.81 ± 13.74	146.15 ± 21.66	134.22 ± 14.42
Control vs. SCR[b]	$P = 0.012$	$P < 0.001$	$P < 0.001$
DBP (mmHg)			
Control	75.11 ± 6.93	74.14 ± 5.78	74.36 ± 5.68
SCR	75.30 ± 9.06	92.07 ± 18.31	84.15 ± 14.82
Control vs. SCR[b]	$P = 0.931$	$P < 0.001$	$P = 0.002$
HR (bpm)			
Control	64.36 ± 8.64	66.5 ± 8.89	68.21 ± 9.06
SCR	63.44 ± 8.70	87.31 ± 16.83	71.04 ± 11.65
Control vs. SCR[b]	$P = 0.698$	$P < 0.001$	$P = 0.319$

Notes: Values are means ± standard deviations. *N* control group = 28; *N* SCR group = 27. Subjective ratings are on a 100 m visual analogue scale. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. Bpm, beats per min. SCR, Stress Cortisol Responders. *P* values are based on [a]unpaired sample *t*-tests or [b]pairwise comparisons following RM ANOVAs. Note that during the SECPT, the measurement prefeet submersion is taken within the stressful context (including video monitoring) that likely contributed to the group difference in SBP observed at baseline.

with our previous study (Dolfen et al. 2019), we classified participants in the stress group with an increase in cortisol from T0′ to T25′ larger than 15.5% and 1.5 nmol/L as stress cortisol responders (SCR), and the others as stress cortisol nonresponders (SCNR) (Miller et al. 2013; see Supplementary Fig. 2 for the distribution of cortisol responses in the current sample). Data acquisition continued until the number of SCR (and control participants) reached the estimated sample size (but see below for noncortisol-related attrition).

Thirty-four (21 females; 62%) and 46 (27 females; 59%) participants were subjected to the control and stress intervention, respectively. In the stress group, 17 and 29 participants were classified as SCNR and SCR, respectively. Four participants in the control group were excluded because they were classified (using the above-mentioned criterion) as cortisol responders. Two participants (one in the SCR group and one in the control group) were discarded because they were statistical outliers (average ± 3SDs) in performance speed and accuracy at the immediate post-training test. One participant in the control group was excluded due to excessive motion during training and retest fMRI sessions (>2 voxels). Lastly, one participant in the SCR group was excluded because he/she presented less than 5 min of sleep during the experimental nap. A total of 72 participants were considered for the analyses (control group [$N = 28$, 17 females]; SCR [$N = 27$, 13 females]; SCNR [$N = 17$, 14 females]). Participant characteristics or for each of the three groups can be found in Supplementary Table 1.

In line with our previous work, the primary group comparison presented in the main text focused on the controls and SCR. However, for the sake of completeness, all results from the relatively small set of SCNR as well as the comparisons between SCNR and the two other groups are reported in the Supplementary Material.

Stress Induction by the SECPT

Subjective and autonomic responses to the intervention are summarized in Table 1. With respect to the subjective response to stress, the SECPT was rated as significantly more stressful, unpleasant, and painful as compared to the control manipulation (unpaired *t*-tests, control vs. SCR, all $Ps < 0.001$).

To investigate the autonomic response to stress in SCR, heart rate (bpm), systolic (SBP), and diastolic (DBP) blood pressure (mmHg) were analyzed using 3 (time: pre- vs. during vs. postintervention) × 2 (groups: control vs. SCR) repeated measures (RM) ANOVAs. Briefly, blood pressure and heart rate significantly increased in response to the SECPT but not in response to the control intervention ([time × group interaction: all $Fs \geq 8.573$, $\eta_p^2 \geq 0.144$, all $Ps < 0.001$], see Table 1 for between-group comparisons for each time point).

With respect to the endocrine response, an 8 (time) × 2 (groups: control vs. SCR) RM ANOVA on cortisol concentration (nmol/L) revealed a significant main effect of time [$F_{(3.568,178.402)} = 24.055$, $\eta_p^2 = 0.325$, $P < 0.001$] and a time × group interaction [$F_{(3.568,178.402)} = 9.115$, $\eta_p^2 = 0.154$, $P < 0.001$]. There was no main effect of group [$F_{(1,50)} = 2.467$, $\eta_p^2 = 0.047$, $P = 0.123$]. As shown in Figure 2 and as expected, cortisol was significantly elevated in SCR as compared to the control group at T25′ ($P < 0.001$) and T60′ ($P < 0.001$) (for all other time points: all $Ps \geq 0.280$). Within the SCR group, peak levels of cortisol were reached approximately 25 min after the stressor and cortisol concentration remained significantly elevated as compared to T0′ for the full duration of MSL training, i.e., up to and including T60′ [time: $F_{(7,44)} = 15.572$, $\eta_p^2 = 0.712$, $P < 0.001$; T25′ vs. T0′/T90′/T2H/T6H, all $Ps \leq 0.025$, T60′ vs. T0′/T90′/T2H/T6H, all $Ps \leq 0.048$]. Within the control group, cortisol levels decreased within the day according to the usual circadian pattern with the lowest concentrations observed at T6H [time: $F_{(7,44)} = 10.378$, $\eta_p^2 = 0.623$, $P < 0.001$]. Altogether, results indicate that the SECPT effectively triggered subjective, autonomic and endocrine responses in SCR.

Finally, and for the sake of completeness, measures of stress effectiveness in SCNR are reported in Supplementary Material Section 2.3. Autonomic and endocrine responses are depicted in Supplementary Figure 3. In summary, the SCNR and SCR groups showed similar subjective and autonomic responses to the SECPT. As expected based on the SCR/SCNR classification

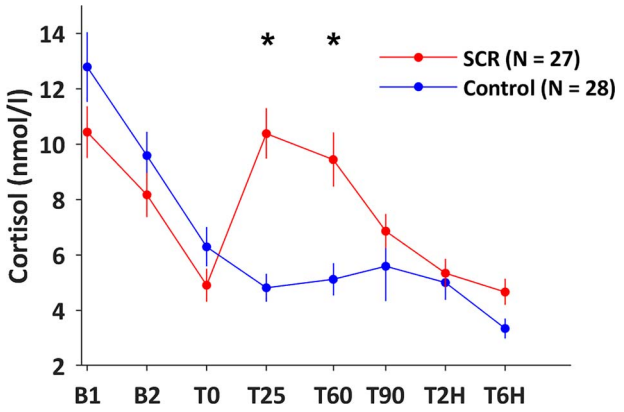

Figure 2. Time course of salivary cortisol concentration (nmol/L). T0 corresponds to the start of the control/stress intervention. In the stress cortisol responder (SCR) group, cortisol levels were significantly elevated at the start of the MSL training (T25) and remained elevated until 60-min postintervention (T60), corresponding to the end of training. Cortisol of two subjects at B1 (1 control, 1 SCR) and of one subject at T6H (control) were missing. See Supplementary Material Section 2.3 and Supplementary Figure 3C for the time course of cortisol concentration in the stress and control groups before cortisol responder/nonresponder classification. B1 and B2, Baseline 1 and 2. (*) Significant group differences at $P < 0.05$. Error bars represent SEM.

(Miller et al. 2013), cortisol concentration in SCNR followed a time course that was different from SCR but comparable with the control group.

Motor Performance

Note that as performance accuracy remained stable with low error rate within and across practice sessions (average accuracy rate of 92.82% ± 5.27 and 95.87% ± 4.61 for MSL training and retest, respectively), the corresponding results are reported in Supplementary Material Section 2.5. Analyses performed on performance speed in control and SCR groups are presented in the main text.

MSL Training

A 20 (blocks of practice during training) × 2 (groups: control vs. SCR) RM ANOVA conducted on performance speed revealed a significant main effect of block [$F_{(4.32,\ 228.97)} = 64.747$, $\eta_p^2 = 0.55$, $P < 0.001$], indicating that speed increased with practice during initial learning. This performance improvement was comparable in both groups [group: $F_{(1,53)} = 1.538$, $\eta_p^2 = 0.028$, $P = 0.220$; block × group interaction: $F_{(4.32,228.97)} = 0.625$, $\eta_p^2 = 0.012$, $P = 0.658$] (Fig. 3A). A 4 (blocks of practice during post-training test) × 2 (groups) RM ANOVA indicated that performance speed further improved during the immediate post-training test, reflected by a main effect of block [$F_{(3,159)} = 2.818$, $\eta_p^2 = 0.05$, $P = 0.041$], and to a similar extent in both groups [group: $F_{(1,53)} = 0.802$, $\eta_p^2 = 0.015$, $P = 0.374$; block × group: $F_{(3,159)} = 1.183$, $\eta_p^2 = 0.022$, $P = 0.318$].

MSL Retest

Similar to the MSL training session, a 20 × 2 RM ANOVA on performance during the retest yielded a main effect of block [$F_{(8.029,425.555)} = 17.646$, $\eta_p^2 = 0.248$, $P < 0.001$], indicating further performance improvements, but no main effect of group nor a block by group interaction [group: $F_{(1,53)} = 0.799$, $\eta_p^2 = 0.015$, $P = 0.375$; block × group: $F_{(8.029,425.555)} = 0.642$, $\eta_p^2 = 0.012$, $P = 0.743$] (Fig. 3A).

Between-Session Changes in Performance

To investigate the effect of the stress intervention on consolidation processes, offline changes in performance were calculated as the percent change from the end of training (average 4 blocks of the immediate post-training test) to the beginning of the retest (average first 4 blocks). Both groups showed similar maintenance in performance speed over the offline period [one sample *t*-test; control: $t_{(27)} = 0.113$, $P = 0.911$; SCR: $t_{(26)} = 0.325$, $P = 0.747$; unpaired *t*-test: $t_{(53)} = -.164$, $P = 0.87$], indicating that stress prior to learning did not influence offline changes in performance in SCR (Fig. 3B).

Altogether, the results indicate that stress induced prior to training had no influence on motor performance during initial motor sequence learning, offline changes in performance between sessions nor performance during the retest.

Correlation Between Offline Changes in Performance and Cortisol

In contrast to our previous work (Dolfen et al. 2019), there was no significant correlation between offline changes in performance and T25′ salivary cortisol levels (i.e., immediately before training) within the SCR group ($N = 27$, $r = -.142$, $P = 0.481$) (for completeness: control, $N = 28$, $r = -.023$, $P = 0.907$).

Similar patterns of behavioral results were observed in the SCNR (see Supplementary Material Section 2.4).

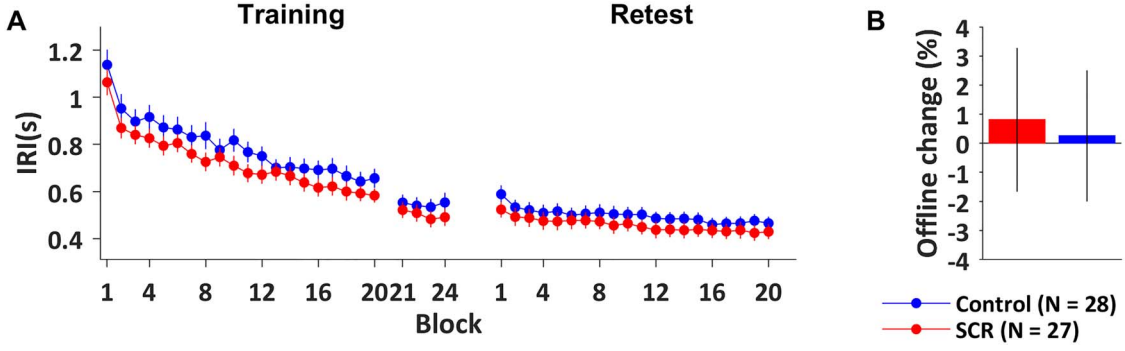

Figure 3. (*A*) Performance speed (average inter-response interval between consecutive correct keypresses, IRI) plotted as a function of blocks of practice during MSL training and retest sessions for the control and stress cortisol responder (SCR) groups. Performance improved with practice similarly in both groups. (*B*) Offline changes (% difference) in performance speed between the end of training (four blocks of the immediate post-test) and the start of the retest (first 4 blocks). There were no group differences in offline changes. See Supplementary Figure 4 for individual offline changes in performance speed and results related to accuracy. Error bars represent SEM.

Table 2 Functional imaging results for the MSL training session—between group comparisons

Area	X mm	Y mm	Z mm	Z	P
1. Main effect of practice [training]					
[Control–SCR] No suprathreshold clusters					
[SCR–control]					
L Postcentral gyrus/S1	−62	−20	42	3.10	0.022
L Postcentral gyrus/S1	−58	−18	26	3.07	0.017
L M1/S1	−42	−32	44	2.8	0.048
L (lateral) PMC	−20	−2	74	2.8	0.045
L (ventral) PMC	−58	10	34	3.16	0.019
R SMA	18	−10	76	2.79	0.049
L SMA	−2	4	62	2.85	0.042
L Cerebellum VIIb/Crus 2	−42	−52	−44	2.85	0.043
2. Modulation in activity by speed [training]					
[Control–SCR]					
M Frontal gyrus	−6	56	40	2.88	0.040
[SCR–control]					
R (lateral) PMC	28	0	66	2.97	0.032
R Insular cortex	44	4	0	3.01	0.029
L Cerebellum VIIB/Crus 2	−38	−68	−48	2.89	0.049
R Hippocampus	28	−36	−2	2.87	0.041
R Parahippocampus	20	−36	−6	2.86	0.042
	18	−38	−10	2.82	0.046
L Supramarginal gyrus	−66	−28	26	2.86	0.042
3. Modulation in R Hippocampus [28–36 −2] connectivity by speed [training]					
[Control–SCR] No differences					
[SCR–control]					
R M1	32	−18	54	3.68	0.005*
L Postcentral gyrus/S1	−60	−18	44	3.24	0.018
L Cerebellum IV	−8	−54	−16	3.14	0.024
L Cerebellum IV	−4	−62	−18	3.13	0.024
R Cerebellum IV	18	−48	−26	3.04	0.031
R Amygdala	28	4	−18	3.08	0.027
	18	0	−24	2.98	0.036

Notes: Significance level set at $P_{corr} < 0.05$ corrected for multiple comparisons (FWE) over small volumes. Voxels of these maps not surviving correction for multiple comparisons and which were not of interest were not reported. SCR, Stress Cortisol Responders; PMC, Premotor Cortex; SMA, Supplementary Motor Area
*$P < 0.05$, Holm-Bonferroni corrected.

Imaging Results

In line with earlier studies on the neural correlates of motor sequence learning (Doyon et al. 2003; Penhune and Doyon 2005; Albouy et al. 2008), our imaging data showed that participants across experimental groups recruited a large set of brain regions including the cerebellum, sensorimotor, (medial) premotor, and parietal cortex to perform the task during initial training. Our results also showed that activity increased as a function of performance improvement in areas including bilateral putamen and the right caudate nucleus (see Supplementary Table 3). Similar to the behavioral analyses, imaging results presented below focus on the comparison between SCR and controls. Results on the SCNR group are detailed in the Supplementary Material Section 2.6 but also briefly summarized in Section 3.4.5 below.

Effect of Stress on Brain Responses During Initial Learning

As compared to the control intervention, stress resulted in increased activity in sensorimotor areas (including the left primary sensory cortex (S1) and left premotor cortex), the supplementary motor area (SMA) and the left cerebellum (VIIb/Crus II) in SCR during MSL training (Table 2.1 and see Fig. 4).

Parametric modulation analyses were performed in order to investigate whether stress also influenced dynamical brain activity during learning, i.e., changes in brain activity as a function of block-to-block performance improvement. In line with our hypotheses, stress altered the pattern of dynamical activity in (para)hippocampo-cortical regions in SCR as compared to controls. Specifically, right hippocampal, parahippocampal and frontal (premotor cortex) activity decreased in proportion to performance speed in SCR and this pattern was significantly different than in controls (Fig. 5A). A similar pattern of results was observed in the right insula, left inferior parietal area (supramarginal gyrus) and left cerebellum (VIIb/Crus II) (Table 2.2).

We then investigated whether stress altered the pattern of connectivity of the right hippocampal cluster reported above as a function of task practice during learning. To do so, functional connectivity of the hippocampus with the rest of the brain was investigated using psychophysiological interaction (PPI) analysis. The analysis revealed that the dynamical pattern of connectivity (i.e., changes in connectivity as a function of practice) of the right hippocampus with a set of regions including the left S1, right primary motor (M1), right amygdala, and right cerebellum (IV) was different between the SCR and control groups (Table 2.3). More specifically, in SCR, the

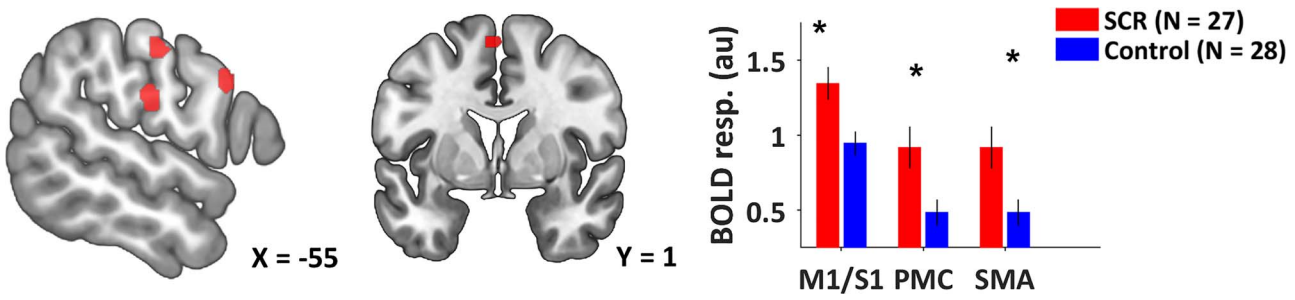

Figure 4. Stress resulted in increased activity in motor cortical regions during task practice ([SCR–control], left M1/S1 [−42–32 44 mm], $Z = 2.8$, $P_{SVC} = 0.048$; left PMC [−58 10 34 mm], $Z = 3.16$, $P_{SVC} = 0.019$; SMA [−2 4 62 mm], $Z = 2.85$, $P_{SVC} = 0.042$). Activations maps are displayed on a T1-weighted template image with a threshold of $P < 0.005$ uncorrected. Error bars indicate SEM. au, arbitrary units; Resp., response.

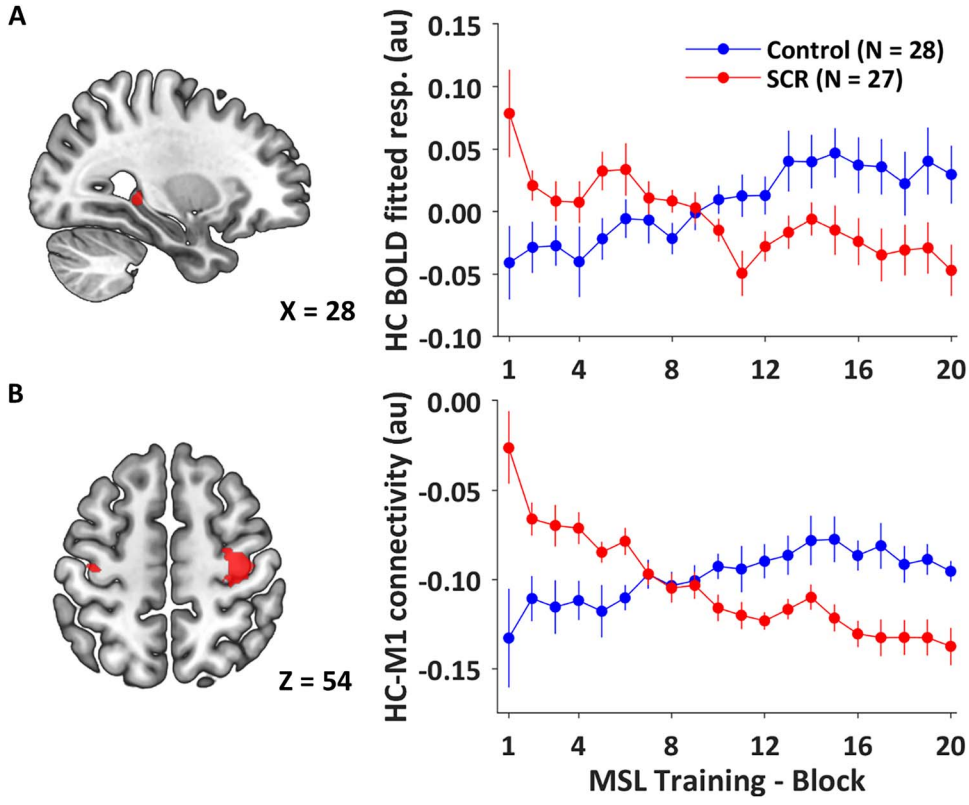

Figure 5. Effects of stress on hippocampal (HC) dynamical activity (*A*) and connectivity (*B*) patterns during MSL training. (*A*) Brain responses in the right hippocampus (left panel) were differently modulated by speed of performance in the stress cortisol responder (SCR) as compared to the control group (HC [28–36 −2 mm], $Z = 2.87$, $P_{SVC} = 0.041$). The averaged BOLD fitted response (resp.) (right panel) shows that hippocampal activity decreased more across blocks of training in the stress group as compared to controls. (*B*) Functional connectivity between the hippocampus seed and the primary motor cortex (M1, left panel) was differentially modulated by performance speed in the SCR as compared to the control group (M1 [32–18 54 mm], $Z = 3.68$, $P_{SVC} = 0.005$). The averaged strength of HC-M1 connectivity (right panel) decreased more across blocks of training in the SCR group as compared to controls. Activations maps are displayed on a T1-weighted template image with a threshold of $P < 0.005$ uncorrected. Error bars indicate SEM. au, arbitrary units.

strength of the functional connectivity of the hippocampus with (sensori)motor-cerebellar regions decreased in proportion to the increase in performance speed and this was different than in controls (Fig. 5*B*). Connectivity between the hippocampus and the amygdala tended to increase in the control but decreased with practice in the SCR group.

Altogether, our results in SCR indicate that stress favored the recruitment of sensorimotor regions during learning and induced a larger disengagement of hippocampal-cortical areas as a function of practice. Importantly, stress also resulted in a progressive disconnection of the hippocampus with sensorimotor regions in proportion to performance improvement.

Effect of Stress on the Relationship Between Brain Responses During Initial Learning and End of Training Performance

As described in the Behavioral Results Section, stress did not, on average, induce differences in motor behavior during MSL training. We nevertheless tested whether interindividual variability in brain responses during training was related to the level of

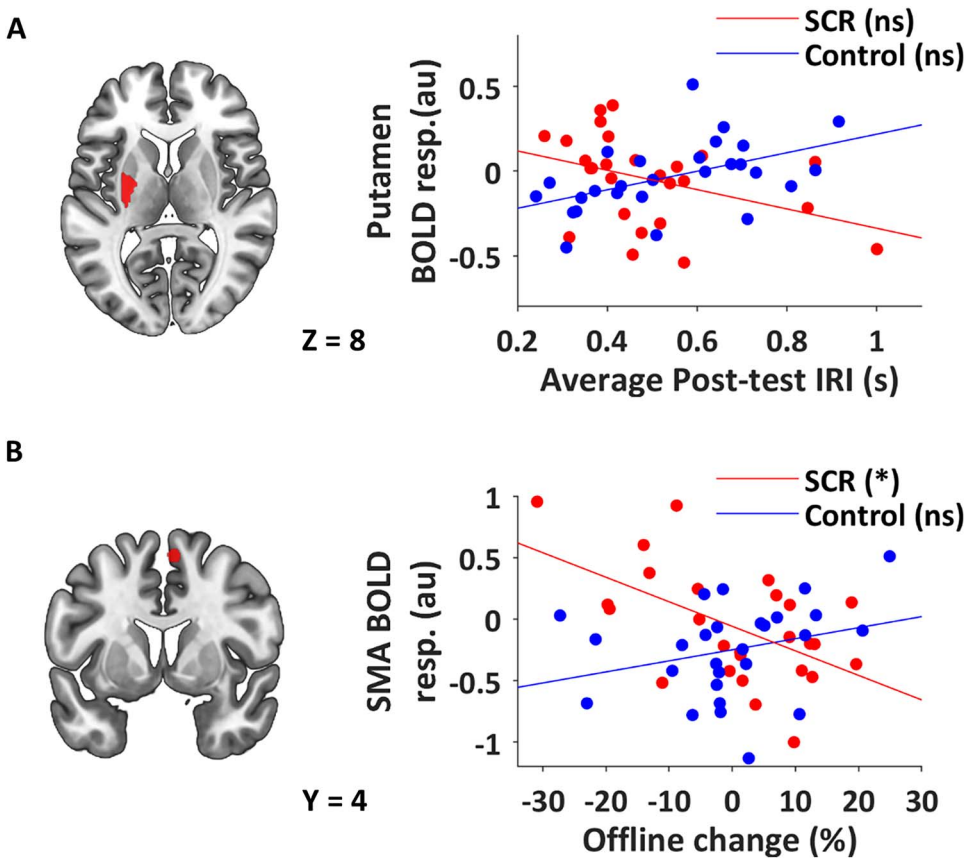

Figure 6. Imaging results of the regression analyses between brain responses during MSL training (TR) and (A) end of training performance (s) (average inter-response interval at the immediate post-test) and (B) offline changes (%) in performance speed. (A) Brain responses during MSL training in striato-motor regions were differently correlated with end of training performance in the stress cortisol responder (SCR) and control groups (left panel). Regression plot of BOLD responses in the left putamen against average performance at the immediate post-test by group (right panel, [SCR–control], Putamen [−30 8 8 mm], $Z = 3.16$, $P_{SVC} = 0.018$). (B) Brain responses in the supplementary motor area (SMA) were differently correlated with offline changes in performance speed in the SCR and control groups. Regression plot of BOLD responses in the SMA against offline changes in performance speed by group (right panel, [SCR–control], SMA [−2 18 60 mm], $Z = 3.00$, $P_{SVC} = 0.039$). *N* SCR group = 27; *N* control group = 28. au., arbitrary units; Resp., response. Activations maps are displayed on a T1-weighted template image with a threshold of $P < 0.005$ uncorrected. (ns) Nonsignificant within-group $P_{uncorr} > 0.001$, (*) significant within-group $P_{uncorr} \leq 0.001$ (see Supplementary Tables 4.1 and 5.1 for within-group regression results for the regression with average post-test speed and offline changes in speed, respectively).

performance reached at the end of training and if these relationships differed between the SCR and control groups. Regression analyses between individual activity maps of the main effect of practice and end of training performance showed that greater activity in striato-cerebello-motor areas during training was correlated to faster end of training performance in the SCR group. This relationship was significantly different from the control group (Fig. 6A) (see Table 3.1 and Supplementary Table 4.1 for between and within-group results, respectively).

Regression analyses between individual modulation maps and end-of-training performance showed that changes in dynamical activity in fronto-parietal and amygdalar regions differently related to end of training performance in the SCR and control groups (Table 3.2). Specifically, a larger practice-related decrease in parietal activity in the SCR as compared to the control group was related to faster performance at the end of training (see Supplementary Table 4.2 for within-group analyses showing that these relationships were not significant within each group). In contrast, a larger increase in frontal activity was related to better performance in the control but not in the SCR group (significant within the control group, Supplementary Table 4.2). Lastly, a learning-related increase in amygdala activity tended to be beneficial for performance in the SCR but detrimental for performance in the control group (not significant within the respective groups, Supplementary Table 4.2).

Regression analyses between individual hippocampal connectivity maps and end-of-training performance showed that the decrease in connectivity observed as a function of practice between the hippocampus and cerebello-(sensori-)motor regions was related to faster performance at the end of training in the SCR as compared to the control group (see Table 3.3 and Supplementary Table 4.3 for between and within-group results, respectively). In addition, a decrease in hippocampo-parietal connectivity was beneficial for end-of-training performance in SCR, but not in control participants (Table 3.3 and Supplementary Table 4.3).

In sum, regression analyses with end of training performance indicate that the recruitment of cerebello- and striato-motor regions during learning in the SCR group supports the development of faster performance. Additionally, larger stress-induced decreases in 1) activity in parietal areas and 2)

Table 3 Results of the regression analysis with average speed at the immediate post-training test—between group comparisons

Area	X mm	Y mm	Z mm	Z	P
1. Main effect of practice [training]					
[Control–SCR]					
L Middle frontal gyrus	−32	54	20	3.67	0.004*
	−40	56	16	3.22	0.016
	−30	38	34	3.14	0.020
L Superior frontal gyrus	−22	64	6	2.8	0.048
L M1/S1	−54	−6	16	3.65	0.004*
L M1/S1	−58	−16	24	3.28	0.014
L Putamen	−30	−8	8	3.16	0.018
R Supramarginal gyrus	68	−34	30	3.03	0.027
L SMA	−18	−10	56	3.01	0.028
M Cerebellum crus II	0	−80	−28	3.00	0.029
L Cerebellum Vermis	−12	−44	−42	2.8	0.048
[SCR–control] No suprathreshold clusters					
2. Modulation in activity by speed [training]					
[Control–SCR]					
L Middle frontal gyrus	−28	40	30	3.28	0.014*
L Inferior parietal lobe	−44	−48	38	2.97	0.032*
[SCR–control]					
R Amygdala	30	2	−28	3.1	0.023*
3. Modulation in right Hippocampus [28–36 −2] connectivity by speed [training]					
[Control–SCR]					
L Inferior parietal lobule/S1	−58	−22	46	4.21	0.002*
L Intraparietal sulcus	−26	−60	62	3.18	0.021
	−32	−50	66	3.45	0.010
L M1/S1	−40	−34	48	3.44	0.010
R M1/S1	46	−26	46	3.3	0.015
	54	−24	44	3.3	0.015
L Cerebellum crus I/lobule VI	−18	−70	−22	3.46	0.009
L Cerebellum V/VI	−4	−66	−16	3.39	0.012
L Cerebellum IV/V	−22	−50	−22	3.45	0.010
R Intraparietal sulcus	30	−56	62	3.21	0.020
	30	−66	56	3.14	0.023
[SCR–control] No suprathreshold clusters					

Notes: Significance level set at $P_{corr} < 0.05$ corrected for multiple comparisons (FWE) over small volumes. Voxels of these maps not surviving correction for multiple comparisons and which were not of interest were not reported. SCR, Stress Cortisol Responders; M1, primary motor cortex; S1, primary sensory cortex; SMA, Supplementary Motor Area *$P < 0.05$, Holm-Bonferroni corrected.

connectivity between the hippocampus and cerebello-(sensori-)motor regions are related to faster performance during initial learning in SCR.

Effect of Stress on the Relationship Between Brain Responses During Initial Learning and Subsequent Consolidation

Similar to initial learning, stress did not influence offline changes in performance. Here, we assessed whether individual brain responses during initial training were differently related to subsequent consolidation between the SCR and control groups. Regressions were significantly different between groups for parietal and supplementary motor areas (Table 4.1 and Fig. 6B). Specifically, larger activity in parietal regions tended to be predictive for subsequent offline gains in performance in the control as compared to SCR (not significant within group, Supplementary Table 5.1). Within-group analyses indicated that the SMA cluster was part of a sensorimotor network (including SMA, S1, M1, and premotor cortex) that was negatively related to offline changes in performance speed in the SCR group (Supplementary Table 5.1).

We next investigated whether and how differential patterns of dynamical activity and connectivity during learning were related to interindividual differences in consolidation. Results indicated that a larger disengagement of frontal, parietal, and cerebellar (Crus I/II) regions during learning was detrimental for subsequent consolidation in the SCR group as compared to controls (see Table 4.2 and Supplementary Table 5.2 for between and within-group analyses, respectively). Regression analyses between hippocampal connectivity maps and subsequent offline changes in performance indicated that connectivity with frontal and cerebellar regions predicted offline gains in performance in the control as compared to the SCR group (Table 4.3). Within-group analyses indicated that while hippocampal connectivity patterns during learning were predictive of consolidation in the control group (especially with fronto-parietal regions and the amygdala), they were not related to such offline changes in performance in the SCR group (Supplementary Table 5.3).

In conclusion, our results in SCR indicate that the stress-induced recruitment of motor regions and the parallel disengagement of fronto-parietal networks during early learning were related to poorer consolidation. Our data further suggest that stress interrupted the relationship between hippocampal connectivity patterns during learning and subsequent optimal consolidation in SCR.

Table 4 Functional imaging results for the regression with offline changes in speed—between group comparisons

Area	X mm	Y mm	Z mm	Z	P
1. Main effect of practice [training]					
[Control–SCR]					
L Intraparietal sulcus	−42	−40	36	3.03	0.027
SMA	−2	−18	60	3.00	0.039
[SCR–control] No suprathreshold clusters					
2. Modulation in activity by speed [training] by speed of performance [training]					
[Control–SCR]					
L Superior parietal	−14	−70	62	2.96	0.033
	−22	−54	52	3.06	0.030
L Precuneus	−10	−64	52	3.50	0.012
R Precuneus	10	−72	63	2.96	0.033
L Cerebellum crus 1	−28	−62	−36	3.25	0.015
L Cerebellum crus 2	−10	−80	−40	2.90	0.038
R Middle frontal gyrus	42	4	60	3.13	0.029
[SCR–control] No suprathreshold clusters					
3. Modulation in right Hippocampus [28–36 −2] connectivity by speed [training]					
[Control–SCR]					
R Cerebellum crus 1	30	−72	−34	3.01	0.033
R Cerebellum VI	36	−42	−28	2.93	0.040
M orbitofrontal	0	42	−14	3.42	0.011
SMA	−14	−4	38	3.27	0.016
L Middle frontal gyrus	−16	24	48	3.02	0.032
L Superior frontal gyrus	−34	18	48	2.89	0.050
[SCR–control]					
R Superior frontal gyrus	18	62	2	3.25	0.017
R Middle frontal gyrus	28	48	6	3.10	0.026

Notes: Significance level set at $P_{corr} < 0.05$ corrected for multiple comparisons (FWE) over small volumes. Note that none of the reported results survived the additional Holm-Bonferroni correction for multiple comparisons. Voxels of these maps not surviving correction for multiple comparisons and which were not of interest were not reported. SCR, Stress Cortisol Responders; SMA, Supplementary Motor Area

Effect of Stress on Between-Session Changes in Brain Responses

The effect of stress on the neural correlates of consolidation was examined using between-session contrasts (but see Supplementary Table 7 for results on the MSL retest session). Our data indicated a larger decrease in activity from training to retest in the left middle frontal gyrus, premotor, medial frontal (including pre-SMA), and superior parietal regions in the SCR as compared to the control group (Table 5).

Regression analyses with offline changes in performance showed that between-session changes in activity in the hippocampus differently correlated with offline changes in performance between groups (Table 5). Inspection of the parameter estimates indicated that an increase in (anterior) hippocampal activity from training to retest was positively related to consolidation in the SCR group, while it correlated negatively with offline changes in performance in the control group (Fig. 7). Within-group analyses indicated that these relationships were not significant within the respective groups (Supplementary Table 5.4).

Stress Cortisol Nonresponders

Imaging results including the SCNR group are detailed in Supplementary Material Section 2.6 (Supplementary Tables 4–6), but the main similarities and differences between SCR and SCNR are summarized here. Results showed that the stress-induced increase in sensory activity observed in SCR during initial learning was larger than in the SCNR group that showed similar brain activity as controls during training. However, as in SCR, hippocampal-cortical activity in SCNR decreased as a function of practice and this significantly more as compared to the control group. Regression with end of training performance yielded no group differences between SCNR and the other groups in sensorimotor cortical regions. However, similar to the SCR, the recruitment of sensorimotor regions (including S1, M1, PMC) during initial training in SCNR was negatively related to consolidation and this relationship was significantly different from the control group. Last, the difference in regression between intersession changes in hippocampal responses and offline changes depicted in Figure 7 was also observed between SCNR and control groups. Altogether, while SCNR did not present the same increase in activity in motor-related networks as compared to controls during initial training, hippocampal modulation was similar as in SCR and (sensori)motor activity during memory acquisition was still related to poor consolidation.

Discussion

In the current study, we investigated whether stress prior to motor sequence learning influences the behavioral and neural substrates underlying motor memory acquisition and subsequent consolidation. Specifically, we used regression analyses in order to examine the relationship between interindividual differences in stress-induced brain responses and motor performance. Note that in the section below, "stress" is used to discuss the results observed in the "stress cortisol responders" unless stated differently. At the behavioral level, our data showed no evidence for an effect of stress on motor performance. At the

Table 5 Functional imaging results for the main effect of session—between group comparisons

Region	X mm	Y mm	Z mm	Z	P
1. Main effect of session [training > retest]					
[SCR–control]					
L Middle Frontal Gyrus	−46	28	38	3.52	0.012
L PMC	−52	18	34	3.34	0.012
	−46	18	32	3.27	0.015
	−46	22	34	3.18	0.020
R (lateral) PMC	32	4	60	3.43	0.009
Pre-SMA	−8	26	46	3.18	0.027
	18	10	70	2.97	0.034
	−14	18	64	3.15	0.021
R Superior parietal	52	−40	58	2.79	0.050
L Cerebellum Crus 1	−20	−66	−32	2.95	0.036
L Cerebellum VIIb	−12	−70	−44	2.85	0.046
[Control–SCR] No suprathreshold clusters					
2. Regression with offline changes in performance [training > retest]					
[Control–SCR]					
R hippocampus	24	−8	−22	2.98	0.033*
[SCR–control] No suprathreshold clusters					

Notes: Significance level set at $P_{corr} < 0.05$ corrected for multiple comparisons (FWE) over small volumes. Voxels of these maps not surviving correction for multiple comparisons and which were not of interest were not reported. SCR, Stress Cortisol Responders; SMA, supplementary motor area; PMC, Premotor cortex *$P < 0.05$, Holm-Bonferroni corrected

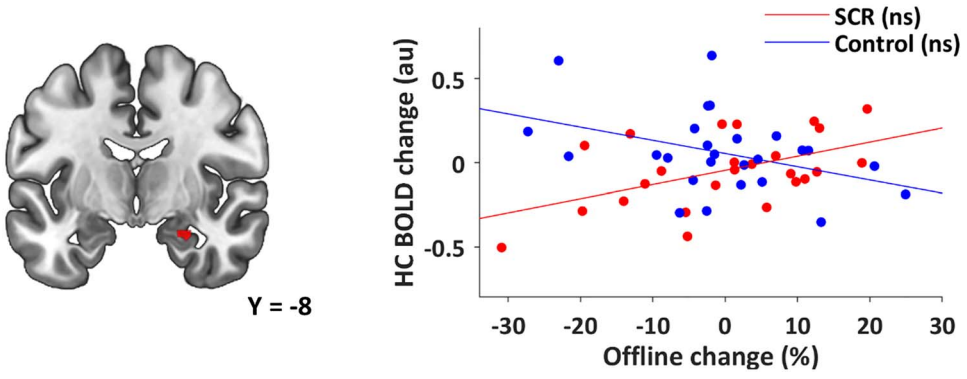

Figure 7. Imaging results of the regression analysis between the magnitude of the between-session changes in brain responses [retest—training] and offline changes in performance speed. Left panel: Between-session changes in hippocampal (HC) activity from training to retest were differently correlated with the subsequent offline change in performance speed in the stress cortisol responder (SCR) and control groups ([control–SCR], HC [24–8 −22 mm], $Z = 2.98$, $P_{SVC} = 0.033$). Right panel: Regression plot of the differences in BOLD response in the right hippocampus [retest—training] against the offline change in performance speed. A positive change represents an increase in activity from training to retest. Activations maps are displayed on a T1-weighted template image at a threshold of $P < 0.005$ uncorrected. (ns) Nonsignificant within-group $P_{uncorr} > 0.001$ (see Supplementary Table 5.4 for within-group regression results for the regression with offline changes in speed). *N* SCR group = 27; *N* control group = 28. au, arbitrary units.

neural level, results indicated that stress favored the recruitment of motor areas and resulted in a progressive disengagement of hippocampo-cortical networks during initial learning. Brain-behavior regression analyses indicated that while stress-induced changes in activity and connectivity were related to better performance at the end of training, these changes related to poorer consolidation. Interestingly, our results further showed that an increase in hippocampal activity after a nap was beneficial for consolidation in the stress as compared to the control group.

Stress Modulates Activity and Connectivity During Motor Memory Acquisition

Previous studies have reported that stress prior to dual-solution tasks (i.e., solvable by either declarative or procedural strategies) induces a shift in activity from hippocampal to striatal networks (for a review see Wirz et al. 2018). In the current study, we found that stress prior to motor sequence learning resulted in an increased task-related activity in motor cortical regions including sensorimotor, premotor, and supplementary motor areas during memory acquisition. Note that against our expectations, striatal activity did not, on average, differ between groups. Our neuroimaging results also showed that stress induced a progressive decrease in hippocampal, parietal, and premotor cortex activity as well as a disconnection of the hippocampus with sensorimotor regions as a function of practice. Given the functional connection between motor cortical regions and the striatum (Johansen-Berg et al. 2004; Lehéricy 2004; Debas et al. 2014), these results are indirectly in line with a stress-induced boost in striatal activity observed in previous studies using nonmotor procedural tasks (Vogel et al. 2015, 2017; Wirz, Wacker, et al.

2017b). Moreover, the decrease in activity in the hippocampus during learning is directly in line with decreases in hippocampal activity observed after stress exposure within this particular time window (Schwabe and Wolf 2012; Schwabe et al. 2013; Wirz, Reuter, et al. 2017a). Although similar learning-related patterns in the hippocampus have been observed in (nonstressed) healthy young adults during motor sequence learning (Grafton et al. 2002; Schendan et al. 2003; Fletcher et al. 2005; Albouy, Sterpenich, et al. 2013b), the absence of such modulation in the control group does point towards a stress-specific effect in the current study. Given the competitive interaction between striatal and hippocampal systems during initial motor learning (Albouy, Sterpenich, et al. 2013b) and evidence to suggest that stress-induced reduction in hippocampal recruitment may allow the striatum to dominate learning under stress (Schwabe 2017), we propose that the decrease in hippocampal activity during practice facilitated the increase in sensorimotor activity in the stress group (but see section on nonresponders below for a more in-depth discussion).

Besides direct modulatory effects of stress on hippocampal and (striato)motor regions, there is considerable evidence in the nonmotor procedural domain for the role of the amygdala in orchestrating the balance between the recruitment of hippocampal and striatal systems under stress (for a review see Vogel et al. 2016). Specifically, stress prior to perceptual learning is thought to favor striatum- over hippocampus-dependent processing through (among other mechanisms) a stress-induced increase in amygdala-striatal and decrease in amygdala-hippocampal connectivity (Schwabe and Wolf 2012; Schwabe et al. 2013; Wirz, Reuter, et al. 2017a; see Wirz et al. 2018 for a review). Moreover, previous work showed that this stress-induced shift in connectivity is mediated by cortisol binding to the mineralocorticoid (MR) receptor (Schwabe et al. 2010, 2013; Wirz, Reuter, et al. 2017a; for a review see Vogel et al. 2016). In line with these observations, our data showed that stress resulted in a progressive decrease in amygdala-hippocampus connectivity in the SCR as opposed to an increase in the control group. This might suggest that the amygdala can mediate the shift from hippocampal to striatal systems also in the motor memory domain. However, this remains hypothetical as amygdala and striatum connectivity were not investigated here.

In the current study, stress did not, on average, result in reduced hippocampal activity (as described in previous studies, Schwabe and Wolf 2012; Schwabe et al. 2013; Wirz, Reuter, et al. 2017a) but rather induced a gradual learning-related decrease (i.e., performance related modulation) in hippocampal activity during practice. It could be argued that the learning-related decrease would eventually result in an average difference and that the process needed more time to develop. This, however, stands in contrast with previous studies reporting reduced hippocampal activity within the exact same time window examined in the current research (Pruessner et al. 2008; Schwabe and Wolf 2012; Wirz, Reuter, et al. 2017a). It is also not in agreement with exploratory analyses performed in the current study showing that the decrease in hippocampal activity was practice dependent rather than time dependent (see Supplementary Table 8). It remains unclear whether the overall decreased hippocampal activity in previous studies resulted from a similar modulation in activity during learning or whether these dynamical responses are specific to procedural tasks acquired progressively through repeated practice. Nonetheless, our data raise the interesting point that stress might alter the competitive interaction between memory systems in a dynamical rather than a static manner.

Stress Alters the Relationship Between Neural Responses and Performance During Initial Motor Learning

Our behavioral data indicated that exposure to stress did not influence motor performance during initial learning. This is in line with a previous behavioral study in our group (Dolfen et al. 2019). Interestingly, regression analyses between brain responses during learning and the level of performance reached at the end of training showed that increased involvement of striato-motor regions as well as decreases in connectivity between the hippocampus and sensori-motor-cerebellar regions were related to faster performance in stressed participants as compared to controls. These results suggest that while stress did not influence initial motor performance on average, it altered the way brain responses during initial learning relate to performance levels reached at the end of learning. In previous studies, observations of increased activity in striato-motor circuits as well as progressive disconnection between the hippocampus and striato-motor networks have consistently been linked to the development of faster performance during initial motor learning (Doyon and Benali 2005; Albouy et al. 2008, 2012, 2015; Steele and Penhune 2010). Our data showed, for the first time, that stress accentuates this link. Importantly, based on the observation that stress reinforces the recruitment of brain responses related to the implementation of faster performance, one could have expected to observe better performance in the stress as compared to the control group at the end of learning. As average performance did not differ between the groups during learning, it can be speculated that these modulations of brain responses were used to compensate for potential stress-induced disruption of performance. This, however, remains hypothetical, as we did not identify stress-induced brain responses showing a detrimental effect on initial performance.

Interestingly, our data also showed that a larger practice-related increase in amygdala activity tended to be beneficial for performance in the stress group but detrimental for performance in the control group (albeit not significant within the respective groups). Given the pattern of stress-induced changes we observed (increase in sensorimotor cortical as opposed to decreases in hippocampal-cortical regions), this might provide further evidence for the role of the amygdala in mediating the stress effect.

Stress and Offline Memory Processes

Previous neuroimaging studies have suggested that the specific combination of hippocampal activity during learning and post-training sleep is necessary to optimize consolidation processes and therefore trigger overnight performance enhancements (for reviews, see Albouy, King, et al. 2013a; King et al. 2017). Importantly, not only hippocampal activity but also its connectivity—and, in particular, the strength of the hippocampal-striatal competitive interaction—during acquisition have been shown to be predictive of overnight gains in performance (Albouy, Sterpenich, et al. 2013b). Given the link between these neural signatures and sleep-dependent consolidation (see Albouy, King, et al. 2013a for a review), we hypothesized that stress-induced modulations of hippocampal activity and connectivity

would forecast a disruption of the subsequent sleep-related consolidation process. Unexpectedly, while our results indicated that stress indeed altered brain processes in regions crucial for memory consolidation such as the hippocampus, we did not observe any direct relationship between the stress-induced modulation of hippocampal activity during training and subsequent consolidation. However, in line with our expectations, regression analyses showed that in contrast to the control group, hippocampal connectivity patterns during learning were not linked to optimal consolidation in the stress group.

Our activation results demonstrate that a stress-related decrease in activity in fronto-parietal networks and a stress-induced increase in motor cortical recruitment during initial learning were related to poorer consolidation. Importantly, these findings cannot be explained by a trade-off between initial learning amplitude and offline changes in performance at the behavioral level, as there was no significant correlation between end of training and between-session changes in performance (see Supplementary Materials Section 2.4). Fronto-parietal networks, and more particularly, hippocampo-fronto-parietal networks, have previously been shown to play a critical role in the encoding of an abstract map of the motor sequence during initial learning (Grafton et al. 1998; Hikosaka et al. 2002; Albouy, King, et al. 2013a). Importantly, we have also shown that the consolidation of motor memory traces supported by such networks depends on sleep (Albouy, King, et al. 2013a). We suggest that the stress-induced modulation of activity in fronto-parietal networks during initial learning compromised the building of a spatial map of the motor sequences, which was then related to poorer consolidation after sleep. This is also in line with our data showing that stress interrupted the link between hippocampo-cortical connectivity patterns during learning and optimal consolidation processes observed in controls. Interestingly, the stress-induced increase in activity in motor regions was also related to poorer consolidation, suggesting that this over-recruitment of motor networks might not rescue the subsequent sleep-related consolidation process. This is in agreement with earlier work showing that (striato-)motor networks support consolidation processes that do not necessarily depend on sleep (Albouy et al. 2015).

Finally, our data indicated that an increase in hippocampal activity from training to retest is differentially related to offline performance improvement in stress as compared to control participants (Note that a similar pattern was observed in SCNR as in SCR). This is in line with evidence that sleep particularly promotes the recruitment of hippocampal-frontal networks during retest on a motor sequence task when performance is enhanced (Walker et al. 2005; Steele and Penhune 2010; Albouy, King, et al. 2013a; King et al. 2013; Fogel et al. 2014). We speculate that the nonoptimal spatial map developed during initial training under stress might be processed during the subsequent sleep episode. Sleep might favor, through increased hippocampal recruitment over the sleeping interval, subsequent improvement in performance. Our design, however, does not allow us to conclude on a specific effect of sleep, as compared to the simple passage of time, on this process as our study did not include a wake control group.

In contrast to previous studies showing either learning-specific hippocampal recruitment (Albouy et al. 2008) or a learning-related decrease in hippocampal activity (Grafton et al. 2002; Schendan et al. 2003; Fletcher et al. 2005), we found no evidence for hippocampal recruitment in the current sample of young healthy controls (i.e., no significant activation or modulation in the hippocampus during initial learning). This is surprising as, compared to previous studies, we used a bimanual task relying on a more complex visuospatial mapping that was hypothesized to favor hippocampal recruitment. It thus remains unclear why hippocampal dynamics were not reproduced in the present study. However, it is important to note that the previously observed relationship between hippocampo-cortical connectivity and subsequent sleep-related consolidation was observed in the control group (Albouy, King, et al. 2013a).

On Stress Cortisol Nonresponders and the Role of Cortisol

In this study, participants in the stress condition with an increase in cortisol smaller than 1.5 nmol/L and 15.5% from baseline to T25′ were classified as stress cortisol nonresponders and not included in the analyses reported in the main text. Although this precise cut-off is based on earlier work using statistical response class allocation (Miller et al. 2013) and is extensively used in the literature (Quaedflieg et al. 2015; Dandolo and Schwabe 2016; Vogel et al. 2018; Smeets et al. 2019), it is worth noting that, in the current sample, the distribution of this variable was rather continuous and thus did not follow a clear bimodal distribution (see Supplementary Fig. 2). As such, results could vary slightly if a different threshold defining a cortisol response was used. Nonetheless, given the unexpectedly large number of stress cortisol nonresponders in the current study (35% as opposed to 15% nonresponders in Dolfen et al. 2019), exploratory analyses including SCNR were performed in order to reflect on the role of the cortisol in the effects reported above. It is worth emphasizing, however, that the current study was not designed nor powered to compare cortisol responders and nonresponders. The points discussed below are therefore rather speculative.

Our results indicate that the gradual disengagement of hippocampo-cortical regions during learning was observed irrespective of whether stress induced an increase in salivary cortisol. Additionally, between-session changes in hippocampal activity were similarly related to consolidation in both stress groups. The observation that hippocampal functioning was equally modulated in both groups is in line with previous evidence that stress-induced modulation of hippocampal activity might not be solely dependent on the presence of cortisol. Indeed, it has been shown that pharmacologically blocking MR receptors after stress exposure does not prevent stress-induced decrease in hippocampal activity (Schwabe 2013). Interestingly, in contrast to cortisol responders, nonresponders did not show a stress-induced boost in sensori(motor) activity during learning. It is, therefore, tempting to speculate that, in contrast to hippocampal modulation, the stress-induced hippocampo-sensorimotor shift observed in responders specifically depends on cortisol. This is in agreement with previous evidence indicating that glucocorticoid activity, through MR receptor binding, is a pre-requisite for the amygdala mediated shift towards striatum-based learning during nonmotor procedural memory tasks (Schwabe et al. 2013; Vogel et al. 2017). Lastly, it is interesting to note that striato-motor activity during initial learning in nonresponders, while not larger than in controls, was related to poorer subsequent consolidation. This suggests that while cortisol might be necessary to induce a shift between memory systems, the stress intervention in nonresponders did modulate the relationship between striato-motor activity

and consolidation, presumably through other stress mediators such as noradrenaline. Note that this remains speculative as no pharmacological intervention was used in the current study.

Stress Does Not Modulate Motor Behavior

Consistent with our previous study, no effects of stress on motor learning nor subsequent memory consolidation processes were found at the group level (see Dolfen et al. 2019 for an extensive discussion on the matter). Our results are also in line with a recent study showing no effect of stress on motor sequence learning using a probabilistic SRT task (Tóth-Fáber et al. 2020 (preprint); note however that stress modulated the statistical component of learning in this study). Importantly, adopting an individual differences approach, our regression analyses indicated that the manner in which the brain responds to stress—in terms of task-related activity and connectivity patterns—was related to performance during initial learning as well as offline memory processing. This suggests that the influence of stress on motor behavior is linked to the effects of stress on brain functioning which varies from individual to individual. It also worth noting that the present study did not replicate our previously reported significant correlation between offline changes in performance and the glucocorticoid response in the SCR group. It is not entirely clear why this correlation was not reproduced in the current research. Several experimental factors that differed between the present work and our previous study may have influenced offline gains in performance, cortisol concentration and their relationship. For example, different consolidation intervals (6 h vs. 24 h), sleep episodes (diurnal vs. nocturnal), and different experimental contexts (real scanner at the hospital vs. mock scanner at the university). However, the exact influence of these methodological differences on these measures of interest and their relationship remains unknown.

Methodological Considerations

It is worth explicitly stating that only a limited number of brain regions survived the additional step of Holm–Bonferroni correction performed at the region level. We acknowledge this limitation and although these are theoretically interesting findings, they need to be interpreted with caution.

Conclusions

The current study provides the first evidence that stress, induced experimentally prior to motor sequence learning, alters brain responses in motor-memory-related networks. Specifically, we showed that stress favored the recruitment of motor areas and resulted in decreased hippocampo-cortical activity and connectivity over the course of learning. While the magnitude of stress-induced brain responses was positively related to motor performance during initial learning, they were negatively related to subsequent consolidation. These results indicate that the competitive nature of the interaction between memory systems observed in other domains (e.g., declarative, perceptual) extends to motor memory and therefore effectively unify mechanisms from diverse memory fields. Importantly, our individual-differences approach revealed that although stress might, on average, not alter motor performance, variability in brain responses to stress explains interindividual differences in the effect of stress on motor learning and subsequent memory consolidation.

Supplementary Material

Supplementary material can be found at *Cerebral Cortex* online.

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Author Contributions

G.A., B.K., L.S., and N.D. designed the experiments. N.D. conducted the experiments in collaboration with M.V., M.G., B.K., G.A. G.A., B.K., N.D., and A.v.L. contributed to the acquisition and analytic tools. N.D., G.A., and BK analyzed the data. All authors contributed to the manuscript.

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