

Default mode and motor networks facilitate early learning of implicit motor sequences: a multimodal MR spectroscopy and fMRI study

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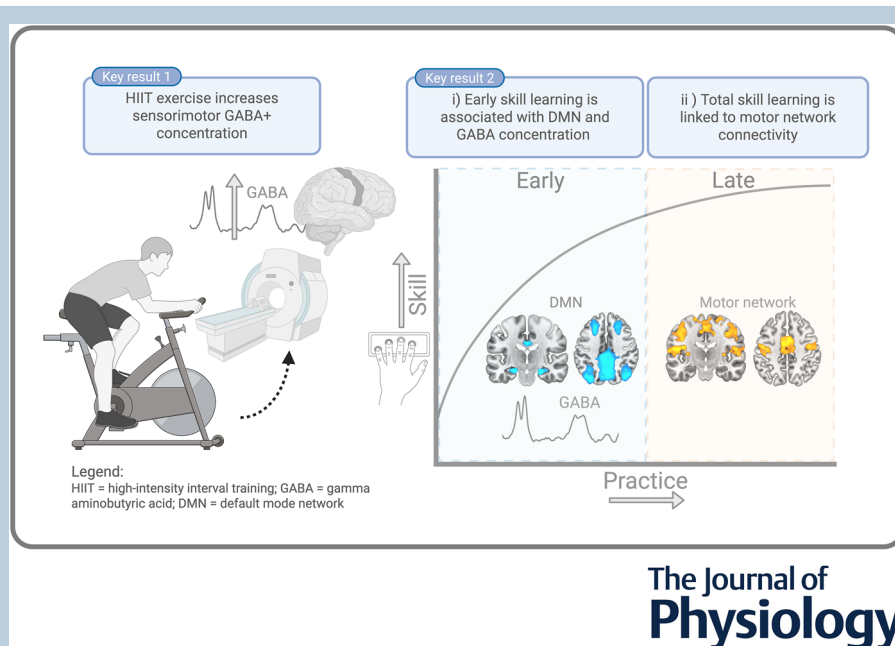
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Abstract figure legend This study investigated the neural correlates of implicit sequence learning and the influence of high-intensity interval training (HIIT). We show that early skill learning is linked to default mode network connectivity, whereas the overall degree of learning is associated with motor network connectivity. Sensorimotor GABA concentration was implicated in the early stages of learning, and an increase in GABA concentration was observed following HIIT. Our results highlight shared involvement of default mode and motor networks in implicit sequence learning and demonstrate the capacity for exercise to modulate neural mechanisms supporting skill learning.

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Abstract Learning new motor skills is a fundamental process that involves the sequencing of actions. Skill develops with practice and time and manifests as performance that is fast and accurate. Although we know that learning can occur through an *implicit* process in the absence of conscious awareness, and across multiple temporal scales, the precise neural mechanisms mediating implicit motor sequence learning remain poorly understood. Similarly the capacity for interventions with known influence on learning and memory, such as cardiovascular exercise, to facilitate implicit learning is yet to be clearly established. Here we investigated the neuroplasticity of implicit motor sequence learning and the effect of acute exercise priming. Healthy adults (39.5% female) aged 22.55 ± 2.69 years were allocated to either a high-intensity interval training (HIIT) exercise group ($n = 16$) or to a very low-intensity control group ($n = 17$). After exercise participants performed a serial reaction time task, magnetic resonance (MR) spectroscopy estimates of sensorimotor GABA were acquired before and after exercise and during task performance, and resting-state functional magnetic resonance imaging (fMRI) was acquired at the end of the protocol. We show that early stages of learning are linked to default mode network connectivity, whereas the overall degree of learning following sustained practice is associated with motor network connectivity. Sensorimotor GABA concentration was linked to the early stages of learning, and GABA concentration was modulated following HIIT, although the two were not related. Overall through the integration of multiple neuroimaging modalities we demonstrate that interactions between hippocampal and motor networks underlie *implicit* motor sequence learning.

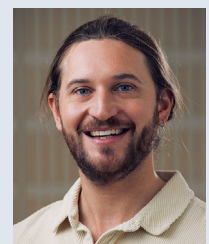
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Key points

- Motor learning occurs across different temporal scales and can arise implicitly in the absence of conscious awareness.
- *Explicit* motor learning is linked to the brain's primary inhibitory neurotransmitter, GABA and interactions across motor and hippocampal networks.
- Whether these same neural mechanisms are implicated in *implicit* learning is unclear. Similarly the capacity to influence implicit learning via priming with cardiovascular exercise is yet to be clearly established.
- We show that early implicit learning is underpinned by default mode network connectivity and sensorimotor GABA concentration, whereas total learning following sustained practice is linked to motor network connectivity. We also found that high-intensity interval training (HIIT) exercise elevated sensorimotor GABA concentration, but not the magnitude of implicit learning.
- Overall our results highlight shared involvement of default mode and motor networks in implicit motor sequence learning.

Joshua Hendrikse is a research fellow in the Turner Institute for Brain and Mental Health and School of Psychological Sciences at Monash University. His work utilises multi-modal neuroimaging methods to investigate the neural mechanisms supporting memory and learning. He is also interested in the capacity for physical exercise and non-invasive brain stimulation (e.g., repetitive transcranial magnetic stimulation and focussed ultrasound stimulation) to influence neuroplasticity and enhance cognitive performance.



Introduction

Motor skills are necessary to perform effectively and efficiently in our everyday lives. Acquiring a new skill (e.g. learning how to operate machinery or play a musical instrument) involves practising a sequence of motor actions, with their execution becoming faster and more accurate with repetition and the passage of time. Research on the neural mechanisms supporting motor sequence acquisition has established a central role of the brain's primary inhibitory neurotransmitter GABA (Coxon et al., 2014; Stagg et al., 2011a). Modulation of the GABA system is a prerequisite for the synaptic plasticity underlying learning and memory (i.e. long-term potentiation) (Bachtar & Stagg, 2014) and is likely a factor contributing to inter-individual differences in learning capacity (Stagg et al., 2011a). GABA concentration within primary sensorimotor (SM) cortex is reduced as a motor sequence is acquired (Floyer-Lea et al., 2006; Kolasinski et al., 2019) and is associated with the degree of learning observed following practice (Kolasinski et al., 2019; Stagg et al., 2011a). SM GABA concentration is also linked to the strength of functional connectivity within the motor network (Stagg et al., 2014), indicating that interactions between the GABAergic system and task-relevant brain networks may underlie the learning of novel motor sequences.

Motor sequence learning occurs across multiple temporal scales, from acquisition during practice to offline consolidation over the minutes to hours and days that follow (Dayan & Cohen, 2011). The consolidation of motor skills over the minutes to hours following practice has been linked to modulation of GABA during acquisition (Stavrinos & Coxon, 2017) and to increased functional connectivity across frontoparietal (Albert et al., 2009), striatal and hippocampal networks (King et al., 2022). Remarkably motor consolidation might begin faster than previously thought, over the time scale of seconds, as evidenced by offline gains during short breaks between initial practice attempts (i.e. micro-consolidation) (Bönstrup et al., 2019, 2020). Evidence indicates that the micro-consolidation of an explicit motor sequence is mediated by activity across networks involving entorhinal and hippocampal regions (Buch et al., 2021; Jacobacci et al., 2020), and is also linked to beta oscillations across frontal-parietal regions (Bönstrup et al., 2019). Collectively this evidence may reflect involvement of a distributed network involving frontal, parietal and hippocampal regions, including the default mode network, which has been proposed to mediate neural replay and consolidation of new memories (Kaefer et al., 2022).

Motor sequence learning can be explicit, investigated using tasks that directly cue the required motor sequence (as per aforementioned studies by Bönstrup et al., 2019,

2020; Jacobacci et al., 2020; Kolasinski et al., 2019), or implicit, where sequence knowledge is developed through repetition without explicit knowledge of the sequence (Robertson, Pascual-Leone, & Miall, 2004; Robertson, Pascual-Leone, & Press, 2004). Our recent work has established that offline micro-consolidation extends to implicit motor sequence learning (Brooks et al., 2024). However the neural mechanisms linking micro-consolidation of implicit motor sequences to overall learning outcomes (following more sustained practice) are yet to be determined. Further it remains unclear whether both motor and default mode networks are involved in the learning of *implicit* motor sequences. Addressing these gaps in knowledge will provide a more comprehensive understanding of how the brain encodes new motor skills across different temporal scales. Advancing knowledge in this regard will likely have implications for optimising human performance across a range of settings, such as in sporting contexts, and/or in the implementation of therapies aimed at rehabilitating motor skills following stroke and brain injury.

A considerable body of prior research has demonstrated that cardiovascular exercise primes neuroplasticity mechanisms implicated in learning and memory (Hendrikse et al., 2017). For example regular exercise is associated with elevated markers of neuronal integrity within the hippocampus (Hendrikse et al., 2022), and a single bout of high-intensity interval exercise (HIIT) alters GABA transmission (Hendy et al., 2022; Neva et al., 2017, 2021) and concentration within non-exercised SM cortex (Coxon et al., 2018). Performing HIIT immediately prior to motor practice has been demonstrated to improve sequence consolidation (Roig et al., 2012; Stavrinos & Coxon, 2017; Taylor et al., 2024). However these interactions may vary as a function of task demands and the time window under which they are assessed (Roig et al., 2013, 2016). The effect of HIIT exercise priming on neural mechanisms underpinning implicit motor sequence learning (i.e. modulation of GABA concentration and functional connectivity) remains unclear.

In summary previous work has established a role of both hippocampal and motor systems in explicit motor sequence learning. The formation of novel motor skills is also linked to the activity of GABA, particularly within primary SM regions (Kolasinski et al., 2019; Stagg et al., 2011a). However the effect of cardiovascular exercise on the neural mechanisms underlying sequence learning is yet to be clearly elucidated. Further our understanding of how these neural mechanisms mediate different stages of *implicit* motor sequence learning (e.g. at the micro-scale *vs.* following sustained practice) remains limited. Therefore in this study we investigated the neuroplasticity of the initial learning of an implicit motor sequence, using magnetic resonance imaging (MR) spectroscopy and resting-state functional magnetic resonance imaging

(fMRI). High-intensity exercise was administered to prime task-relevant plasticity mechanisms in one group and compared to a very-low-intensity control group. We expected that high-intensity exercise priming would modulate SM GABA concentration and facilitate motor sequence learning. We also hypothesised that early micro-offline consolidation of an implicit motor sequence would be associated with the co-ordinated activity of resting-state networks involving the hippocampus, i.e. functional connectivity of default mode network (as per Bönstrup et al., 2019; Jacobacci et al., 2020), but not anatomically and functionally distinct networks (i.e. salience and visual networks). Lastly we hypothesised that latter stages of motor skill learning (i.e. following more sustained practice, characterised by a reduced slope of the learning curve) would be associated with motor network connectivity and GABA concentration within SM cortex, and that this may be more pronounced in the HIIT group.

Methods

Ethical approval

This study was approved by the Monash University Human Research Ethics Committee (project # 27 724), and all participants provided their written informed consent. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database. Participants were remunerated \$30 for their participation.

Participants

Thirty-eight healthy adults (39.5% female), aged 22.55 ± 2.69 years (mean $\pm$ SD; range 19–28), were recruited into the study. Participants reported no contraindications to MRI or cardiovascular exercise, no current use of psychoactive medication and no history of psychological or neurological disorders. All participants were required to complete the long format of the International Physical Activity Questionnaire (IPAQ) prior to participation to assess self-reported physical activity. Handedness was determined by the Edinburgh Handedness Inventory. All participants were right-hand dominant ($M = +90.42$, $SD = 23.72$). Data from five participants were excluded due to developing explicit awareness of the repeating motor sequence during completion of the serial reaction time task ($n = 4$, see motor sequence learning for further detail), and an error in the MRI console software ($n = 1$). Thus analyses were conducted with $N = 33$ (HIIT group: $n = 16$, 31% female, $M_{\text{age}} = 21.69 \pm 2.15$, $M_{\text{handedness}} = 91.64 \pm 19.02$; control group: $n = 17$, 35.3% female, $M_{\text{age}} = 22.35 \pm 2.60$, $M_{\text{handedness}} = 86.13 \pm 30.82$).

Experimental design

A mixed experimental design was used to investigate interactions between exercise, implicit motor sequence learning and MRI measures. Participants completed a 20-min acute bout of either high-intensity interval cycling (HIIT group, $n = 16$) or a matched period of very-low-intensity cycling (control group, $n = 17$). After this participants performed a serial reaction time task. MR spectroscopy (MRS) estimates of GABA concentration were acquired at rest, both before and after exercise, and during the performance of two runs of a serial reaction time motor task (Fig. 1). Resting-state fMRI was also acquired at the end of the protocol to assess associations between network connectivity and learning. Self-report measures relating to exercise tolerance (Ekkekakis et al., 2005) and lifestyle factors that impact learning and memory (e.g. sleep quality) were also assessed (Buysse et al., 1989).

Exercise

Participants were pseudo-randomised into HIIT or control groups using an algorithm that minimised variance between groups with regard to biological sex and age. Exercise intensity was tailored to each individual based on their heart rate reserve (HRR), where $HRR = \text{age predicted maximum heart rate} - \text{resting heart rate}$ (Tanaka et al., 2001). Heart rate was continuously monitored throughout the exercise session using a Polar H7 monitor (Polar Electro, Kempele, Finland). HIIT exercise involved cycling on a stationary cycle ergometer (Wattbike, Geelong, Australia) at alternating epochs of moderate intensity (3 min, target heart rate of 50%–60% HRR) and high intensity (2 min, target heart rate of 90% HRR for the final epoch) for a total duration of 20 min, as per our previous work (Cadwallader et al., 2024; Coxon et al., 2018; Curtin et al., 2023; Stavrinou & Coxon, 2017; Taylor et al., 2024). The control group was instructed to turn the pedals at a very low cadence (target heart rate was to remain below 20% HRR), requiring minimal exertion. Here we were interested in assessing the systemic effects of HIIT exercise on motor plasticity. As such in line with a previous study by Coxon et al. (2018) we utilised lower limb-based stationary cycling and recorded GABA concentration from a non-exercised upper limb (i.e. the hand representation in primary motor cortex).

Motor sequence learning – serial reaction time task

To assess motor sequence learning we utilised a customised version of the serial reaction time task (SRTT) programmed in PsychoPy (version 2020.1.3) (Brooks et al., 2024). The SRTT was commenced approximately 20–25 min following exercise (Fig. 1). Participants lay

supine in the scanner and viewed four white circles arranged horizontally on a monitor screen, with their non-dominant (left) hand positioned over a four-button box. Their little finger aligned to the left most button (and left most circle #1) and index finger to the right most button (and right most circle #4). Participants

were instructed to respond to the circle that turned black by pressing the corresponding button as quickly and accurately as possible. Undisclosed to participants the visual cues followed a 12-item repeating sequence (2-3-1-4-3-2-4-1-3-4-2-1) (Fig. 1B). A correct response was required to progress to the next cue. The task

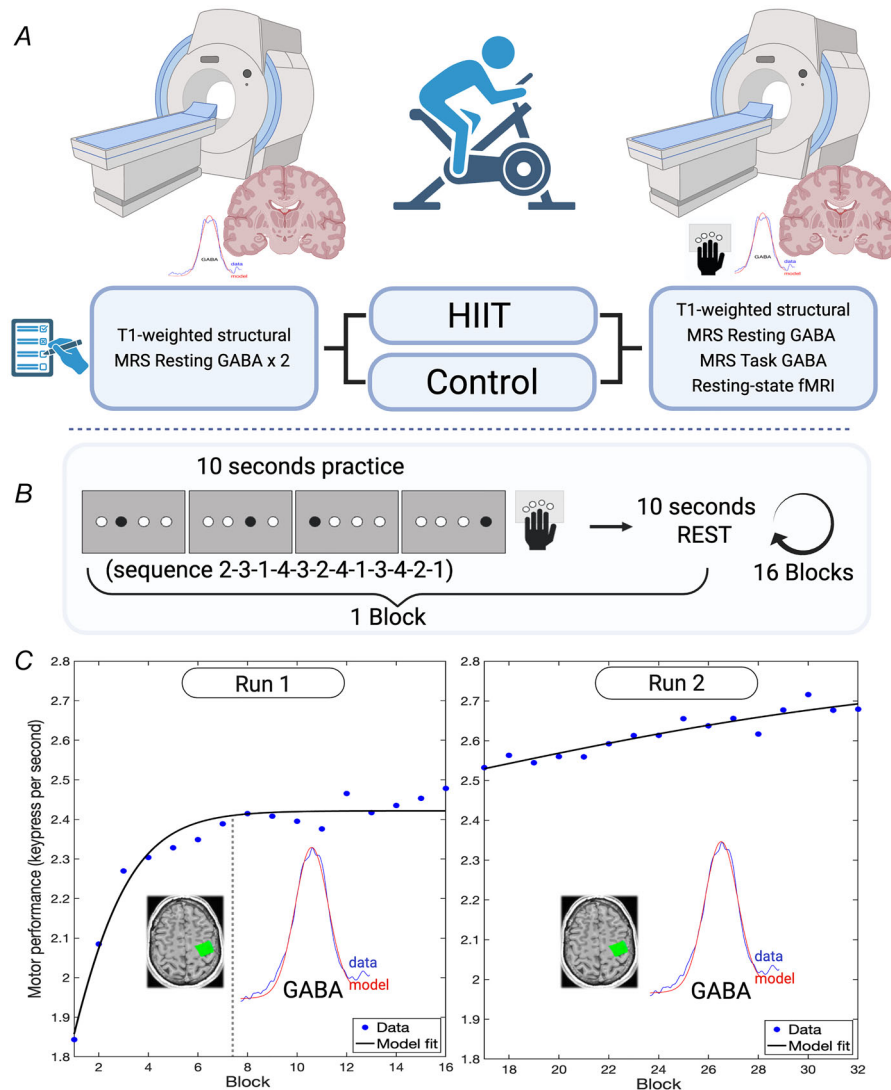


Figure 1.

A, experimental overview. A mixed experimental design was used to investigate associations between exercise, motor learning and magnetic resonance imaging (MRI) measures. Participants completed a 20-min acute bout of either high-intensity interval cycling (HIIT group, $n = 16$) or a matched period of very-low-intensity cycling (control group, $n = 17$). B, following this participants performed two runs of a serial reaction time task, which included an implicit 12-item repeating sequence. Each run comprised 16 blocks of 10 s periods of practice and rest. MR spectroscopy estimates of GABA+ concentration were acquired before and after exercise and during performance of the motor task. Resting-state functional MRI (fMRI) was also acquired to assess associations between network connectivity and learning. C, motor task performance is represented as the mean increase in correct keypresses per second across blocks for all participants (blue circles), modelled separately for each run of the motor task with an exponential function, $B(t) \sim L(t) = k_1 + (k_2 / 1 + e^{-k_3 t})$, where k_1 and k_2 determine learning plateau, k_3 the steepness of the learning function and $t \in (1, +\infty)$ represents trial (t) starting from 1 and extending to infinity (black line, Bönstrup et al., 2019). The model was fit separately to each task run, and the '95% early learning' phase cut-off was delineated for the first run (Bönstrup et al., 2019). This is represented by the vertical dotted line. GABA+ concentration was measured across both runs of the motor task.

alternated every 10 s between task practice and rest for a total of 16 practice blocks, 320 s in total. Participants completed two separate runs of the task (i.e. a total of 32 practice blocks, 640 s) to assess the initial learning of an implicit motor sequence and associated changes in GABA concentration.

After completion of the session participants were questioned about their level of explicit sequence awareness. Participants who were able to accurately replicate the first five or more sequence items were determined to have gained some degree of explicit sequence awareness (HIIT, $n = 2$, control, $n = 2$) and were removed from subsequent analyses (Brooks et al., 2024).

Magnetic resonance imaging

MRI data were acquired using a Siemens 3 T Skyra scanner and 32-channel head coil. T1-weighted structural images (magnetisation-prepared rapid gradient echo, TR = 2.3 s, TE = 2.07 ms, voxel size 1 mm³, flip angle 9°, 192 slices) and single voxel GABA-edited Mescher-Garwood Point Resolved (MEGA-PRESS) spectra were acquired at baseline prior to exercise. T1-weighted structural data were inspected to localise each participant's hand knob region of the central sulcus. MEGA-PRESS data were acquired from a 3 × 3 × 3 cm voxel positioned in SM cortex, centred over the hand knob in the right hemisphere (i.e. contralateral hemisphere to left hand used to complete the motor task). Two assessments of resting GABA concentration were acquired prior to exercise (TE = 68 ms, TR = 1.5 s, edit pulse bandwidth = 45 Hz, editing pulses at 1.9 ppm and 7.5 ppm, 105 on-off averages

at each acquisition). Repeat assessments of resting-state GABA were conducted to establish the stability of MRS measures prior to exercise, as per our previous work (Coxon et al., 2018). A reference spectrum, comprising 16 on-off averages with unsuppressed water, was also obtained using the same parameters.

After exercise a second T1-weighted image was acquired to inform accurate voxel placement relative to the baseline assessment (see Fig. 2, voxel overlap). SM GABA concentration was assessed again at rest (105 averages) and across both runs of the SRTT (105 averages each run, 210 averages total). Resting-state echo planar images (TR = 2.46 s, TE = 30 ms, voxel size 3 mm³, flip angle 90°, 44 slices, 7.34 min duration) with whole-brain coverage were acquired at the end of the protocol, as per Hendrikse et al. (2020, 2021). This was acquired at the end of the protocol to avoid any impact of gradient coil heating from the echo planar imaging sequence on magnetic field homogeneity and the stability of MRS estimates (Hui et al., 2021). During the resting-state scan participants were instructed to keep their eyes open and focus on a black fixation cross presented on a white background, while trying not to think of anything in particular.

Data analysis

Motor sequence learning. As per previous work (Bönstrup et al., 2019; Brooks et al., 2024) motor skill performance was conceptualised as number of correct responses per second. The learning trajectory was modelled at the group level ($N = 33$) separately for each run of the serial reaction time task, using an

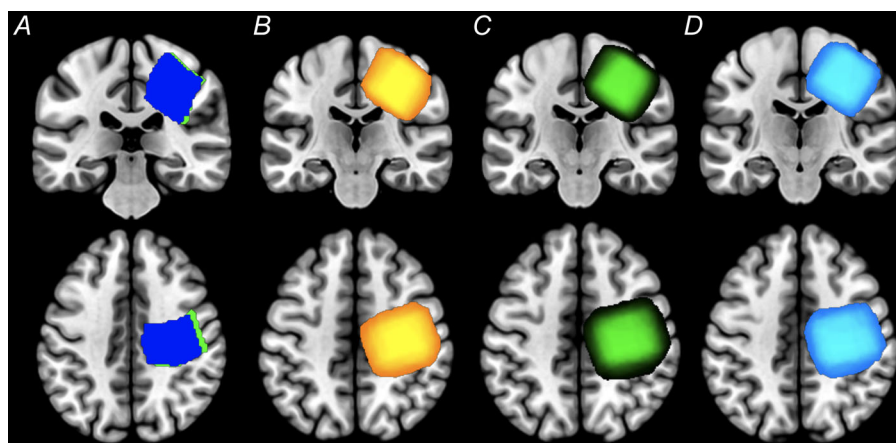


Figure 2. Positioning of magnetic resonance spectroscopy voxel centred over hand region of sensorimotor cortex

MEGA-PRESS data were acquired from a 3 × 3 × 3 cm voxel before and after exercise and during performance of a serial reaction time task. The overlap in voxel position pre-post exercise is shown for a representative participant (A), all participants ($N = 33$) (B), and separately for HIIT ($n = 16$) (C) and control ($n = 17$) (D) groups, where greater spatial overlap is represented by lighter colouration (B–D). The top row depicts the voxel position in the coronal plane, and the bottom row depicts the voxel position in the axial plane.

exponential function (Bönstrup et al., 2019; Brooks et al., 2024; Fig. 1C).

Motor learning was assessed at the micro-scale (i.e. micro-consolidation) according to our previous work (Brooks et al., 2024). In brief we investigated rapid improvements in performance during both active practice (i.e. micro-online gains) and the brief 10 s periods of rest (i.e. micro-offline gains). To assess micro-online and offline changes, analysis was restricted to an ‘early learning’ phase, where 95% of learning occurred (i.e. blocks 1–7 on the first run of the task, Fig. 1C) (Brooks et al., 2024). A sliding average window of four correct responses was used (Matlab movmean function, 2-item overlap), and the mean tapping speed (quantified as keypresses/s) across the first and last four correct key presses in each block represented performance at the start and end of each block, respectively. Modelling performance across four-item intervals was informed by the approach of Bönstrup et al., 2019, and was a natural derivative of the 12-item repeating sequence featured in our paradigm. Micro-offline changes were quantified as the summed change between blocks, i.e. the summed change in tapping speed between the start of block $n+1$ and the end of block n . Micro-online changes were the summed change within blocks, i.e. the summed change in tapping speed between start and end of block n . Early learning was calculated by summing micro-online and micro-offline values. We also assessed the total degree of learning across performance of both task runs. Total learning was defined as the change in tapping speed from the first four correct keypresses of block 1 to the last four correct keypresses of block 32 (i.e. end of second run).

Quantification of GABA±. Gannet (version 3.2.0) (Edden et al., 2014) was used to analyse MEGA-PRESS spectra. Estimates of GABA concentration (3.0 ppm) were calculated relative to the unsuppressed water signal from the voxel centred over right SM. Pre-processing of MRS data (GannetLoad module) included steps for phased-array channel combination of raw Siemens ‘twix’ files, exponential line broadening (3 Hz), Fourier transformation to yield time-resolved frequency-domain spectra, robust spectral registration for frequency and phase correction (Mikkelsen et al., 2020), outlier rejection (phase, frequency, area or creatine signal full width at half maximum (FWHM) > 3 SD from mean) and time averaging of the edited difference spectrum. The area of the edited GABA peak at 3 ppm was quantified using a single Gaussian peak model and a non-linear least-squares fitting approach (GannetFit module). To acknowledge a possible macromolecule contribution to the edited spectra GABA+ is referred to in place of GABA for all relevant analyses (Edden et al., 2014). As an internal reference GABA+ concentrations are expressed in institutional

units, relative to the unsuppressed water signal estimated with a Gaussian-Lorentzian model, according to the following equation (Edden et al., 2014):

where,

$$C_{corr} = \frac{I_M}{I_W} \cdot \frac{H_W}{H_M} \cdot \frac{MM}{\kappa} \cdot C_W \cdot W_{vis} \cdot \frac{\exp\left(-\frac{TE_W}{T_{2W}}\right) \left[1 - \exp\left(-\frac{TR_W}{T_{1W}}\right)\right]}{\exp\left(-\frac{TE_M}{T_{2M}}\right) \left[1 - \exp\left(-\frac{TR_M}{T_{1M}}\right)\right]}$$

C_{corr}	Estimated metabolite concentration in i.u.
I_M	Metabolite signal integral
I_W	Water signal integral
H_M	Number of ^1H protons that give rise to the metabolite signal
H_W	Number of ^1H protons that give rise to the water signal
MM	Correction factor for the contribution of the co-edited macromolecule signal in the metabolite signal
κ	Editing efficiency
C_W	Molal concentration of pure water
W_{vis}	Approximate relative MR visibility of water in brain tissue
TE_W	Echo time of the water reference acquisition
TR_W	Repetition time of the water reference acquisition
TE_M	Echo time of the metabolite acquisition
TR_M	Repetition time of the metabolite acquisition
T_{2W}	Average transverse relaxation time of water in GM and WM
T_{1W}	Average longitudinal relaxation time of water in GM and WM
T_{2M}	Transverse relaxation time of metabolite
T_{1M}	Longitudinal relaxation time of metabolite

Resting GABA+ was quantified at baseline prior to exercise and, again, following exercise (HIIT or control condition). GABA+ was also quantified during different phases of motor skill learning. To yield an estimate of GABA+ during the ‘early learning’ phase the mean of the spectra acquired during the first 52 MEGA-PRESS on–off acquisitions was calculated (i.e. the midpoint of the MRS sequence encompassing blocks 1–8 of the SRTT). To examine change in GABA+ concentration across the SRTT task early learning GABA+ was subtracted from late learning GABA+ (the last 52 MEGA-PRESS on–off acquisitions, corresponding to blocks 24–32 of the SRTT).

Partial volume correction was also performed. The MRS voxel was co-registered to each subject’s anatomical T1-weighted image using the GannetCoRegister module,

and partial volume segmentation of the T1-weighted anatomical image within the SM voxel was then conducted using GannetSegment, with segmentation of the structural image into constituent tissue types performed via the SPM12 algorithm. Partial volume correction of GABA+ concentration was then performed by removing cerebrospinal fluid fractions (GannetQuantify) to yield corrected GABA+/H₂O ratios. The full width at half maximum (FWHM) of GABA+ signals and GABA+ signal fit error (relative to the unsuppressed water peak, EGABA) were used to assess the quality of GABA+ model fit. Spectra with an EGABA value of > 20% or exceeding a z-score of 3.29 were labelled as outliers and removed from the analysis. FSLmaths was used to calculate the percentage of voxel overlap between baseline and post-exercise assessments (as depicted in Fig. 2).

Resting-state fMRI analysis

Data were pre-processed using fMRIPrep (version 1.1.1), with default parameters, including ICA-AROMA denoising and susceptibility-derived distortion estimation (AFNI 3dqwrap). This resulted in spatially realigned echo planar images in normalised space (normalised to the MNI152Nlin6Asym template). To examine associations between functional connectivity and motor performance we then conducted a dual-regression analysis in FSL (Jenkinson et al., 2012), according to the approach outlined in Stagg et al. (2014). Briefly each pre-processed resting-state fMRI scan was temporally concatenated across participants to yield a single four-dimensional (4-D) dataset. Data were then split into 25 components using independent component analysis (Stagg et al., 2014). Resting-state networks of interest were identified

via a spatial comparison with previously defined networks (Beckmann et al., 2005). The motor and default mode network were the primary networks of interest, given previous evidence implicating hippocampal and motor regions in motor sequence learning at both micro-scale (Buch et al., 2021; Jacobacci et al., 2020) and macro-scale (Dupont-Hadwen et al., 2019; Kolasinski et al., 2019). The salience network and visual network were also identified as control networks to assess anatomical specificity (see Fig. 3). The dual-regression analysis was then conducted to establish subject-specific functional networks. This analysis involves a two-stage regression sequence, in which a spatial regression was first performed against single-subject data using all 25 components, followed by a temporal regression of the resulting normalised time course to generate subject-specific maps. This map was taken as a subject-specific estimate of functional connectivity. Binarised masks were then generated for each resting-state network of interest, derived from the group average of pre-processed 4-D images (multiple comparison corrected, $P < 0.05$). The mean value was calculated for each masked network for each subject to yield estimates of average network connectivity.

Statistical approach

Statistical analysis was conducted using JASP (version 0.10.2). An alpha level of 0.05 was adopted for all inferential statistics conducted under the null hypothesis significance testing framework. To quantify the relative evidence for the alternative vs. the null hypothesis equivalent Bayesian statistics were also determined (the prior was set in support of the alternative over the null hypothesis, i.e. BF_{10}). For Bayesian tests, BF_{10} values

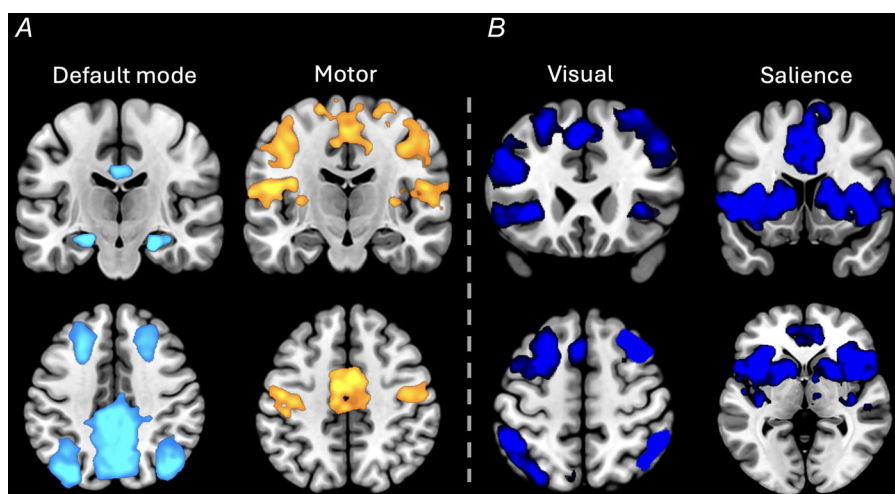


Figure 3. Visualisation of resting-state networks

Functional maps derived from all participants ($N = 33$) are shown for primary networks of interest (A), including default mode (light blue colouration) and motor networks (yellow-orange colouration) and control networks (B), including visual and salience networks (dark blue colouration).

between 3 and 10 were considered moderate evidence against the null, and $BF_{10} > 10$ was considered strong evidence against the null. A $BF_{10} < 0.33$ was classified as moderate evidence for the null hypothesis. For Bayesian t tests the Cauchy parameter was set to a conservative default value of 0.707 (Ly et al., 2016; Rouder et al., 2009).

Comparisons between HIIT and control conditions

To assess the effect of HIIT on measures of sequence learning and MRI measures (i.e. GABA+ and functional connectivity) independent-samples t tests were conducted between HIIT and control groups. In the case of violations of normality the non-parametric equivalent (i.e. Mann-Whitney U test) was used. In our previous work we showed that a single dose of HIIT did not significantly impact consolidation at the micro-scale (Brooks et al., 2024). Therefore in this study we restrict our group comparisons to imaging measures and total learning outcomes. To account for differences in baseline GABA+ concentration difference scores were calculated by subtracting baseline GABA+ estimates from post timepoint (i.e. post exercise – baseline).

Relationship between motor plasticity and learning

Based on past evidence linking hippocampal dynamics to micro-offline gains in explicit learning (Buch et al., 2021; Jacobacci et al., 2020), bivariate correlations were conducted to investigate associations between resting-state functional connectivity and micro-offline consolidation of an implicit motor sequence (data pooled across exercise groups, bonferroni-adjusted for the number of included networks, α -level = 0.013). Multiple linear regression models were conducted to examine how micro-offline consolidation and total learning related to functional connectivity and change in GABA+ concentration measured across motor practice. Separate models were conducted on learning measures at the micro- and overall (macro-) scale.

Results

Exercise was well tolerated by participants, and no adverse events were reported following HIIT. There were no significant differences between HIIT and control groups in terms of age ($P = 0.68$), biological sex ($P = 0.75$), pre-existing physical activity ($P = 0.30$) or sleep quality ($P = 0.21$) (see Table 1).

Baseline assessment of sensorimotor GABA+ concentration

Two assessments of GABA+ were acquired prior to exercise to verify stability and reliability of the measure.

Table 1. Comparison of participant characteristics between high-intensity cardiorespiratory exercise (HIIT) and control groups (mean $\pm$ SD)

	HIIT	Control	<i>P</i> -value
<i>N</i> (% female)	19 (36.84%)	19 (42.11%)	0.75
Age (years)	22.37 $\pm$ 2.65	22.74 $\pm$ 2.79	0.68
BMI (kg/m ²)	22.06 $\pm$ 2.21	23.3 $\pm$ 4.14	0.26
IPAQ (MET)	4800 $\pm$ 3220	3783 $\pm$ 2739	0.30
PRETIE-Q T	26 $\pm$ 5.08	23.95 $\pm$ 4.92	0.21
PRETIE-Q P	26.32 $\pm$ 5.09	23.37 $\pm$ 4.22	0.06
PSQI	5.05 $\pm$ 2.53	4.16 $\pm$ 1.77	0.21
RHR (bpm)	64.26 $\pm$ 9.75	65.37 $\pm$ 10.07	0.92
Exercise HR (bpm)	180.68 $\pm$ 7.10	89.95 $\pm$ 10.96	3.271 $\times 10^{-27}$
%HRR	93.9 $\pm$ 3.3	49.6 $\pm$ 13.3	1.094 $\times 10^{-31}$

Note: For exercise heart rate the peak of the last minute of exercise is reported for HIIT, and the 20 min average is reported for the low-intensity control group.

Abbreviations: BMI, body mass index; bpm, beats per minute; %HRR, percentage of heart rate reserve achieved during exercise, where HRR = age-predicted maximum heart rate – resting heart rate; IPAQ, International Physical Activity Questionnaire; METS, metabolic equivalent minutes per week, with higher values indicate greater physical activity levels; PSQI, Pittsburgh Sleep Quality Index global score, with higher values indicating poorer sleep quality; PRETIE-Q, Preference for and Tolerance of the Intensity of Exercise Questionnaire, higher exercise intensity tolerance (PRETIE-Q T) and preference (PRETIE-Q P) are indicated by higher values; RHR, resting heart rate (seated).

No significant differences were observed between GABA+ baseline assessments when compared across the entire sample [$t(1,32) = 0.66$, $P = 0.52$, Cohen's $d = 0.11$, $BF_{10} = 0.23$] nor within the HIIT [$t(1,15) = 1.50$, $P = 0.15$, Cohen's $d = 0.38$, $BF_{10} = 0.65$] or active control [$t(1,16) = -0.53$, $P = 0.61$, Cohen's $d = 0.13$, $BF_{10} = 0.28$] groups separately. We also observed a positive correlation between GABA+ baseline assessments across the sample ($r = 0.39$, $P = 0.015$, $BF_{10} = 3.58$). Therefore the average of the two baseline GABA+ assessments was calculated and used for subsequent analysis (mean FWHM (M_{FWHM}) = 20.40 $\pm$ 0.86). No significant differences in baseline SM GABA+ concentration were observed between HIIT and control groups [$t(1,31) = -0.96$, $P = 0.35$, Cohen's $d = 0.33$, $BF_{10} = 0.47$], indicating that groups were appropriately matched. There were also no differences in the unsuppressed water signal ($M_{FWHM} = 8.69 \pm 0.52$), which was used as a reference for GABA+ quantification, nor in the quality of the GABA+ model fit (EGABA). There were no differences when comparing the groups at baseline [water: $t(1,31) = -0.47$, $P = 0.65$, Cohen's $d = 0.16$, $BF_{10} = 0.36$; EGABA: $t(1,31) = 0.33$, $P = 0.74$, Cohen's $d = 0.12$, $BF_{10} = 0.35$] or following exercise [water: $t(1,31) = -0.78$, $P = 0.44$,

Cohen's $d = 0.27$, $BF_{10} = 0.42$; EGABA: $t(1,31) = -1.05$, $P = 0.30$, Cohen's $d = 0.37$, $BF_{10} = 0.51$].

Effect of HIIT on GABA $\pm$ and learning

To examine changes in GABA $\pm$ following exercise baseline concentration was subtracted from post-assessment (Δ GABA $\pm$ = GABA-post - GABA-pre). In line with our previous findings (Coxon et al., 2018) we observed modulation of resting SM GABA $\pm$ concentration following a bout of HIIT. A greater increase in GABA $\pm$ concentration was observed following HIIT exercise, relative to the control group [HIIT $M_{diff.} = 0.12 \pm 0.57$; control $M_{diff.} = -0.30 \pm 0.51$, $t(1,31) = 2.23$, $P = 0.033$, Cohen's $d = 0.78$], with anecdotal evidence in favour of the alternative hypothesis ($BF_{10} = 2.09$). An increase was also observed when GABA $\pm$ concentration was quantified relative to the creatine signal [$t(1,31) = 1.79$, $P = 0.084$, Cohen's $d = 0.62$, $BF_{10} = 1.10$], albeit with a comparatively smaller effect size. We also examined whether exercise-related changes in neurotransmission were specific to GABA $\pm$. As per our previous work (Coxon et al., 2018) we observe no change in the combined glutamate-glutamine-glutathione (Glx) concentration in SM cortex following exercise [HIIT $M_{diff.} = -0.31 \pm 1.71$; control $M_{diff.} = -0.27 \pm 1.29$; $t(1,31) = -0.09$, $P = 0.93$, Cohen's $d = 0.03$, $BF_{10} = 0.33$]. Despite the exercise-related change in GABA $\pm$, the groups did not differ behaviourally in terms of their learning on the SRTT. Specifically there were no differences between HIIT and control groups for early learning [task run 1: $t(1,31) = 0.18$, $P = 0.86$, Cohen's $d = 0.06$, $BF_{10} = 0.34$] or total learning [across runs: $t(1,31) = 0.29$, $P = 0.77$, Cohen's $d = 0.10$, $BF_{10} = 0.34$] (Fig. 4). Similarly there were no differences observed in GABA $\pm$ concentration between groups during motor task performance (all timepoints $P > 0.05$). Overall we provide evidence that

HIIT modulates resting SM GABA $\pm$ but not implicit motor sequence learning at the micro-scale (Brooks et al., 2024), or when total learning across this task is considered.

Micro-offline consolidation and default mode network connectivity

Previous evidence has linked the micro-consolidation of an explicit motor sequence to the activity of networks involving the hippocampus (Buch et al., 2021; Jacobacci et al., 2020). In our recent work we demonstrate that micro-consolidation generalises to implicit motor sequence learning (Brooks et al., 2024). Here we were interested in whether micro-consolidation of an implicit motor sequence was also associated with default mode network connectivity, in which the hippocampus is a primary node. We observed a positive association between micro-offline gains and resting-state connectivity in the default mode network, with moderate evidence in favour of the alternative hypothesis ($r = 0.44$, $p_{BonF} = 0.044$, $BF_{10} = 4.73$; data pooled across groups). This relationship was specific to the default mode network; no other resting-state networks showed significant associations with micro-offline gains (motor: $r = 0.35$, $p_{BonF} = 0.24$, $BF_{10} = 1.29$, salience: $r = 0.24$, $p_{BonF} = 0.68$, $BF_{10} = 0.53$; visual: $r = 0.22$, $p_{BonF} = 0.88$, $BF_{10} = 0.45$) (Fig. 5). In summary default mode network activity is associated with the rapid micro-consolidation of implicit motor sequences occurring during brief periods of rest.

Motor sequence learning, GABA concentration and functional connectivity

GABA regulates the plasticity underlying motor learning (Stagg et al., 2011a), with change in SM GABA $\pm$

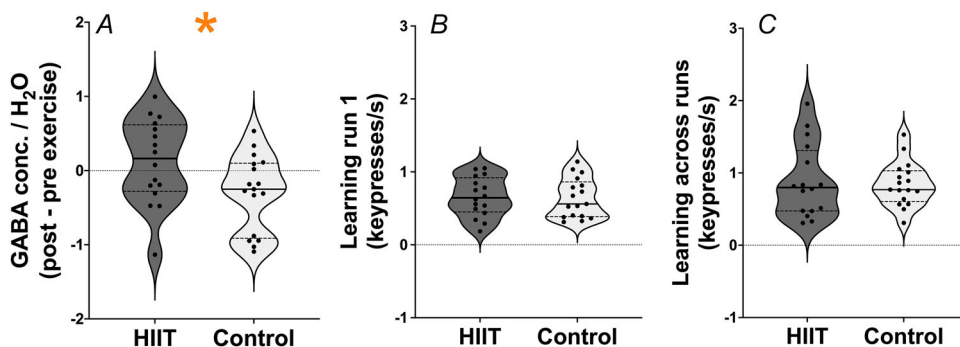


Figure 4. Comparison of sensorimotor (SM) GABA $\pm$ concentration and motor learning between high-intensity interval training (HIIT) and control groups

A, comparison of GABA $\pm$ concentration between groups. B, comparison of learning across the first practice run of the serial reaction time task (SRTT) between groups. C, comparison of learning across both runs of the SRTT between groups. For all plots filled circles indicate individual participants. For each exercise group median values are indicated by the horizontal bolded line, and higher and lower quartiles are indicated by dashed lines. * $P < 0.05$.

concentration associated with motor sequence learning magnitude (Kolasinski et al., 2019). Here we extend on these findings and examine how resting-state network connectivity and GABA+ are involved across early and later stages of implicit sequence learning, and whether a single dose of HIIT exercise influences these relationships.

We first assessed the relationship between early learning, GABA+ and hippocampal network connectivity. GABA+ measured during early learning and resting-state default mode connectivity explained a significant proportion of the variance in early sequence learning [model summary: $R^2 = 0.27$, adjusted $R^2 = 0.19$, $F(3,32) = 3.51$, $P = 0.028$, $BF_{10} = 3.39$]. We observed a negative correlation between early learning and GABA+ [$B = -0.14$ ($-0.26, -0.03$ 95% confidence interval (CI), $P = 0.015$, $BF_{10} = 1.95$, 13% unique variance explained)] and a positive correlation between early learning and default mode connectivity [$B = 0.709$ (0.12, 1.51 95% CI), $P = 0.021$, $BF_{10} = 1.87$, 7% unique variance explained]. Notably, these associations were specific to GABA+ measured during early learning and default mode connectivity; no other resting-state connectivity measure nor estimates of GABA+ across other timepoints explained a significant proportion of variance in early motor sequence learning (all $P > 0.05$). Exercise condition (HIIT vs. control) was not a significant predictor in the model [$B = -0.09$ ($-0.21, 0.06$ 95% CI), $P = 0.248$, 3% unique variance explained]. Overall we provide evidence that default mode network connectivity and GABA+ measured during learning support early motor skill consolidation.

Next we investigated the neurophysiological correlates of sequence learning across the entire practice period, i.e. total learning across task blocks 1–32. Two motor connectivity estimates were identified as outliers (z -score > 2.7) and removed from the analysis. Previous work has linked sequence learning across similar timespans to that seen here with motor network plasticity (Pascual-Leone et al., 1994; Tzvi et al., 2014). Indeed a

regression model with motor network connectivity and Δ GABA+ explained a significant proportion of the variance in total sequence learning [model summary: $R^2 = 0.27$, adjusted $R^2 = 0.19$, $F(3,28) = 3.13$, $P = 0.044$, $BF_{10} = 3.84$]. We observed a positive correlation between total learning and motor network connectivity [$B = 1.59$ (0.63, 3.07 95% CI), $P = 0.002$, $BF_{10} = 7.04$, 18% unique variance explained]. This relationship was specific to motor connectivity; no other resting-state network explained a significant proportion of variance in total learning when substituted or added as an additional predictor in the model (all $P > 0.05$). Non-significant associations were observed between total learning and Δ GABA+ [$B = 0.11$ ($-0.10, 0.37$ 95% CI), $P = 0.29$, $BF_{10} = 0.84$, 3% unique variance explained], and total learning and exercise condition [$B = 0.02$ ($-0.43, 0.28$ 95% CI), $P = 0.92$, 0% unique variance explained]. Overall we provide evidence implicating GABA and both motor and default mode network connectivity in the consolidation of an implicit motor sequence.

Discussion

In this study we investigated the neural mechanisms supporting implicit motor sequence learning and the influence of high-intensity cardiorespiratory exercise (HIIT) priming. Previous evidence has established a central role of SM GABA in motor learning (Kolasinski et al., 2019; Stagg et al., 2011a) and involvement of hippocampal and motor networks in *explicit* motor sequence learning at both the micro-scale (Buch et al., 2021; Jacobacci et al., 2020) and following more sustained practice (Stagg et al., 2011a). Here we report evidence that these systems support the learning of an *implicit* motor sequence. We show that micro-offline gains in implicit sequence learning are linked to default mode connectivity. Further we demonstrate that the early stages of implicit sequence learning are linked to default mode connectivity and SM GABA+ concentration, and that total learning

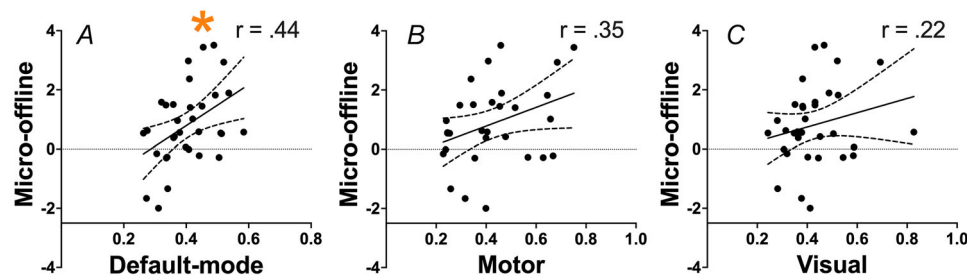


Figure 5. Associations between micro-offline consolidation and resting-state functional connectivity
A, significant positive correlation between micro-offline gains and default mode network connectivity. No other significant correlations were observed between micro-offline consolidation and other resting-state networks: (B) the motor network and (C) the visual network. Circles represent individual values; continuous line shows regression line of best fit; dashed line depicts 95% confidence interval. Micro-offline gains in implicit sequence learning are represented on the y-axis, and averaged functional network connectivity is represented on the x-axis. * $P < 0.05$.

following more sustained practice is associated with the functional connectivity of the motor network. Our results indicate that both default mode and motor networks underlie initial *implicit* motor learning. Investigation of the priming effects of HIIT exercise replicated our previous study (Coxon et al., 2018), showing that SM GABA+ is modulated by HIIT. However in contrast to our hypothesis the priming effect of HIIT on SM GABA+ did not influence the magnitude of sequence learning.

The effect of high-intensity interval training on MRI measures of motor plasticity

A growing body of evidence indicates that cardiorespiratory exercise impacts GABAergic neurotransmission (Hendrikse et al., 2017). For instance increased GABA+ concentration has been reported in visual cortex following 8–17 min of moderate-/high-intensity exercise (Maddock et al., 2016). Further our previous work established that there is a consistent elevation in SM GABA+ concentration following a single session of HIIT (Coxon et al., 2018). Several studies have demonstrated modulation of GABA-mediated synaptic inhibition in motor cortex with transcranial magnetic stimulation measures following both moderate (Singh et al., 2014) and high-intensity exercises (Cadwallader et al., 2024; Curtin et al., 2023; Hendy et al., 2022). In the present study we replicate our previous spectroscopy study (Coxon et al., 2018), demonstrating increased resting SM GABA+ concentration following a 20 min dose of HIIT relative to a control condition. We show this in a larger sample than previously reported, establishing the effect is robust.

Modulation of GABA+ has been associated with the HIIT exercise-related increases in blood lactate concentration (Coxon et al., 2018). Systemic concentrations of lactate increase following high-intensity exercise (Brooks et al., 2005), and lactate is implicated in signalling pathways regulating the *de novo* synthesis of GABA in the brain (Maddock et al., 2016). The increase in GABA+ may also relate to circulation of brain-derived neurotrophic factor, which is increased in response to high-intensity exercise (Saucedo Marquez et al., 2015), and involved in regulation of GABAergic neurotransmission (Cash et al., 2017). MRS provides an estimate of the overall tissue concentration of GABA, which on a functional level likely reflects extrasynaptic GABAergic tone (Stagg et al., 2011b). Therefore collectively these findings highlight the effect of high-intensity cardiovascular exercise on tonic GABA activity within SM cortex. Future work is required to delineate the specific physiological mechanisms mediating these effects.

In contrast to our hypotheses modulation of SM GABA+ by HIIT-exercise priming did not influence

implicit sequence learning. We observed no differences between HIIT and control conditions for the earliest micro-phase of learning ('fast' learning) nor when comparing the total magnitude of learning across more extended practice (total task duration ~11 min). Similarly no associations were observed between learning and exercise in the regression analyses. This result extends upon our recent work (Brooks et al., 2024) but conflicts with some previous work reporting facilitation of implicit sequence learning following exercise (Roig et al., 2013). For example improved encoding (Mang et al., 2014) and retention (Mang et al., 2016) of implicit motor sequences have been demonstrated following a single dose of HIIT exercise. The reason for these inconsistent outcomes is unclear, though this may relate to differences in task demands across studies. In the current study we utilised a serial reaction time task as a measure of implicit sequence learning. We observed a rapid improvement in skill, with 95% of learning occurring by block 7 at the group level (i.e. following 140 s of practice). In comparison studies reporting an effect of HIIT priming on implicit learning have utilised more complex tasks with comparatively protracted learning trajectories (Mang et al., 2016). Plausibly the additional benefit of HIIT priming on sequence learning may be more likely to manifest in the context of more challenging sequence learning paradigms, though future work is required to assess the validity of this interpretation.

An alternative explanation for these inconsistent outcomes across studies relates to the temporal coupling between exercise and learning. Previous studies reporting exercise-related facilitation of implicit learning have commenced motor skill practice immediately following exercise (Mang et al., 2014, 2016). In the current study owing to the timing of our MRI protocol in which a T1-weighted structural image and resting estimates of GABA+ concentration were acquired prior to the task, motor practice was commenced ~20–25 min after exercise cessation. Indeed the temporal proximity of exercise to learning has been cited as a factor that may influence subsequent outcomes (Roig et al., 2016). In this sense a closer temporal coupling of HIIT to motor learning may have maximised exercise-related benefits. However on the contrary both fatigue and high levels of physiological arousal (e.g. as commonly experienced immediately following HIIT) are also known to impact memory performance (Park, 2005) and may otherwise mask the cognitive benefits of exercise. For example a single bout of moderate-high-intensity exercise performed 4 h after encoding improved retention on declarative memory tasks, whereas exercise immediately after encoding did not (van Dongen et al., 2016). This result indicates that exercise-related effects on learning may vary across time depending on physiological states, phase of memory formation and the brain regions

involved. The intensity and duration of exercise may also influence subsequent effects on learning. Collectively the findings suggest a complex relationship between exercise and memory formation, likely influenced by task demands and the time window under which they are assessed.

Default mode and motor systems support implicit sequence learning

In line with our expectations we provide the first evidence that micro-offline consolidation of an implicit motor skill is linked to the activity of brain networks involving the hippocampus. We observed a significant positive correlation between default mode functional connectivity strength and individual micro-offline skill gain. This relationship was specific to the default mode network; no other relationships between offline skill gain and functional connectivity were observed across motor, salience or visual networks. This finding extends on previous work which has linked micro-offline consolidation of explicit motor sequence learning to hippocampal dynamics, including neural replay bursts across a cortical-hippocampal network (Buch et al., 2021), and changes in both blood-oxygen level-dependent activity and microstructure within hippocampus and precuneus (Jacobacci et al., 2020). The default mode network acts as a primary hub for neural replay (Higgins et al., 2021) and may mediate the encoding of novel experience within the hippocampus to longer-term consolidation across a distributed cortical representation (Kaefer et al., 2022). Our finding adds to a growing body of literature linking short-term consolidation of procedural motor memories during wakefulness to the co-ordinated activity of brain networks involving the hippocampus – a region classically associated with declarative learning taxonomies (Henke, 2010). Our finding is correlational, and further work is required to establish causal links between default mode network dynamics and the micro-consolidation phenomenon.

Converging lines of evidence indicate that explicit motor learning is underscored by interactions across both SM and hippocampal systems (Deleglise et al., 2023; Della-Maggiore, 2024; Döhring et al., 2017; Jacobacci et al., 2020). Explicit sequence learning has also been linked to dynamic changes between networks, with a decrease in SM GABA concentration during learning (Floyer-Lea et al., 2006; Kolasinski et al., 2019) and a shift towards decreased hippocampal connectivity and increased SM connectivity in the time window following practice (Deleglise et al., 2023). In line with these findings we demonstrate that greater ‘fast’ learning during initial learning of an implicit motor sequence is associated with both increased default mode network connectivity and decreased SM GABA+ concentration. In line with our

analysis of micro-offline periods this finding indicates that explicit sequence awareness may not be a necessary condition for involvement of hippocampal networks across early learning, aligning with models which propose that the hippocampus performs a more general ‘event sequence generating’ role in memory formation (Buzsáki & Tingley, 2018).

When examining learning outcomes across the entire practice period (i.e. 32 task blocks), we observed that the total degree of learning was linked with greater motor network connectivity. These findings add to a growing body of literature demonstrating dual involvement of hippocampal and motor networks in the initial ‘fast’ phase of motor learning (Buch et al., 2021; Deleglise et al., 2023; Jacobacci et al., 2020) that has traditionally been linked to interactions between cortico-striatal and cortico-cerebellar systems (Dayan & Cohen, 2011). Using a combination of MRS and fMRI we also provide evidence linking tonic GABA inhibition to motor network functional connectivity strength. Overall our findings indicate that implicit sequence learning is related to local inhibitory and large-scale network connectivity strength. Considered in conjunction with our finding of default mode network involvement in micro-scale consolidation during ‘fast/early’ (but not total) learning our results may also suggest differential involvement of brain networks at distinct stages of learning. Future work is required to establish how these mechanisms may generalise to acquisition of more complex skills and/or are implicated in consolidation and stabilisation processes across longer timeframes.

Limitations and future directions

In this study we demonstrate that both default mode and motor network functional connectivity strength are associated with the development of implicit motor skills. We show that the early phases of learning relate to local SM inhibition, which may be modulated via engagement in high-intensity interval exercise. However there are certain methodological limitations which should be considered when interpreting these findings. First we link network connectivity and SM GABA+ concentration to learning outcomes but did not assess the regional specificity of GABA+ in this study. Our spectroscopy analysis focused on SM GABA+ concentration based on our previous work, which reported a regionally specific change in resting-state GABA+ concentration in this region following HIIT exercise (Coxon et al., 2018). We also acknowledge that our measure of GABA+ is not specific to GABA and may contain a macromolecule contribution, as is the case for all studies utilising MEGA-PRESS acquired on a 3 T scanner (Puts & Edden, 2012). Second the evidence provided is correlational.

Future work using dynamic causal modelling approaches or perturbation techniques (e.g. non-invasive brain stimulation methods) could be informative in establishing the causal contribution across temporal phases of implicit sequence learning and interrogating the anatomical specificity of our findings. Third we acquired resting-state fMRI at the end of the experimental protocol. We acknowledge that measuring resting-state connectivity at baseline would have eliminated any possible influence of the exercise protocol. However to ensure that we obtained valid MRS estimates it was necessary to avoid any impact of gradient coil heating from the resting-state fMRI echo planar imaging sequence (Hui et al., 2021). We also did not observe any significant differences in resting-state connectivity between exercise groups, increasing our confidence that our results have not been inadvertently influenced by this aspect of the experimental design. Finally we focused our analysis on the effect of exercise on motor learning across practice. However our work and that of others suggest that exercise may have greater impact on the *retention* of motor skills relative to acquisition (Roig et al., 2012; Stavrinou & Coxon, 2017; Taylor et al., 2024). Future work may consider the addition of retention tests across longer timeframes to establish time-dependent effects of HIIT priming on implicit learning.

Conclusions

Overall through the analysis of multiple neuroimaging modalities we provide novel insights into the network and neurochemical mechanisms supporting implicit motor skill learning. High-intensity interval exercise is an effective method of modulating tonic concentrations of SM GABA but may not lead to consistent facilitation of implicit sequence learning. Future research is required to elucidate causal relationships between network nodes and implicit learning across temporal scales.

References

- Albert, N. B., Robertson, E. M., & Miall, R. C. (2009). The resting human brain and motor learning. *Current Biology: CB*, **19**(12), 1023–1027.
- Bachtiar, V., & Stagg, C. J. (2014). The role of inhibition in human motor cortical plasticity. *Neuroscience*, **278**, 93–104.
- Beckmann, C. F., DeLuca, M., Devlin, J. T., & Smith, S. M. (2005). Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**(1457), 1001–1013.
- Bönstrup, M., Iturrate, I., Hebart, M. N., Censor, N., & Cohen, L. G. (2020). Mechanisms of offline motor learning at a microscale of seconds in large-scale crowdsourced data. *Nature Partner Journals Science of Learning*, **5**(1), 1–10.
- Bönstrup, M., Iturrate, I., Thompson, R., Cruciani, G., Censor, N., & Cohen, L. G. (2019). A rapid form of offline consolidation in skill learning. *Current Biology: CB*, **29**(8), 1346–1351.e4.
- Brooks, E., Wallis, S., Hendrikse, J., & Coxon, J. (2024). Micro-consolidation occurs when learning an implicit motor sequence, but is not influenced by HIIT exercise. *Nature Partner Journals Science of Learning*, **9**(1), 23.
- Brooks, G. A., Fahey, T. D., & Baldwin, K. M. (2005). *Exercise physiology: Human bioenergetics and its applications*.
- Buch, E. R., Claudino, L., Quentin, R., Bönstrup, M., & Cohen, L. G. (2021). Consolidation of human skill linked to waking hippocampo-neocortical replay. *Cell Reports*, **35**(10), 109193.
- Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research*, **28**(2), 193–213.
- Buzsáki, G., & Tingley, D. (2018). Space and time: The Hippocampus as a sequence generator. *Trends in Cognitive Sciences*, **22**(10), 853–869.
- Cadwallader, C. J., Curtin, D., Taylor, E. M., de Moel, T., Jarvis, H., Hutchison, C., Hendrikse, J., Chong, T. T.-J., & Coxon, J. P. (2024). Exercise-induced cortical disinhibition mediates the relationship between fitness and memory in older adults. *The Journal of Physiology*, **602**(12), 2945–2959.
- Cash, R., Udupa, K., Gunraj, C., Mazzella, F., Daskalakis, Z. J., Wong, A. H., Kennedy, J. L., Fitzgerald, P. B., & Chen, R. (2017). Influence of the BDNF Val66Met polymorphism on the balance of excitatory and inhibitory neurotransmission and relationship to plasticity in human cortex. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, **10**(2), 502.
- Coxon, J. P., Cash, R. F. H., Hendrikse, J. J., Rogasch, N. C., Stavrinou, E., Suo, C., & Yücel, M. (2018). GABA concentration in sensorimotor cortex following high-intensity exercise and relationship to lactate levels. *The Journal of Physiology*, **596**(4), 691–702.
- Coxon, J. P., Peat, N. M., & Byblow, W. D. (2014). Primary motor cortex disinhibition during motor skill learning. *Journal of Neurophysiology*, **112**(1), 156–164.
- Curtin, D., Cadwallader, C. J., Taylor, E. M., Andrews, S. C., Stout, J. C., Hendrikse, J. J., Chong, T. T.-J., & Coxon, J. P. (2023). Ageing attenuates exercise-enhanced motor cortical plasticity. *The Journal of Physiology*, **601**(24), 5733–5750.
- Dayan, E., & Cohen, L. G. (2011). Neuroplasticity subserving motor skill learning. *Neuron*, **72**(3), 443–454.
- Deleglise, A., Donnelly-Kehoe, P. A., Yeffal, A., Jacobacci, F., Jovicich, J., Amaro Jr, E., Armony, J. L., Doyon, J., & Della-Maggiore, V. (2023). Human motor sequence learning drives transient changes in network topology and hippocampal connectivity early during memory consolidation. *Cerebral Cortex*, **33**(10), 6120–6131.
- Della-Maggiore, V. (2024). The human hippocampus beyond episodic memory. *Nature Reviews Neuroscience*, **25**(4), 211–211.

- Döhring, J., Stoldt, A., Witt, K., Schönfeld, R., Deuschl, G., Born, J., & Bartsch, T. (2017). Motor skill learning and offline-changes in TGA patients with acute hippocampal CA1 lesions. *Cortex; A Journal Devoted to the Study of the Nervous System and Behavior*, **89**, 156–168.
- Dupont-Hadwen, J., Bestmann, S., & Stagg, C. J. (2019). Motor training modulates intracortical inhibitory dynamics in motor cortex during movement preparation. *Brain Stimulation*, **12**(2), 300–308.
- Edden, R. A. E., Puts, N. A. J., Harris, A. D., Barker, P. B., & Evans, C. J. (2014). Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *Journal of Magnetic Resonance Imaging: JMIR*, **40**(6), 1445–1452.
- Ekkekakis, P., Hall, E. E., & Petruzzello, S. J. (2005). Some like it vigorous: Measuring individual differences in the preference for and tolerance of exercise intensity. *Journal of Sport and Exercise Psychology*, **27**(3), 350–374.
- Floyer-Lea, A., Wylezinska, M., Kincses, T., & Matthews, P. M. (2006). Rapid modulation of GABA concentration in Human sensorimotor cortex during motor learning. *Journal of Neurophysiology*, **95**(3), 1639–1644.
- Hendrikse, J., Chye, Y., Thompson, S., Rogasch, N. C., Suo, C., Coxon, J. P., & Yücel, M. (2022). Regular aerobic exercise is positively associated with hippocampal structure and function in young and middle-aged adults. *Hippocampus*, **32**(3), 137–152.
- Hendrikse, J., Coxon, J. P., Thompson, S., Suo, C., Fornito, A., Yücel, M., & Rogasch, N. C. (2020). Multi-day rTMS exerts site-specific effects on functional connectivity but does not influence associative memory performance. *Cortex; A Journal Devoted to the Study of the Nervous System and Behavior*, **132**, 423–440.
- Hendrikse, J., Kandola, A., Coxon, J., Rogasch, N., & Yücel, M. (2017). Combining aerobic exercise and repetitive transcranial magnetic stimulation to improve brain function in health and disease. *Neuroscience & Biobehavioral Reviews*, **83**, 11–20.
- Hendrikse, J., Thompson, S., Suo, C., Yücel, M., Rogasch, N. C., & Coxon, J. P. (2021). The effects of multi-day rTMS and cardiorespiratory fitness on working memory and local GABA concentration. *Neuroimage: Reports*, **1**(4), 100049.
- Hendy, A. M., Andrushko, J. W., Della Gatta, P. A., & Teo, W.-P. (2022). Acute effects of high-intensity aerobic exercise on motor cortical excitability and inhibition in sedentary adults. *Frontiers in Psychology*, **13**, 814633.
- Henke, K. (2010). A model for memory systems based on processing modes rather than consciousness. *Nature Reviews Neuroscience*, **11**(7), 523–532.
- Higgins, C., Liu, Y., Vidaurre, D., Kurth-Nelson, Z., Dolan, R., Behrens, T., & Woolrich, M. (2021). Replay bursts in humans coincide with activation of the default mode and parietal alpha networks. *Neuron*, **109**(5), 882–893.e7.
- Hui, S. C. N., Mikkelsen, M., Zöllner, H. J., Ahluwalia, V., Alcauter, S., Baltusis, L., Barany, D. A., Barlow, L. R., Becker, R., Berman, J. I., Berrington, A., Bhattacharyya, P. K., Blicher, J. U., Bogner, W., Brown, M. S., Calhoun, V. D., Castillo, R., Cecil, K. M., Choi, Y. B., ... Edden, R. A. E. (2021). Frequency drift in MR spectroscopy at 3T. *Neuroimage*, **241**, 118430.
- Jacobacci, F., Armony, J. L., Yeffal, A., Lerner, G., Amaro, E., Jovicich, J., Doyon, J., & Della-Maggiore, V. (2020). Rapid hippocampal plasticity supports motor sequence learning. *Proceedings of the National Academy of Sciences*, **117**(38), 23898–23903.
- Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *Neuroimage*, **62**(2), 782–790.
- Kaefer, K., Stella, F., McNaughton, B. L., & Battaglia, F. P. (2022). Replay, the default mode network and the cascaded memory systems model. *Nature Reviews Neuroscience*, **23**(10), 628–640.
- King, B. R., Gann, M. A., Mantini, D., Doyon, J., & Albouy, G. (2022). Persistence of hippocampal and striatal multivoxel patterns during awake rest after motor sequence learning. *Isience*, **25**(12), 105498.
- Kolasinski, J., Hinson, E. L., Divanbeighi Zand, A. P., Rizov, A., Emir, U. E., & Stagg, C. J. (2019). The dynamics of cortical GABA in human motor learning. *The Journal of Physiology*, **597**(1), 271–282.
- Ly, A., Verhagen, J., & Wagenmakers, E.-J. (2016). Harold Jeffreys's default Bayes factor hypothesis tests: Explanation, extension, and application in psychology. *Journal of Mathematical Psychology*, **72**, 19–32.
- Maddock, R. J., Casazza, G. A., Fernandez, D. H., & Maddock, M. I. (2016). Acute modulation of cortical glutamate and GABA content by physical activity. *Journal of Neuroscience*, **36**(8), 2449–2457.
- Mang, C. S., Snow, N. J., Campbell, K. L., Ross, C. J. D., & Boyd, L. A. (2014). A single bout of high-intensity aerobic exercise facilitates response to paired associative stimulation and promotes sequence-specific implicit motor learning. *Journal of Applied Physiology (Bethesda, Md: 1985)*, **117**(11), 1325–1336.
- Mang, C. S., Snow, N. J., Wadden, K. P., Campbell, K. L., & Boyd, L. A. (2016). High-intensity aerobic exercise enhances motor memory retrieval. *Medicine & Science in Sports & Exercise*, **48**(12), 2477–2486.
- Mikkelsen, M., Tapper, S., Near, J., Mostofsky, S. H., Puts, N. A. J., & Edden, R. A. E. (2020). Correcting frequency and phase offsets in MRS data using robust spectral registration. *Nuclear Magnetic Resonance in Biomedicine*, **33**(10), e4368.
- Neva, J. L., Brown, K. E., Mang, C. S., Francisco, B. A., & Boyd, L. A. (2017). An acute bout of exercise modulates both intracortical and interhemispheric excitability. *European Journal of Neuroscience*, **45**(10), 1343–1355.
- Neva, J. L., Brown, K. E., Peters, S., Feldman, S. J., Mahendran, N., Boisgontier, M. P., & Boyd, L. A. (2021). Acute exercise modulates the excitability of specific interneurons in Human motor cortex. *Neuroscience*, **475**, 103–116.
- Park, J. (2005). Effect of arousal and retention delay on memory: A meta-analysis. *Psychological Reports*, **97**(2), 339–355.
- Pascual-Leone, A., Grafman, J., & Hallett, M. (1994). Modulation of cortical motor output maps during development of implicit and explicit knowledge. *Science*, **263**(5151), 1287–1289.

- Puts, N. A. J., & Edden, R. A. E. (2012). In vivo magnetic resonance spectroscopy of GABA: A methodological review. *Progress in Nuclear Magnetic Resonance Spectroscopy*, **60**, 29–41.
- Robertson, E. M., Pascual-Leone, A., & Miall, R. C. (2004). Current concepts in procedural consolidation. *Nature Reviews Neuroscience*, **5**(7), 576–582.
- Robertson, E. M., Pascual-Leone, A., & Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology*, **14**(3), 208–212.
- Roig, M., Nordbrandt, S., Geertsen, S. S., & Nielsen, J. B. (2013). The effects of cardiovascular exercise on human memory: A review with meta-analysis. *Neuroscience & Biobehavioral Reviews*, **37**(8), 1645–1666.
- Roig, M., Skriver, K., Lundbye-Jensen, J., Kiens, B., & Nielsen, J. B. (2012). A single bout of exercise improves motor memory. *PLoS ONE*, **7**(9), e44594.
- Roig, M., Thomas, R., Mang, C. S., Snow, N. J., Ostadan, F., Boyd, L. A., & Lundbye-Jensen, J. (2016). Time-dependent effects of cardiovascular exercise on memory. *Exercise and Sport Sciences Reviews*, **44**(2), 81.
- Rouder, J. N., Speckman, P. L., Sun, D., Morey, R. D., & Iverson, G. (2009). Bayesian t tests for accepting and rejecting the null hypothesis. *Psychonomic Bulletin & Review*, **16**(2), 225–237.
- Saucedo Marquez, C. M., Vanaudenaerde, B., Troosters, T., & Wenderoth, N. (2015). High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise. *Journal of Applied Physiology*, **119**(12), 1363–1373.
- Singh, A. M., Duncan, R. E., Neva, J. L., & Staines, W. R. (2014). Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BioMed Central Sports Science, Medicine and Rehabilitation*, **6**(1), 23.
- Stagg, C. J., Bachtiar, V., Amadi, U., Gudberg, C. A., Ilie, A. S., Sampaio-Baptista, C., O'Shea, J., Woolrich, M., Smith, S. M., Filippini, N., Near, J., & Johansen-Berg, H. (2014). Local GABA concentration is related to network-level resting functional connectivity. *eLife*, **3**, e01465.
- Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011a). The role of GABA in Human motor learning. *Current Biology*, **21**(6), 480–484.
- Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011b). What are we measuring with GABA Magnetic resonance spectroscopy? *Communicative & Integrative Biology*, **4**(5), 573–575.
- Stavrinou, E. L., & Coxon, J. P. (2017). High-intensity interval exercise promotes motor cortex disinhibition and early motor skill consolidation. *Journal of Cognitive Neuroscience*, **29**(4), 593–604.
- Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology*, **37**(1), 153–156.
- Taylor, E. M., Cadwallader, C. J., Curtin, D., Chong, T. T.-J., Hendrikse, J. J., & Coxon, J. P. (2024). High-intensity acute exercise impacts motor learning in healthy older adults. *Nature Partner Journals Science of Learning*, **9**(1), 9.
- Tzvi, E., Münte, T. F., & Krämer, U. M. (2014). Delineating the cortico-striatal-cerebellar network in implicit motor sequence learning. *Neuroimage*, **94**, 222–230.
- van Dongen, E. V., Kersten, I. H. P., Wagner, I. C., Morris, R. G. M., & Fernández, G. (2016). Physical exercise performed four hours after learning improves memory retention and increases hippocampal pattern similarity during retrieval. *Current Biology*, **26**(13), 1722–1727.

Additional information

Data availability statement

De-identified behavioural and MRS data are available at <https://osf.io/guzs8/>. The conditions of our ethical approval do not permit open sharing of participant structural MRI data without prior informed consent. Therefore we are unable to publicly archive the raw structural MRI images used in this study. Readers seeking access to the data and/or task materials should contact the lead author (Joshua.hendrikse@monash.edu). Access to structural MRI data will be granted to named individuals in accordance with ethical procedures governing the reuse of sensitive data. Specifically requestors must complete a formal data sharing agreement and make clear the process by which participant consent would be sought. All code used for cognitive and MRI analysis is available at <https://github.com/jhendrikse/>.

Competing interests

None of the authors have any competing interests to declare.

Author contributions

J.H., E.B. and J.C. were involved in the study design. J.H., E.B., S.W., R.M. and J.C. collected the data. J.H., E.B., D.C., N.C.R., M.Y., M.B., C.J.S., M.A.B., C.S. and J.C. were involved in the analysis and interpretation of the data. J.H., E.B., S.W., D.C., N.C.R., M.Y., M.B., C.J.S., M.A.B., R.M., C.S. and J.C. contributed to writing and/or revising the manuscript. All authors approved the submitted manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

cardiovascular exercise, GABA, hippocampus, micro-consolidation, motor cortex, motor learning, MR spectroscopy, neuroplasticity

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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