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The Reciprocal Relationship Between Short- and Long-Term Motor Learning and Neurometabolites

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ABSTRACT

Skill acquisition requires practice to stimulate neuroplasticity. Changes in inhibitory and excitatory neurotransmitters, such as gamma-aminobutyric acid (GABA) and glutamate, are believed to play a crucial role in promoting neuroplasticity. Magnetic resonance spectroscopy (MRS) at 3 T, using the MEGA-PRESS sequence, and behavioral data were collected from 62 volunteers. Participants completed a 4-week protocol, practicing either complex ($n = 32$) or simple ($n = 30$) bimanual tracking tasks (BTT). Neurotransmitter levels and skill levels at baseline, after 2 and 4 weeks of motor training were compared for the left and right primary sensorimotor cortex (SM1) and the left dorsal premotor cortex (PMd). Furthermore, task-related modulations of neurotransmitter levels in the left PMd were assessed. The study yielded that baseline neurotransmitter levels in motor-related brain regions predicted training success. Furthermore, lower GABA+ ($p = 0.0347$) and higher Glx (glutamate + glutamine compound) levels ($p = 0.0234$) in left PMd correlated with better long-term learning of simple and complex tasks, respectively, whereas higher GABA+ in right SM1 correlated with complex task learning ($p = 0.0064$). Resting neurometabolite levels changed during the intervention: Left SM1 Glx decreased with complex training toward Week 4 ($p = 0.0135$), whereas right SM1 Glx was increased at Week 2 ($p = 0.0043$), regardless of training type. Group-level analysis showed no task-related neurometabolite modulation in the left PMd. However, individual baseline GABA+ and Glx modulation influenced short-term motor learning (interaction: $p = 0.0213$). These findings underscore the importance of an

Abbreviations: BLOCK_BTT1/BTT2, MRS acquisition in left PMd during execution of the BTT (first/last 11 min, respectively); BLOCK_RESTafter, MRS acquisition in left PMd at rest after task execution; BLOCK_RESTbefore, MRS acquisition in left PMd at rest before task execution; BTT, bimanual tracking task; CSF, cerebrospinal fluid; E-I balance, excitatory-inhibitory balance; fMRI, functional magnetic resonance imaging; GABA, gamma-aminobutyric acid; GABA+, gamma-aminobutyric acid plus co-edited macromolecules; Glu, glutamate; Glx, compound measure of glutamate and glutamine; GM, gray matter; LMM, linear mixed model; LTD, long-term depression; LTP, long-term potentiation; M1, primary motor cortex; MEGA-PRESS, Mescher–Garwood point resolved spectroscopy; MID, measurement session after 2 weeks of motor training; MOIST, Multiply Optimized Insensitive Suppression Train; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NM, neurometabolite; PET, positron emission tomography; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; POST, measurement session after 4 weeks of motor learning; PRE, measurement session at baseline; PT, progress test; PT_BTT, progress tests of the bimanual tracking task; S1, primary somatosensory cortex; SM1, primary sensorimotor cortex; SMA, supplementary motor area; SNR, signal-to-noise ratio; SRTT, serial reaction time task; tDCS, transcranial direct current stimulation; TE, echo time; TMS, transcranial magnetic stimulation; TR, repetition time; VOI, volume of interest; WM, white matter.

interplay between inhibitory and excitatory neurotransmitters during motor learning and suggest potential for future personalized approaches to optimize motor learning.

1 | Introduction

Motor learning, the process of acquiring and refining motor skills through practice, is essential for daily activities and rehabilitation. Bimanual movements, such as driving a car or getting dressed, necessitate coordination beyond the simple summation of independently moving both limbs, requiring practice to overcome basic inherent movement constraints (such as mirroring movements) (Swinnen 2002). This motor-learning process consists of early or fast learning, characterized by rapid performance gains typically observed within the first practice session, and late or slow learning, marked by gradual skill development and consolidation of performance over weeks or months (Dayan and Cohen 2011; Karni et al. 1998). Despite the significance of motor learning in health and disease, the neurochemical mechanisms are yet to be explored, potentially informing future rehabilitation strategies for movement disorders.

Motor learning relies on synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), which respectively refer to the strengthening or weakening of neural connections based on activity (Castillo et al. 2011; Rosenkranz et al. 2007; Sanes and Donoghue 2000; Ziemann et al. 2004). Gamma-aminobutyric acid (GABA) and glutamate (Glu), the brain's primary inhibitory (Watanabe et al. 2002) and excitatory (Zhou and Danbolt 2014) neurotransmitters, play key roles in modulating synaptic transmission and plasticity within the motor cortex by regulating the excitability of neuronal circuits and hence enabling motor learning (Johnstone et al. 2021; Jongkees et al. 2017; McDonnell et al. 2007). Alterations in GABAergic and glutamatergic neurotransmission are also linked to motor deficits and rehabilitation of neurological disorders such as stroke (Blicher et al. 2015; Chen et al. 2020; Grigoras and Stagg 2021), highlighting their significance in shaping motor performance and adaptation.

Magnetic resonance spectroscopy (MRS) enables noninvasive quantification of neurometabolites (NMs), offering insights into the neurochemical basis of motor learning (Buonocore and Maddock 2015). In the context of motor learning, resting-state Mescher–Garwood point resolved spectroscopy (MEGA-PRESS) (Mescher et al. 1998, 1996; Mullins et al. 2014) MRS enables the measurement of baseline GABA and Glx (a compound measure of Glu and glutamine) levels, reflecting the steady-state neurochemical environment in the motor cortex or other regions. Task-related or functional MRS (fMRS), on the other hand, allows for the real-time assessment of neurotransmitter dynamics during task performance, capturing transient changes in neurotransmitter concentrations associated with motor task execution and learning processes (Pasanta et al. 2022). These measures allow for investigation of both short-term (within-session) and long-term (across sessions) changes in NMs during learning.

The primary sensorimotor cortex (SM1) and dorsal premotor cortex (PMd) are central to bimanual motor control and learning. SM1, comprising the primary motor cortex (M1) and primary somatosensory cortex (S1), is crucial for motor execution and sensory processing (e.g., Borich et al. 2015; Huang et al. 2024; Ogawa et al. 2019; Pearson 2000; Sanes and Donoghue 2000), whereas PMd integrates sensory input and modulates M1 output (Chouinard and Paus 2006; Dum and Strick 1991; Genon et al. 2018), particularly in complex bimanual coordination (Beets et al. 2015; Debaere et al. 2004; Karabanov et al. 2023; Van Ruitenbeek et al. 2023; Verstraelen et al. 2021). The left PMd is especially important, as it encodes movements of both upper limbs and is heavily involved in movement planning and skill acquisition (Debaere et al. 2004; Fujiyama et al. 2016; Hardwick et al. 2013; Merrick et al. 2022).

Previous MRS studies on motor learning have mainly focused on SM1 but not PMd, and have inconsistently reported Glx and GABA changes during task performance or training. While performing a motor task, an increase in Glu/Glx levels in SM1 has been reported repeatedly (Chen et al. 2017; Eisenstein et al. 2023; Schaller et al. 2014; Volovyk and Tal 2020; for meta-analysis of fMRS studies, see Pasanta et al. 2022). Furthermore, higher Glu levels at rest and at the start of executing a motor task have been associated with better learning of a serial reaction time task (SRTT) (Bell et al. 2023). However, other studies reported no change in Glu levels during motor learning (Bell et al. 2023; Floyer-Lea et al. 2006; Kolasinski et al. 2019; Maruyama et al. 2021), during the performance of a bimanual interference task (Rasooli et al. 2024) or after initial motor learning of an SRTT (Eisenstein et al. 2024).

For GABA levels in SM1, evidence is even less unanimous. A motor task (Chen et al. 2017) and motor learning-related (Floyer-Lea et al. 2006; Kolasinski et al. 2019) decrease in GABA levels has been reported by some authors. In contrast, other studies reported no change in GABA levels during motor learning (Bell et al. 2023; Maruyama et al. 2021), after motor learning (Chalavi et al. 2018; Eisenstein et al. 2023, 2024), or during task execution (Rasooli et al. 2024; Schaller et al. 2014). Moreover, baseline GABA levels in SM1 have been linked to motor learning, with lower (Chalavi et al. 2018) or higher (Li et al. 2024) baseline GABA levels in SM1 predicting a better initial performance, but not the training progress over several days on a bimanual tracking task (BTT) (Chalavi et al. 2018; Li et al. 2024). Another study indicated that lower baseline GABA levels in M1 were associated with better subsequent motor learning on an SRTT (Kolasinski et al. 2019). Interestingly, one study in which anodal transcranial direct current stimulation (tDCS) was employed showed that the ability to modulate (i.e., decrease) GABA levels in M1 was associated with better motor learning on an SRTT (Stagg et al. 2011).

Although several studies have investigated the immediate effects of motor learning on Glu/Glx and GABA levels in SM1, there are no reports of neurotransmitter levels in PMd in the context of motor learning or working memory. Only one study has looked into long-term changes in neurotransmitter levels in SM1, revealing a decrease in GABA levels at rest after 6 weeks of low intensity (i.e., 5 days per week for 15 min), but not high intensity (30 min), juggling training (Sampaio-Baptista et al. 2015).

Summary

- Neurotransmitter dynamics predict training success
  - Baseline levels of GABA+ and Glx in motor-related brain regions (left PMd and right SM1) were found to predict long-term learning success, with specific patterns correlating with better performance in both simple and complex motor tasks.
- Training-induced changes in neurometabolites
  - Resting levels of Glx in SM1 changed significantly during the motor training, with a decrease in left SM1 Glx after 4 weeks of complex training and an increase in right SM1 Glx after 2 weeks for both the simple and complex training groups, indicating dynamic adaptations in response to motor training.
- Personalized motor-learning potential
  - The interaction between baseline levels and task-related modulation of GABA+ and Glx influenced short-term motor-learning outcomes.

Since evidence suggests that LTP- and LTD-like neuroplasticity processes are mediated by changes in GABA (Kim et al. 2014) and/or Glu (Lüscher and Malenka 2012) neurotransmitter levels, understanding the neurochemical changes within these cortical regions during bimanual motor learning provides valuable insights into the neural mechanisms underlying the acquisition and refinement of complex motor skills. Changes in the excitatory/inhibitory (E-I) balance might lead to increased or decreased communication between neurons (Butefisch et al. 2000; Ziemann et al. 2001), which may ultimately facilitate synaptogenesis (Kleim et al. 2002, 2004) and synaptic pruning (Wenger et al. 2017; Yang et al. 2009). This process of synaptic reorganization is vital for motor learning.

In this study, we aimed to explore the temporal dynamics of GABA and Glx changes within the SM1 and PMd during bimanual motor learning using a longitudinal MRS approach. By investigating both resting (bilateral SM1, left PMd) and functional (left PMd) neurotransmitter profiles, we unraveled the excitatory and inhibitory neurotransmitter dynamics underlying skill acquisition and consolidation. We hypothesized that the resting levels of neurotransmitters would change in the context of a 4-week motor-learning intervention and that the task-related modulation of excitatory and inhibitory neurotransmitters in left PMd would be more pronounced in the initial stage as compared to the final stage of the motor training, reflecting the need for more modulation of the motor output via the PMd when task proficiency is still low. Finally, we expected these neurotransmitter correlates to be linked to the short- and long-term performance gains.

2 | Materials and Methods

2.1 | Participants

Sixty-two volunteers (aged 25.94 ± 4.02 [mean ± SD] years, range 19–34; $n = 37$ [59.7%] female) participated in this MRS experiment: Initial inclusion: $n = 68$; exclusion because of brain anomalies on initial structural magnetic resonance imaging (MRI) scan: $n = 3$; exclusion due to nonadherence to training protocol: $n = 2$; no MRS data because of technical difficulties: $n = 1$. Participants were right-handed according to the Edinburgh Handedness Inventory laterality quotient (EHI LQ; 86.61% ± 13.45%, range 50–100) (Oldfield 1971) and showed no indication of cognitive impairments as assessed with the Montreal Cognitive Assessment (MoCA; 28.39 ± 1.50 points, range 25–30) (Rossetti et al. 2011). The study protocol was approved by the local ethics committee (Ethics Committee Research UZ/KU Leuven; reference S65077) and participants gave full written informed consent prior to study initiation, according to the latest amendment of the Declaration of Helsinki (World Medical Association 2013).

2.2 | Overview of the Experiment

A 4-week training on the BTT was conducted with three comprehensive measurement sessions at baseline (PRE), after 2 weeks (MID), and after 4 weeks of motor training (POST). During a screening session, participants' safety and eligibility to participate in this research were checked. Further, participants were presented with a brief familiarization with the BTT (15 trials of the simplest 1:1 frequency ratio task, ~3 min), while lying supine in a mock MRI scanner. Then a pretest of the BTT was performed, and participants were pseudo-randomly (stratified by sex) assigned to either a complex or a simple training group of the BTT (task and training details, see below). During the PRE and POST measurement, a SynVesT1 positron emission tomography (PET) scan to assess synaptic density (subsample of $n = 22$; scan of 30 min), an MRI protocol (~2 h), and a dual-site transcranial magnetic stimulation (TMS) protocol to assess the intra- and interhemispheric PMd–M1 connectivity (~4 h) were conducted. At the MID measurement, only the MRI protocol was assessed. A single MRI session (conducted at PRE, MID and POST session) consisted of the following scans (in order): Resting-state fMRI (~15 min), high-resolution T1- and T2-weighted anatomical images (~10 min), resting MRS of right and left SM1 (~22 min), break outside of scanner (5 min), low-resolution T1-weighted anatomical scan (~2 min), resting and task-related MRS of left PMd (~45 min), diffusion-weighted imaging (~10 min). All MRI sessions followed the exact same protocol and order. Importantly, none of the used measurement methods is of an interventional nature, meaning that the PET, MRI or TMS measurements themselves have no influence on the participant's skill acquisition or brain characteristics (as opposed to, e.g., certain repetitive TMS protocols). In the present research, we will only describe the MRS data (and the T1-weighted anatomical data in function of it) of this research (for an overview of the experimental design regarding the MRS acquisition, see Figure 1).

2.3 | Bimanual Motor Training and Behavioral Measures

2.3.1 | Motor Training Intervention

All participants followed a motor-learning paradigm that lasted for 4 weeks, consisting of four 30-min (6 × 5 min, with short self-paced breaks after each 5-min block) training

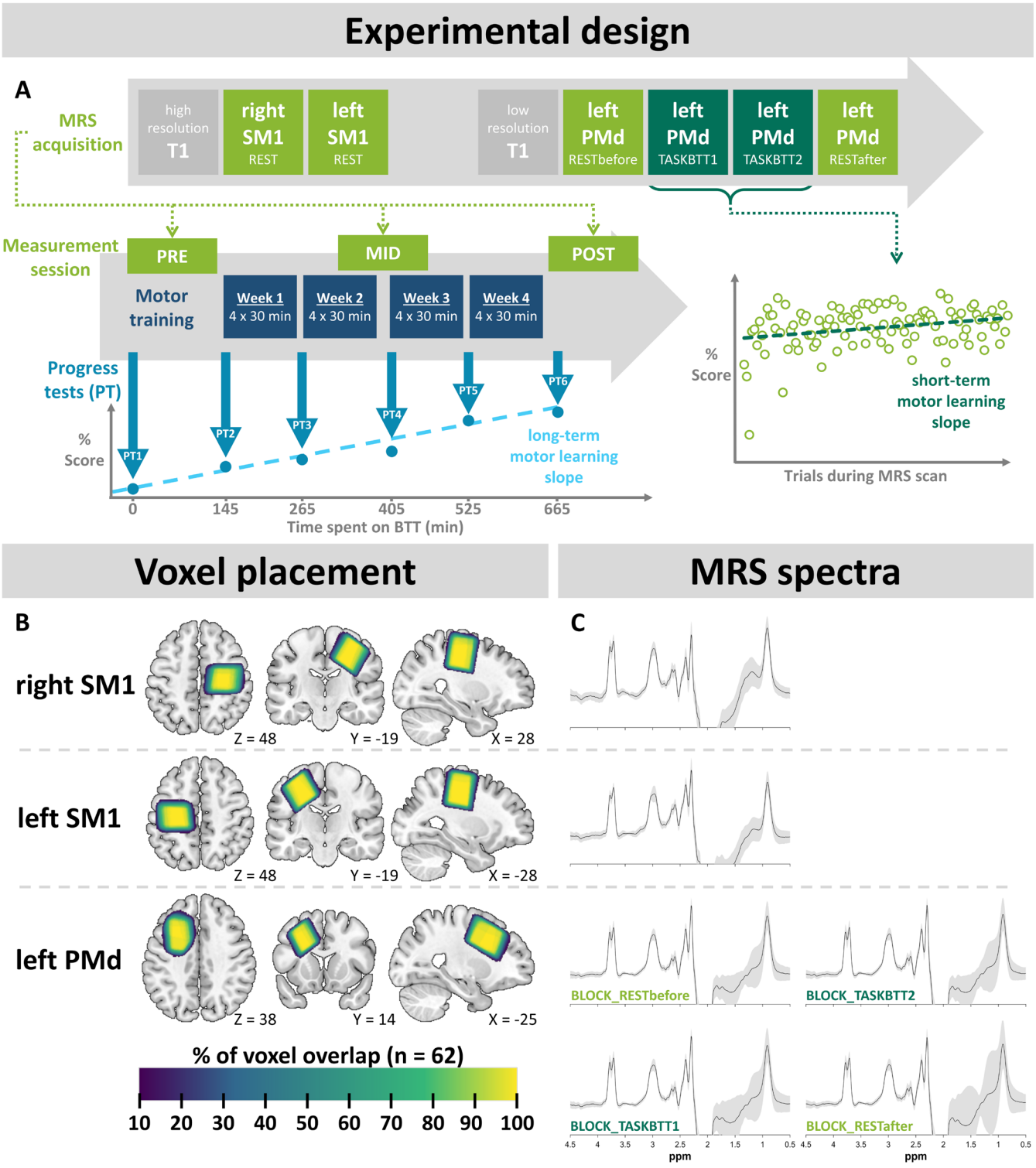

FIGURE 1 | Overview of the experimental design. (A) Timing of the three measurements at baseline (PRE session), after 2 weeks (MID session), and after 4 weeks of motor training (POST session) on a bimanual tracking task (BTT). During each magnetic resonance (MR) measurement session, neurometabolite levels in the right and left primary sensorimotor cortex (SM1) were acquired at rest (each ~8 min acquisition time), and for the left dorsal premotor cortex (PMd) both resting and task-related MRS were collected in four blocks (~11 min each, see top arrow): One at rest (BLOCK_RESTbefore), two during the BTT (BLOCK_TASKBTT1, BTT2), and one at rest after the task (BLOCK_RESTafter). Short-term motor-learning progress was defined by the linear slope of BTT scores within a session (lower right plot), whereas long-term progress was defined by the linear slope of the standardized progress test (PT) scores assessed at six time points over the 4-week training period (lower left plot). (B) Voxel placement (warped to MNI space) during the PRE session, serving as the reference for placement during the MID and POST sessions, and percentage overlap between participants. (C) Obtained MR spectroscopy (MRS) spectra (mean ± SD) from all three measurement sessions per region and task block.

sessions per week, with maximally one training session per day and the four sessions spread as much as possible over the week. To induce training-related plasticity, the BTT (Fujiyama et al. 2016; Sisti et al. 2011; Zivari Adab et al. 2020) was selected. This is a novel task that does not belong to the daily motor repertoire but involves similar neural mechanisms like those required for everyday bimanual movements. Besides, the task allows an easy adaptation of the difficulty level based on the individual performance level to ensure optimal challenge and motivation during motor learning.

During the BTT training, participants were seated in an upright position facing a 15-inch laptop and the task setup positioned in front of them (see Figure 2A). Participants were instructed to accurately track a visual target (white dot) presented on the screen. After 2 s from the beginning of each trial, the target started to move at a constant speed over the target line (blue line). Participants were asked to track the target as accurately as possible by rotating two dials with the left and right index finger to control, respectively, the vertical and horizontal movements of a cursor, resulting in a red path (reflecting the actual trajectory covered) (see Figure 2B) (Fujiyama et al. 2016; Sisti et al. 2011; Zivari Adab et al. 2020). After each trial, participants received brief on-screen visual feedback about their performance, expressed as a percentage ranging from 0% to 100%. The score reflected the overall accuracy considering speed, movement direction, and distance from the target (see Appendix 1 for all BTT details and the score calculation).

After successful screening, participants were pseudo-randomly assigned to a complex or simple training group, stratified by sex. In both training groups, participants performed the BTT four times per week for 30 min, with short self-paced breaks every 5 min. In the complex training group, maximal motor learning was pursued by continuously adjusting the task difficulty based on the individual performance level, using an adapting staircase-like level paradigm. More specifically, this training consisted of 280 different trials, combined and arranged into 5-min levels starting with easier levels (i.e., consisting of straight-line trials) and progressing to more difficult levels (i.e., consisting of curves, zig–zags, waves and complex figures and various combinations including variations in speed). When performing well on a level (i.e., reaching on average > 65%), then participants would progress to the next level, whereas medium performance (40%–65%) or bad performance (< 40%) would lead to stagnation or regression in levels (see Appendix 1 for details). In contrast, the simple training group spent the same amount of time on the same task setup but only trained the simplest variant (1:1 frequency ratio line requiring both hands have to move equally fast, see Figure 2D, first panel), limiting the complexity level and thus the amount of motor learning as compared to the complex group. To compare motor learning independently of the training condition (complex vs. simple) and individual progress on the level scheme, participants of both training groups were presented with a progress test (PT). This test was administered six times (at baseline, at the beginning of each training week, and after finalizing the training paradigm) and consisted of a standardized set of tasks (see Figure 2D, and mirrored counterparts, total of 18 tasks, duration: ~5 min). Furthermore, the performance (Score in %) on the PTs was used to quantify the learning progress in both groups. Specifically, the linear slope of the PT scores over time was estimated by a linear regression using the average scores of each of the PTs as the dependent variable and the time spent on the BTT (BTT_{time}; summed over all training and measurement sessions, expressed in minutes) as the independent variable (see Figure 1A). To obtain a detailed picture of the long-term learning progress over the 4-week training period, linear slopes were calculated for the average scores on the BTT progress tests (PT_BTT) of: (1) All straight-line trials, that is, 1:1, 1:3, and 3:1 (left:right hand) frequency ratios ($PT\_BTTslope_{Lines}$ derived from $PT\_BTTscore_{Lines}$), and (2) the three complex task variants, that is, zig–zag, waves, and flamingo trials ($PT\_BTTslope_{Complex}$ derived from $PT\_BTTscore_{Complex}$) (see Figure 2D). These two groups of subtasks were analyzed separately, because they reflect the skill level and motor-learning progress on simpler and more complex tasks, respectively.

2.3.2 | BTT During MRS Scans

During the task-related MRS acquisition of the left PMd, participants performed the BTT inside of the MR scanner (see Section 2.4 below; duration: 20 min, about 100 trials in total). To do so, an adapted nonferromagnetic version of the BTT setup was used, which could be placed over the participants' hips in a bridge-wise manner and could effortlessly be operated in supine position (see Figure 2C). The visual input of the BTT was presented to the participant via a projection at the cranial end of the MR scanner and a mirror (~14 × 9 cm) placed in front of (~13 cm distance) the participant's eyes (LCD projector: NEC NP-PA500U, 1920 × 1200 pixels; see red line in Figure 2C, representing the path of the visual input). During the MRS sessions, participants performed only straight-line tasks with the frequency ratios 1:1, 1:3, and 3:1 (left:right hand movements), with the target moving in the four possible directions (toward upper right/upper left/lower right/lower left corner of the screen). Linear slopes for the progress made during the training session within the MR scanner were calculated with trial number as independent variable and score as dependent variable. Since only straight-line trials were performed in the scanner (i.e., 1:1, 1:3, and 3:1 frequency ratios), slopes were calculated for all tasks ($MR\_BTTslope_{allLines}$) to quantify short-term learning progress during the scanning session, representing performance progression as a result of learning (see Figure 1A, right panel). For a more detailed behavioral analysis, the motor-learning progress slopes for 1:1 frequency ratio trials ($MR\_BTTslope_{11}$), and 1:3/3:1 frequency ratio trials together ($MR\_BTTslope_{13,31}$) were calculated as well.

2.4 | MR Acquisition

MRI and spectroscopy data were acquired using a 3 T Philips Achieva dStream scanner (University Hospital Leuven, Gasthuisberg) with a 32-channel receiver head coil (Philips; Best, the Netherlands). A T1-weighted high-resolution anatomical image was collected using a three-dimensional turbo field echo (3DTFE) sequence (echo time [TE] = 4.6 ms, repetition time [TR] = 9.7 ms, flip angle = 8°, field of view = 256 × 242 × 182 mm, 182 sagittal slices, voxel size = 1 × 1 × 1 mm^3, and acquisition time ~6 min), based on which the MRS VOIs for the right and left SM1 were placed.

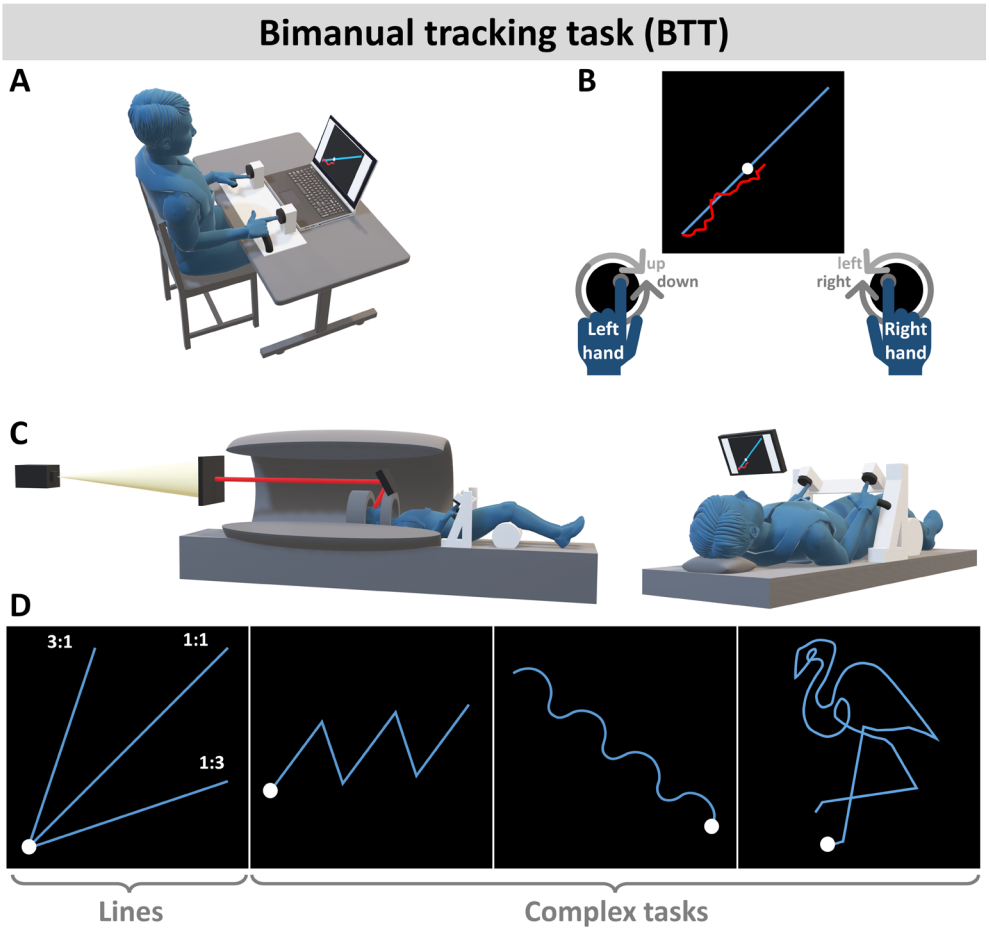

FIGURE 2 | Overview of the bimanual tracking task (BTT). (A) BTT training setup for home training and progress tests. Participants were comfortably seated, the task setup and a laptop placed in front of them, with their hands resting on the handles of the task setup and index fingers in the grooves of the BTT dials. (B) The main goal of the BTT was to trace a white dot, moving at a constant speed along a blue target line, as accurately as possible. To do so, participants were instructed to rotate the left and right dial with their index fingers in order to respectively manipulate the vertical and horizontal movements of their own trajectory, indicated by a red line. (C) Magnetic resonance (MR) compatible BTT setup for task execution during MR spectroscopy. Participants were in supine position, elbows well-supported, hands resting on the BTT handles and index fingers in the grooves of the BTT dials. Visual task input was provided via a projector and a screen that participants could view via a mirror placed in front of their eyes (see mirror path displayed in red). (D) Standardized progress tests to evaluate each individual's skill level throughout task practice. The set of easier straight-line tasks (frequency ratios of the left:right hands are indicated purely for illustrative purposes) and complex tasks (zig–zag, waves, flamingo) were assessed six times in total: At baseline (PRE), at the beginning of each of the four training weeks, and after finalizing the training paradigm (POST). The lines were presented four times per frequency ratio (once per quadrant of movement direction, to cover each movement speed and direction for each hand), the complex tasks were presented twice (original and vertically mirrored).

Since there was a brief break in the middle of the MRI session including participant removal from and replacement in the scanner bore (see Section 2.2) an additional lower-resolution T1-weighted 3DTFE (TE = 4.6 ms, TR = 9.6 ms, flip angle = 8°, field of view = 256 × 244 × 182 mm, 182 sagittal slices, voxel size = 1.5 × 1.5 × 1.5 mm^3, and acquisition time ~2 min) was acquired before MRS acquisition in the left PMd to inform volume of interest (VOI) placement. During processing of the MRS data, high-resolution T1-weighted images were used for all voxels (see Section 2.5 below).

GABA-edited MRS spectra were acquired using a MEGA-PRESS sequence (Edden and Barker 2007; Mescher et al. 1998) (TE = 68 ms, TR = 2000 ms, samples = 1024, spectral bandwidth = 2 kHz), with ON and OFF spectra being collected in an interleaved fashion and editing pulses (basing pulse duration = 14 ms) being applied at 1.9 or 7.46 ppm, respectively. An automatic first-order pencil-beam (PB) shimming procedure was performed, and Multiply Optimized Insensitive Suppression Train (MOIST; bandwidth 140 Hz) water suppression was used. For each MRS acquisition, an additional 16 unsuppressed water averages were acquired and used for online interleaved water referencing. The edited signal detected at 3 ppm contains co-edited contributions from both macromolecules (MM) and homocarnosine, and hence it will be referred to as GABA+ rather than GABA.

During the first session, MRS VOIs were placed based on anatomical landmarks, individually defined on the T1-weighted MRI (see Figure 1B,C for VOI visualization and obtained spectra, respectively). For follow-up scans, views of each VOI in all three planes and superimposed on the T1-weighted image acquired at the first session were used to guide the positioning of each voxel during the second and third sessions. MRS VOIs were placed in

the right and left SM1 [voxel dimensions = 30 × 30 × 30 mm^3; 112 edit-ON/112 edit-OFF spectra acquired; ~8 min acquisition time (Mikkelsen et al. 2018)], with the center of the VOI placed over the respective hand knob (Yousry et al. 1997) in the axial view and alignment of the VOI with the cortical surface in the coronal and sagittal views. A third MRS VOI was placed in the left PMd (voxel dimensions = 40 × 25 × 25 mm^3; 160 ON/160 OFF spectra acquired; ~11 min acquisition time per acquisition block). The VOI was placed in the axial view just anterior to the left hand knob, with the posterior surface of the VOI aligned with the precentral sulcus, the medial surface parallel to the longitudinal fissure (Greenhouse et al. 2016; Maes et al. 2021), and the longitudinal center of the VOI over the superior frontal sulcus (Maes et al. 2020). The VOI was then aligned to the cortical surface in the sagittal and coronal views. For the left PMd, four directly adjacent acquisitions were conducted (total of ~45 min) with the participant at rest during the first acquisition block (BLOCK_RESTbefore), performing the BTT inside of the MR scanner during the second and third acquisition blocks (BLOCK_BTT1 and BLOCK_BTT2, respectively), and resting again during the fourth block (BLOCK_RESTafter) (see Figure 1A).

2.5 | MRS Data Processing

MRS data were analyzed using the MATLAB-based (vR2022a, MathWorks, Natick, MA) software toolkit "Gannet" (version 3.3.1) (Edden et al. 2014), specifically designed to analyze edited single-voxel MRS data. GABA+ and Glx quantification were conducted according to the following steps: (1) Frequency and phase alignment of free induction decays with spectral registration in the time domain (Near et al. 2015); (2) subtraction of aligned and averaged edit-ON from edit-OFF spectra to obtain GABA+-edited (3.0 ppm) and Glx (Glu + glutamine; 3.75 ppm) co-edited difference spectra; (3) fitting a three-Gaussian function using nonlinear least-squares fitting to quantify the GABA+ and Glx peak areas between 4.1 and 2.79 ppm, and a Gaussian–Lorentzian model to fit the water signal between 5.6 and 3.8 ppm, which was used as a reference (Mikkelsen et al. 2019); (4) water-scaling using the unsuppressed water reference signal and tissue correction of GABA+ and Glx levels based on gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) fractions within each VOI. To obtain these, the three-dimensional T1-weighted high-resolution images were segmented using SPM 12 (Statistical Parametric Mapping v7771, Wellcome Trust Centre for Human Neuroimaging, University College; London, UK), and the individual MRS VOIs were coregistered to the structural image in Gannet (Edden et al. 2014). Since PMd VOIs were placed on a lower-resolution T1-weighted image, a coregistration of the high-resolution to the lower-resolution anatomical image was performed, and further analysis of the PMd MRS data was done based on the segmentation of that coregistered high-resolution T1-weighted image. Tissue correction was performed and included correction for CSF (i.e., assumption of negligible GABA in CSF) and α-correction (i.e., assumption of twice as much GABA in GM as in WM) [see (Harris et al. 2015), their Equation 5].

Data quality of individual MRS spectra was assessed by visual inspection of the GannetLoad and GannetFit output (screening for lipid contamination, unsuccessful water suppression, overall spectral and fit quality) and quantitatively excluding data with high fit errors [cutoff fit error > 12% (Puts et al. 2018)] or low signal-to-noise ratio (SNR) of the GABA+ or Glx signal [cutoff SNR_{GABA+} < mean-3*SD (Maes et al. 2021)].

2.6 | MRS Data Quality and VOI Placement

2.6.1 | MRS Data Quality

Of the 1140 acquired spectra, two (right and left SM1 of the same participant from PRE measurement) were excluded based on low SNR of the GABA+ signal (outlier: SNR < mean–3*SD). No further data points were excluded. An overview of the data quality can be found in Table 1, displaying the linewidth of GABA+, Glx, water, and creatine in full-width half maximum (FWHM) as a measure of spectral resolution and the degree of signal overlap, the SNR for the metabolites of interest (GABA+, Glx), the FitError for the GABA+:water and Glx:water quantification as fitted by the Gannet toolbox, and the voxel fractions of GM, WM, and CSF in each VOI.

2.6.2 | Within-Participant VOI Overlap Between Sessions

To evaluate the accuracy of VOI placement, the overlap between sessions was calculated. Within participants, VOI overlap between sessions was consistently high. Average VOI overlap was 91.99% ± 6.27% between PRE and MID measurement, and 92.69% ± 4.52% between PRE and POST measurement (left SM1: 92.21% ± 7.81% and 93.36% ± 3.98%; right SM1: 92.14% ± 6.50% and 93.04% ± 4.00%; left PMd: 91.63% ± 3.99% and 91.67% ± 5.34% for PRE vs. MID and PRE vs. POST, respectively). The part of the VOIs that overlapped between all three measurement sessions was 87.55% ± 7.17% (left SM1: 88.05% ± 8.28%, right SM1: 87.82% ± 7.49%, left PMd: 86.79% ± 5.51%).

2.6.3 | VOI Overlap Between Left SM1 and PMd

On average, the left PMd VOI overlapped with 6.15% ± 5.71% (i.e., 1.66 ± 1.54 mL) of the left SM1 VOI, indicating that the overlap was minimal, and that two VOIs carry primarily different information despite their adjacent location.

2.7 | Statistical Analysis

Behavioral and MRS data were analyzed using R Studio (R version 4.2.2, RStudio 2022.12.0 Build 353; α set to 0.05 unless specified otherwise). If necessary, to comply with model assumptions, a transformation of the dependent variable was performed. Models were manually simplified according to the stepwise backward procedure, where nonsignificant effects (based on type III analysis of variance (ANOVA) fixed effect test, $p > 0.05$) were removed from the model one-by-one, keeping main effects that were still included in an interaction in the model. This procedure is described in detail in the Appendix S1 for significant results. Where applicable, post hoc *t*-tests with Bonferroni correction were applied.

TABLE 1 | Magnetic resonance spectroscopy (MRS) data quality measures for left primary sensorimotor cortex (SM1), right SM1, and left dorsal premotor cortex (PMd). Data are pooled over the three measurement sessions (PRE, MID, and POST).

	Left SM1		Right SM1		Left PMd	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**
FWHM GABA+	18.54 ± 1.48	15.51–24.69	17.01 ± 1.41	12.82–21.09	18.91 ± 1.31	15.12–22.51
FWHM Glx	14.06 ± 1.75	11.93–32.91	14.56 ± 1.40	12.06–20.14	14.25 ± 1.21	11.99–19.52
FWHM Water	9.83 ± 0.74	8.30–11.96	9.66 ± 0.75	7.93–13.18	9.93 ± 0.80	8.42–12.94
FWHM Cr	9.39 ± 0.72	8.02–12.20	9.54 ± 0.81	7.69–14.39	9.20 ± 0.83	7.68–12.20
SNR GABA+	16.45 ± 3.49	9.72–29.49	14.48 ± 3.05	8.15–23.76	17.79 ± 3.51	9.62–30.10
SNR Glx	18.85 ± 3.60	10.05–34.78	17.50 ± 3.28	9.27–28.91	20.25 ± 4.91	7.99–36.56
FitError GABA+:water	5.55 ± 1.72	2.26–10.79	5.75 ± 1.46	2.97–11.13	4.22 ± 1.10	2.00–10.60
FitError Glx:water	4.74 ± 1.21	1.88–9.62	4.71 ± 1.08	2.67–9.06	3.75 ± 0.97	1.86–7.25
Voxel fraction GM	0.33 ± 0.03	0.22–0.39	0.33 ± 0.03	0.19–0.40	0.34 ± 0.04	0.23–0.42
Voxel fraction WM	0.60 ± 0.04	0.51–0.74	0.59 ± 0.04	0.50–0.77	0.62 ± 0.05	0.49–0.74
Voxel fraction CSF	0.07 ± 0.02	0.02–0.13	0.08 ± 0.02	0.02–0.14	0.05 ± 0.02	0.01–0.10

Abbreviations: Cr, creatine; CSF, cerebrospinal fluid; FWHM, full-width half maximum; GABA+, gamma-aminobutyric acid plus co-edited macromolecules; Glx, glutamate and glutamine compound measure; GM, gray matter; PMd, dorsal premotor cortex; SD, standard deviation; SM1, primary sensorimotor cortex; SNR, signal-to-noise ratio; WM, white matter.

2.7.1 | Behavioral Data

To investigate progress in motor skill over the 4-week training period, linear mixed models (LMM) were used with BTT scores of the PTs as the dependent variable (one LMM for each, simple and complex subtasks respectively: $PT\_BTTscore_{Lines}$ and $PT\_BTTscore_{Complex}$), SUBJECT as a random effect, and GROUP (simple vs. complex training group) and BTT_{time} (in min) as independent variables. Furthermore, the interaction $GROUP \times BTT_{time}$ was tested: $PT\_BTTscore \sim GROUP + BTT_{time} + GROUP \times BTT_{time} + (1|SubjectID)$, with two levels for GROUP (simple vs. complex training group) and BTT_{time} being a continuous variable. Since BTT_{time} was a continuous variable, classic post hoc testing was not possible. Nevertheless, the exact timing of training progress and differences between groups can be informative to understand changes in NMs. Hence, *t*-tests/Wilcoxon rank sum tests with Bonferroni correction were used to compare scores on consecutive PTs and, where applicable, the effect of the training group on the performance ($PT\_BTTscore_{Lines}$ and $PT\_BTTscore_{Complex}$). Lastly, for the learning slopes as measures of learning progress ($PT\_BTTslope_{Lines}$ and $PT\_BTTslope_{Complex}$), a group comparison (simple vs. complex training group) was performed using independent Welch *t*-tests to assess differences in learning speed.

For in-scanner learning on the BTT, the learning slopes during the MRS scans ($MR\_BTTslope_{allLines}$, $MR\_BTTslope_{11}$, $MR\_BTTslope_{13,31}$) were compared per training group between measurements (PRE, MID, POST) using paired *t*-tests, and per measurement between training groups (complex vs. simple training) using independent Welch *t*-tests. Only the compound measure $MR\_BTTslope_{allLines}$ was used for analyzing associations between short-term learning and NM levels. Additionally, the 1:1 and 1:3/3:1 frequency ratio learning slopes were behaviorally analyzed in order to investigate whether there were training group effects for each of the subtask groups.

2.7.2 | Baseline NM Levels to Predict Motor-Learning Progress

The predictive value of GABA+ and Glx levels at rest during the PRE measurement was assessed using six different multiple linear regressions. Each motor-learning outcome ($MR\_BTTslope_{allLines}$ of the PRE measurement, $PT\_BTTslope_{Lines}$, and $PT\_BTTslope_{Complex}$ respectively for motor-learning progress in the short-term (i.e., within the first MRS session), in the long-term (i.e., over the 4-week motor-learning intervention) on simple subtasks, and in the long-term on complex subtasks) was regressed by the NM levels in the three VOIs (left SM1, right SM1, left PMd), resulting in two linear models per motor-learning outcome: Short-term motor-learning outcome ~ NM_L-SM1 + NM_R-SM1 + NM_L-PMd, with NM = GABA+ for linear model 1, and NM = Glx for linear model 2. For short-term motor learning (where only PRE measurement data was included), the GROUP assignment was not of interest for the model, because a group-specific intervention was only applied afterwards. For the long-term motor-learning outcome measures concerning the whole training phase, GROUP was additionally added to the model, as well as the interaction effects between GROUP and the NM level in each VOI: Long-term motor-learning outcome ~ NM_L-SM1 + NM_R-SM1 + NM_L-PMd + GROUP + NM_L-SM1 × GROUP + NM_R-SM1 × GROUP + NM_L-PMd × GROUP, with two levels for GROUP (simple vs. complex training group), and the NM levels being continuous variables.

2.7.3 | Changes in Resting NM Levels as a Result of Motor Learning

To assess changes in resting GABA+ and Glx levels per VOI associated with motor learning, six LMMs (one per NM [GABA+, Glx] and per VOI [left SM1, right SM1, left PMd]) were constructed according to the formula: NM_VOI ~ GROUP + SESS

ION + GROUP × SESSION + (1|SubjectID), with two levels for GROUP (simple vs. complex training group) and three levels for SESSION (PRE vs. MID vs. POST).

2.7.4 | Modulation of Left PMd NM During Task Execution and Changes in Modulation With Motor Learning

The modulation of left PMd GABA+ and Glx levels was investigated for the PRE measurement using an LMM with the NM levels in left PMd during the PRE measurement (GABA+ or Glx) as the dependent variable, GROUP with two levels (simple vs. complex training group), TASK with four levels (11-min blocks of MRS acquisition: BLOCK_RESTbefore, BLOCK_BTT1, BLOCK_BTT2, BLOCK_RESTafter), and their interaction as fixed effects of interest, the linewidth of the water signal as a covariate of no interest [since changes in BOLD have previously been associated with changes in linewidth at 7 T (Stanley and Raz 2018), which despite lacking evidence might influence the GABA+ and Glx quantifications at 3 T (Dwyer et al. 2021)], and SubjectID as a random effect: NM_L-PMd ~ GROUP + TASK + GROUP × TASK + Water_FWHM + (1|SubjectID). Changes in NM modulation associated with the 4-week motor training intervention were assessed with LMMs as stated above but including data from all three measurements and SESSION respective interactions up to the second degree as fixed effects: NM_L-PMd ~ GROUP + SESSION + TASK + GROUP × SESSION + SESSION × TASK + GROUP × TASK + Water_FWHM + (1|SubjectID).

2.7.5 | Relationship Between the Modulation of Left PMd NM and Motor Learning

To investigate whether the modulation of NMs was related to the short-term or long-term motor-learning progress, an additional measure of the maximal modulation per subject and session was calculated. The absolute maximal modulation (|MODmax|) was calculated as |MODmax| = max(|NM[-BLOCK_BTT]−NM[BLOCK_RESTbefore]|). In other words, the NM level at baseline (BLOCK_RESTbefore) was subtracted from the task-related block with the maximal change in NM (NM[BLOCK_BTT], corresponding to either NM[BLOCK_BTT1] or NM[BLOCK_BTT2]). In addition, a directional measure of |MODmax| was used (MODmax), where decreases in NM levels from baseline to task were indicated by a negative sign, and increases by a positive sign (i.e., MODmax = NM[BLOCK_BTT]−NM[BLOCK_RESTbefore]). Hence, to summarize, the absolute maximal modulation |MODmax| focuses on the magnitude of the modulation, ignoring the direction, providing insight into the overall flexibility of the neurometabolic system to adapt to task demands. In contrast, the directional maximal modulation MODmax captures the direction of this change in NM levels, allowing for an assessment of whether excitatory or inhibitory shifts are associated with motor-learning outcomes. By considering both measures, we aimed to distinguish between general neurometabolic adaptability and specific excitation/inhibition dynamics in relation to short-term and long-term motor learning.

To evaluate the influence of NM modulation during the first session on short- and long-term motor-learning progress, multiple linear regressions were used to model each motor-learning outcome ($MR\_BTTslope_{allLines}$ of the PRE measurement, $PT\_BTTslope_{Lines}$, and $PT\_BTTslope_{Complex}$ for overall short-term, simple long-term, and complex long-term motor-learning progress, respectively) by the GABA+ and Glx modulation in left PMd during the PRE measurement: Motor-learning outcome ~ GABA_MODmax + Glx_MODmax + GABA_MODmax × Glx_MODmax. The same was done for |MODmax| instead of MODmax to investigate the influence of the strength of modulation on motor learning, resulting in a total of six multiple linear regressions (3 motor-learning outcomes × 2 modulation measures).

3 | Results

3.1 | Behavioral Outcomes of Motor-Learning Paradigm

Training adherence was high, with an overall 99.75% ± 0.79% of the 5-min training blocks being completed. There was no significant difference between training groups (complex group: $n = 32$, 99.90% ± 0.42%; simple group: $n = 30$, 99.75% ± 0.79%; independent t-test: $t(60) = 0.9493$, $p = 0.3463$).

3.1.1 | Performance Improvements but No Group Difference for Simple Task Variants With Motor Training

Participants' performance on the PTs improved significantly when more time was spent on the BTT for both simpler and more complex task variants, with a difference between the performance and motor-learning progress of the two training groups only being present for the complex task variants. Furthermore, for the simpler straight-line tasks of the progress tests ($PT\_BTTscore_{Lines}$), cubic transformation of the dependent variable was necessary to comply with the assumption of normally distributed residuals. The stepwise model simplification resulted in a final model with a significant effect of time spent on the BTT on the cubic transformed $PT\_BTTscore_{Lines}$ ($\beta = 660.71$, SE = 16.65, $t(310.0) = 39.671$, $p < 0.0001$) (see Figure 3A; for model details and stepwise procedure, see Appendix 2A). All subsequent PT pairs showed a significant increase in $PT\_BTTscore_{Lines}$ (all, $p < 0.0007$ with Bonferroni correction $\alpha = 0.05/5 = 0.01$; for tabular results, see Appendix 2B). In accordance with the nonsignificant effect of GROUP on $PT\_BTTscore_{Lines}$, comparing the motor-learning slopes for the straight-line tasks ($PT\_BTTslope_{Lines}$) yielded no significant difference between the groups ($p = 0.9263$, $t(55.4) = -0.093$, mean ± SD for simple/complex training group respectively: 0.076 ± 0.018/0.076 ± 0.025; see Figure 3B).

3.1.2 | Higher Improvements on Complex Subtasks for the Complex Training as Compared with the Simple Training Group

In contrast, training group assignment did show an influence on performance and motor-learning progress on the complex

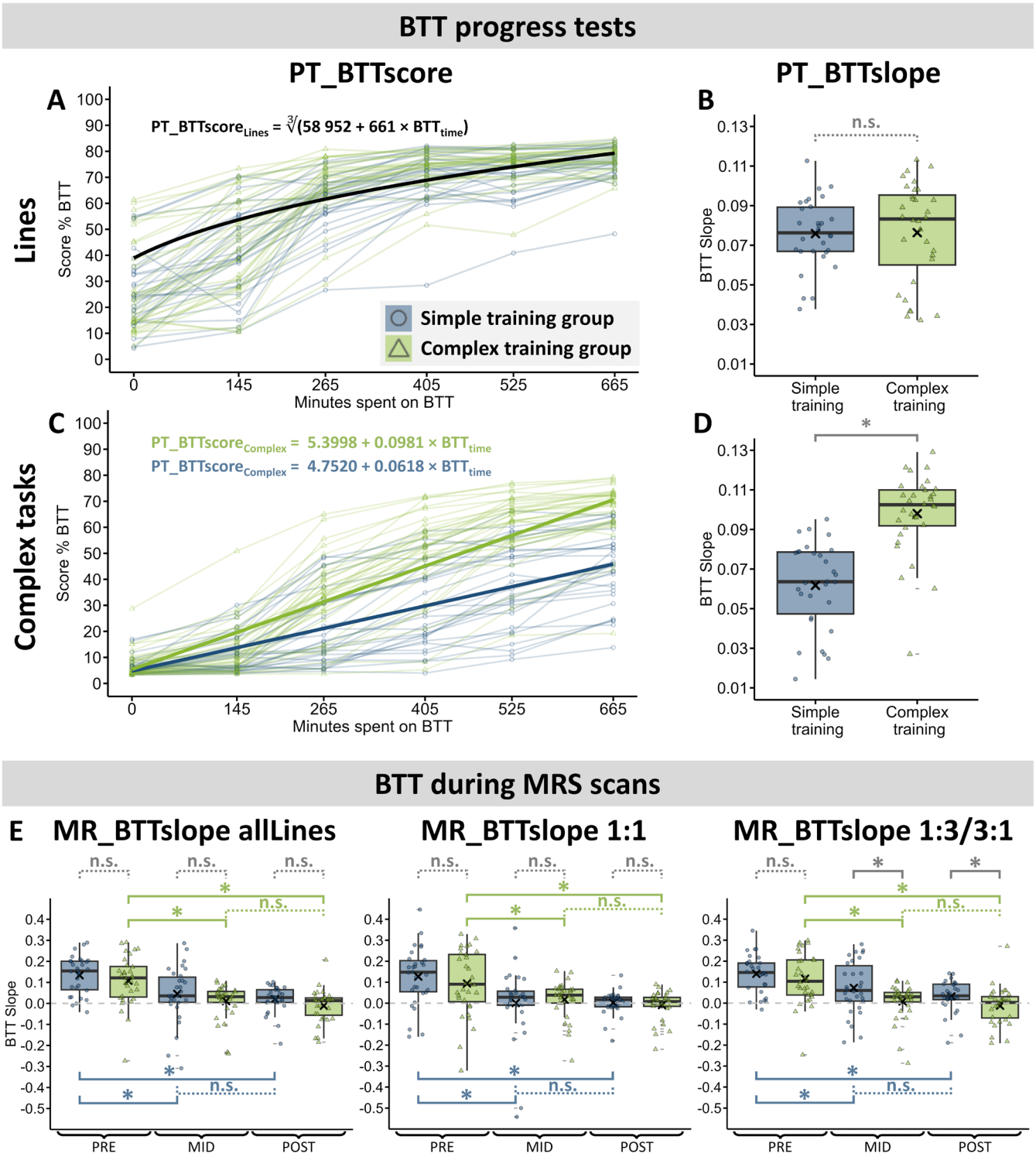

FIGURE 3 | Results of the behavioral analysis of the bimanual tracking task (BTT). (A) and (C) Regression analysis of the BTT scores over the time spent on the BTT (in minutes) for the easier straight-line tasks and the complex tasks, respectively. (B) and (D) Long-term motor-learning progress expressed as slopes of individual linear regressions over the progress test scores during the 4-week motor-learning paradigm, that is, expressing the average improvement on the BTT score (%) per minute training on the BTT. (E) Short-term motor-learning progress expressed as slopes of individual linear regressions over the individual trials performed during the MRS acquisition at PRE, MID, and POST measurement. Slopes indicate the average improvement on the BTT score (%) per minute. Slopes close to 0 (gray dashed line) indicate no learning. *Boxplots*: The box and horizontal bar indicate the interquartile range (Q1–median–Q3), the whiskers span over the min and max values (up to 1.5×IQR, outliers marked by a dash), and "×" marks the mean. Asterisks indicate a significant difference in BTT slope as compared with nonsignificant (n.s.) results.

subtasks of the BTT PT. For $PT\_BTTscore_{Complex}$, the time spent on the BTT ($\beta = 0.0618, SE = 0.0027, t(310.00) = 22.967, p < 0.0001$) and the interaction $GROUP \times BTT_{time}$ ($GROUP \times BTT_{time}$: $F_{1,310} = 93.766$, $p < 0.0001$; $GROUP[complex] \times BTT_{time}$: $\beta = 0.0363$, $SE = 0.0037$, $t(310.00) = 9.683$, $p < 0.0001$) had a significant effect on task performance (see Figure 3C; for model

details, see Appendix 2C). When comparing the mean performances of the two groups for each PT, group differences started to emerge from the third PT onwards, but not the first two PTs during which participants had not yet started the motor training program (PT1: $p = 0.4733$; PT2: $p = 0.3462$; PT3–6: All $p < 0.004$; with Bonferroni correction $\alpha = 0.05/6 = 0.0083$; tabular results, see Appendix 2D; overview of measurements and PTs, see Figure 1A). Moreover, all subsequent PTs showed a significantly better performance for the later as compared with the earlier test moment in each of the groups separately (all, $p < 0.0001$, with Bonferroni correction $\alpha = 0.05/5 = 0.01$ for each group; for tabular results, see Appendix 2D). The result of a significant GROUP × BTT_{time} effect on the PT scores was supported by a significant GROUP difference in the learning slopes of the complex tasks ($PT\_BTTslope_{Complex}$; $p < 0.0001$, $t(58.9) = -6.733$, mean ± SD for simple/complex training group respectively: 0.062 ± 0.022/0.098 ± 0.020; see Figure 3D), indicating a steeper slope and consequently a faster learning progress for participants training on the complex training paradigm as compared with the simple one.

3.2 | Behavioral Outcomes During MRS Acquisition

For the BTT learning progress during MRS scans, learning slopes ($MR\_BTTslope_{allLines}$, $MR\_BTTslope_{11}$, and $MR\_BTTslope_{13,31}$) for each group were steeper during the PRE measurement as compared with the MID and the POST measurement (all, $p \leq 0.0165$, with Bonferroni correction $\alpha = 0.05/3 = 0.0167$ for each group of three tests), but not different between the MID and POST measurement (all, $p \geq 0.0758$). When comparing the two training groups at each of the three sessions across time, the learning slopes of the simple training group are steeper than those of the complex training group at the MID and POST sessions for the $MR\_BTTslope_{13,31}$ (both $p \leq 0.0398$, not surviving Bonferroni correction $\alpha = 0.05/3 = 0.0167$), but not during the PRE session or for the $MR\_BTTslope_{11}$ or $MR\_BTTslope_{allLines}$ (see Figure 3E; tabular results, see Appendix 2E).

3.3 | Baseline NM Levels to Predict Motor-Learning Progress

3.3.1 | No Relationship Between Short-Term Motor Learning and GABA+ or Glx Levels

There was no relationship between short-term motor learning during the PRE measurement (i.e., in-scanner change in BTT performance, quantified as $MR\_BTTslope_{allLines}$) and the resting GABA+ or Glx levels in any of the VOIs during the PRE measurement.

3.3.2 | Long-Term Motor Learning on Simple Tasks Is Negatively Associated With GABA+ Levels in Left PMd but Has No Relationship With Glx Levels

Long-term motor-learning progress over the 4-week training period on the simpler straight-line tasks ($PT\_BTTslope_{Lines}$) was negatively associated with GABA+ levels in left PMd (see Figure 4A). When regressed on GABA+ levels, the dependent variable first required a cubic transformation to ensure normality of the residuals and hence comply with model assumptions. After the stepwise modelling procedure, the final model $(PT\_BTTslope_{Lines})^3 \sim GABA\_L\text{-}PMd$ ($F_{1,60} = 4.667$, $p = 0.0348$) showed a significant effect of GABA_L-PMd ($\beta = -0.00027$, SE = 0.00013, $t(60) = -2.160$, $p = 0.0347$), indicating that lower baseline GABA+ levels in left PMd are associated with better motor learning on the simpler task variants, independent of the training group (see Appendix 3A for model details). In comparison, there was no significant influence of Glx levels in either of the VOIs on $PT\_BTTslope_{Lines}$.

3.3.3 | Long-Term Motor Learning on Complex Tasks Was Positively Associated With GABA+ Levels in Right SM1 and Glx Levels in the Left PMd

For the long-term motor-learning progress on the complex task variants ($PT\_BTTslope_{Complex}$), GABA+ levels in right SM1 at baseline were positively associated with better motor learning (see Figure 4B). To comply with model assumptions, first, a quadratic transformation of the dependent variable was conducted. Then, the model was simplified, and the final model $(PT\_BTTslope_{Complex})^2 \sim GABA\_R\text{-}SM1 + GROUP$ ($F_{2,58} = 35.810$, $p < 0.0001$; see Appendix 3B for model details) showed a significant main effect of GROUP (GROUP[complex]: $\beta = 0.00575$, SE = 0.00073, $t(58) = 7.856$, $p < 0.0001$) and GABA_R-SM1 ($\beta = 0.0024$, SE = 0.00086, $t(58) = 2.828$, $p = 0.0064$). The main effect of GROUP indicates a steeper learning slope for the complex compared with the simple learning group (see Figure 3D). The main effect of GABA_R-SM1 indicates that higher GABA+ levels in right SM1 were linked to a steeper learning slope. Likewise, baseline Glx levels in the left PMd were positively associated with better motor learning (see Figure 4C). Again, a quadratic transformation of the dependent variable was necessary to comply with model assumptions. The model was simplified, resulting in the final model $(PT\_BTTslope_{Complex})^2 \sim Glx\_L\text{-}PMd + GROUP$ ($F_{2,59} = 32.640$, $p < 0.0001$; see Appendix 3C for model details). Motor-learning progress on the complex task variants was significantly positively influenced by higher Glx levels in left PMd at baseline ($\beta = 0.00050$, SE = 0.00021, $t(59) = 2.328$, $p = 0.0234$), and was better for the complex as compared with the simple training group (GROUP[complex]: $\beta = 0.00567$, SE = 0.00074, $t(59) = 7.655$, $p < 0.0001$).

3.4 | Changes in Resting NM Levels as a Result of Motor Learning

3.4.1 | Left SM1 GABA+ Levels: No Change

For the left SM1 VOI, resting GABA+ levels were not significantly influenced by motor learning. In contrast, the final model $GABA\_L\text{-}SM1 \sim GROUP + (1|SubjectID)$ (observations = 185, groups in random effects = 62) only indicated a significant effect of GROUP (GROUP[complex]: $\beta = 0.170$, SE = 0.849, $t(62.15) = 2.005$, $p = 0.0493$). Since this result was not of interest but rather an overall group difference persisting over all three measurements, no further model details are being reported.

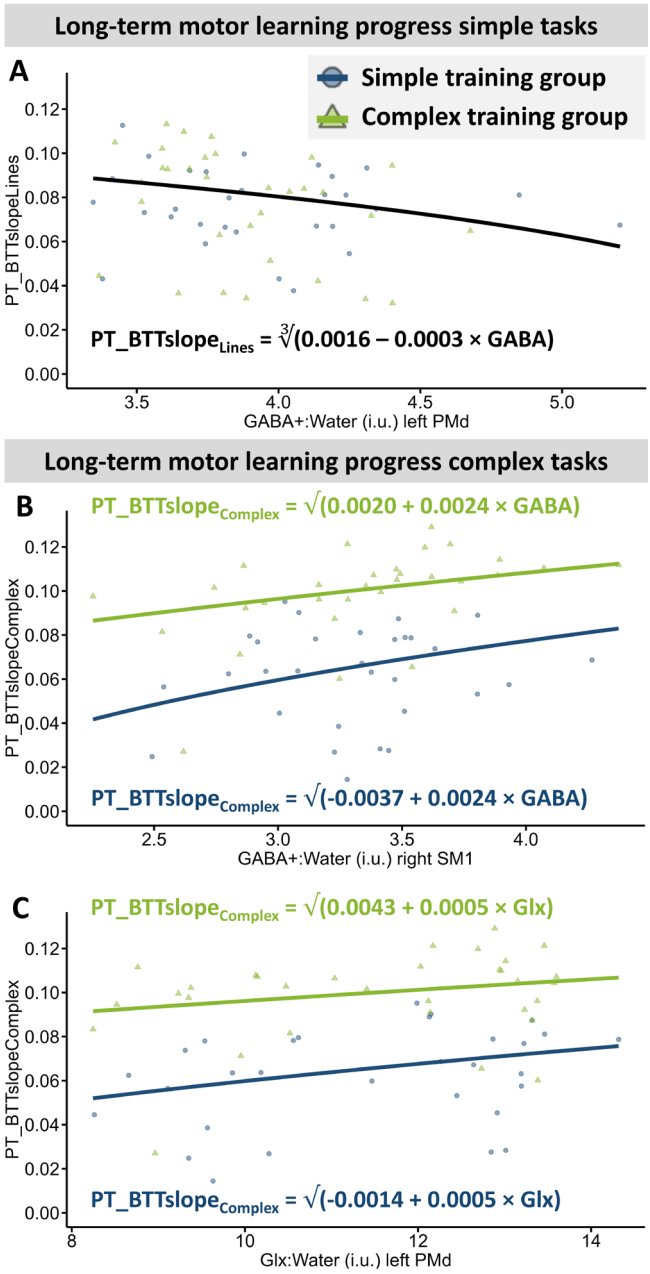

FIGURE 4 | Effect of baseline neurometabolite levels on long-term motor-learning progress. (A) Effect of GABA+ levels in left dorsal premotor cortex (PMd) on motor-learning progress on simple tasks (PT_BTTslopeLines). Since there was no effect of training group, only one regression line is displayed. (B, C) Effect of GABA+ levels in right primary sensorimotor cortex (SM1) (Panel B) and Glx levels in left PMd (Panel C) on motor-learning progress on complex tasks (PT_BTTslopeComplex). Since there was a significant effect of group, regressions for the simple (blue) and complex (green) learning groups are shown.

3.4.2 | Left SM1 Glx Levels: Decrease Over 4 Weeks of Training, but Only for the Complex Training Group

The Glx levels in the left SM1 VOI were related to a GROUP × SESSION interaction (see Figure 5A). The model assumptions were fulfilled without any transformation, and since the model yielded an interaction effect, no further simplification of the model was performed (final model: Glx_L-SM1 ~ GROUP + SESSION + GROUP × SESSION + (1|SubjectID) [observations = 185, groups in random effects = 62]). The interaction effect of GROUP × SESSION had a significant influence on Glx levels in left SM1 (GROUP × SESSION: $F_{2,123.13} = 4.769$, $p = 0.0101$; GROUP[complex] × SESSION[MID]: $\beta = -0.604$, SE = 0.310, $t(123.24) = -1.945$, $p = 0.0540$; GROUP[complex] × SESSION[POST]: $\beta = -0.947$, SE = 0.310, $t(123.24) = -3.053$, $p = 0.0028$), but neither the main effect GROUP ($p = 0.4287$) nor SESSION ($p = 0.5505$) had a significant influence. To explore this interaction effect, one LMM per GROUP was constructed according to the formula Glx_L-SM1 ~ SESSION + (1|SubjectID). This model showed a significant effect of SESSION for the complex training group (SESSION: $F_{2,63.15} = 3.673$, $p = 0.0310$; SESSION[MID]: $\beta = -0.208$, SE = 0.218, $t(63.26) = -0.955$, $p = 0.3435$; SESSION[POST]: $\beta = -0.581$, SE = 0.218, $t(63.26) = -2.669$, $p = 0.0097$), but not for the simple training group ($p = 0.1441$). Post hoc tests within the complex training group revealed that the effect of SESSION on the Glx levels of the left SM1 resulted from a difference between PRE and POST measurement ($p = 0.0135$, $t(30) = 2.624$, mean difference = 0.607, 95% CI = 0.135–1.080; surviving Bonferroni correction $\alpha = 0.05/3 = 0.0167$), but that neither PRE nor MID ($p = 0.3369$) nor MID and POST ($p = 0.0986$) measurements significantly differed from each other, indicating that the Glx levels at rest in the left SM1 of the complex training group decreased significantly when looking at the full 4 weeks of motor training, but that the effect was not strong enough to reach significance for the intermediate comparisons of 2 weeks each (PRE vs. MID, MID vs. POST; for details on the LMMs and post hoc tests, see Appendix 4A).

3.4.3 | Right SM1 GABA+ Levels: No Change

For the right SM1, resting GABA+ levels did not change significantly with motor training.

3.4.4 | Right SM1 Glx Levels: Increase During the First 2 Weeks of Motor Training

For the right SM1 VOI, the Glx levels changed with motor training, independent of the group assignment (see Figure 5B). After the stepwise backwards simplification, the final model Glx_R-SM1 ~ SESSION + (1|SubjectID) (observations = 185, groups in random effects = 62) was obtained. SESSION significantly influenced resting Glx_levels in right SM1 (SESSION: $F_{2,123.2} = 4.159$, $p = 0.0179$; SESSION[MID]: $\beta = 0.474$, SE = 0.165, $t(123.28) = 2.884$, $p = 0.0046$; SESSION[POST]: $\beta = 0.234$, SE = 0.165, $t(123.28) = 1.421$, $p = 0.1578$), and post hoc tests revealed a significant increase in Glx levels from PRE to MID session ($p = 0.0043$, $t(60) = -2.968$, mean difference = −0.476, 95% CI = −0.797 to −0.155; surviving Bonferroni correction $\alpha = 0.05/3 = 0.0167$), but no significant differences between PRE and POST ($p = 0.1499$) or MID and POST ($p = 0.1933$) measurements. This indicates an increase in resting Glx levels of the right SM1 in the first 2 weeks of motor training, independent of the training group, that partially renormalized toward the POST measurement (for details on LMMs and post hoc tests, see Appendix 4B).

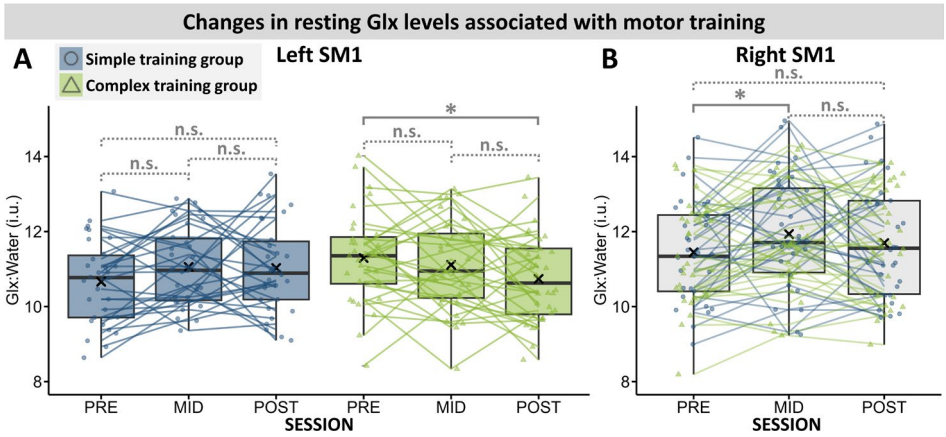

FIGURE 5 | Changes in resting Glx levels associated with motor training intervention. (A) Changes in resting Glx levels in the left primary sensorimotor cortex (SM1). Groups are displayed separately due to the significant effect of the group. (B) Changes in resting Glx levels in the right SM1, with means pooled for the two training groups. *Boxplots*: The box and horizontal bar indicate the interquartile range (Q1–median–Q3), the whiskers span over the min and max values (up to 1.5×IQR, outliers marked by a dash), and "×" marks the mean. Asterisks indicate a significant difference in Glx levels as compared with nonsignificant (n.s.) results.

3.4.5 | Left PMd GABA+ Levels: No Change

For the left PMd, resting GABA+ levels did not change significantly with 4 weeks of motor training.

3.4.6 | Left PMd Glx Levels: No Change

Resting Glx levels in left PMd did not change either as a result of the motor training intervention.

3.5 | Modulation of Left PMd NM Levels During Task Execution and Changes in Modulation With Motor Learning

3.5.1 | No Modulation of the GABA+ or Glx Levels in Left PMd Associated With Task Performance in the Scanner During the PRE Measurement

For GABA+, the model was simplified and yielded neither an effect of the training paradigm nor of the task execution in the scanner on GABA+ levels in left PMd during the PRE measurement. Similarly, Glx levels were not influenced by the training group or task execution.

3.5.2 | No Change in GABA+ or Glx Modulation Associated With Motor Training

Stepwise backwards regression using LMMs yielded no significant influence of measurement session, task execution, or training group assignment on GABA+ levels in left PMd when investigating data from all three measurement sessions. Likewise, no influence of task execution on Glx levels in left PMd was found. However, the LMM yielded changes in Glx levels of left PMd when pooled over the four MRS acquisition blocks (BLOCK_RESTbefore, BLOCK_BTT1, BLOCK_BTT2, BLOCK_RESTafter; due to the removal of the TASK main and interaction effects). More specifically, stepwise simplification resulted in the final model: Glx_L-PMd ~ GROUP + SESSION + GROUP × SESSION + Water_FWHM + (1|SubjectID) (observations = 744, groups in random effects = 62). This model showed a significant influence of SESSION ($p = 0.0005$), the GROUP×SESSION interaction ($p = 0.0017$), and the covariate Water_FWHM ($p = 0.0140$), but not the main effect of GROUP ($p = 0.5015$). Since this result corresponds to a change in Glx levels (and more specifically, a decrease in Glx levels of the complex training group toward the POST measurement) when pooling the data over the four MRS acquisition blocks in the left PMd (which was not a main question of this research), the results of this LMM analysis including a visualization are reported in Appendix 5A. The task-related changes in GABA+ and Glx per subject and session are visualized in Appendix 5B.

3.6 | Relationship Between Baseline Maximal Modulation of NM Levels and Motor Learning

3.6.1 | The Absolute Maximal Modulation at the PRE Measurement Is Predictive of Short-Term but Not Long-Term Motor Learning

Short-term motor learning within the PRE measurement session ($MR\_BTTslope_{allLines}$) was related to the absolute maximal modulation (|MAXmod|) of NMs in left PMd. After the removal of one outlier with a highly negative learning slope that distorted the residuals' normality in a way that could not be corrected by transformations, no further model simplification was necessary: $MR\_BTTslope_{allLines} \sim GABA\_|MODmax| + Glx\_|MODmax| + GABA\_|MODmax| \times Glx\_|MODmax|$ ($F_{3,57} = 3.645$, $p = 0.0178$), with a significant influence of GABA_|MODmax| ($\beta = 0.2451$, SE = 0.0913, $t(57) = 2.686$, $p = 0.0095$), Glx_|MODmax| ($\beta = 0.1662$, SE = 0.0565, $t(57) = 2.942$, $p = 0.0047$), and the interaction GABA_|MODmax| × Glx_|MODmax| ($\beta = -0.2442$, SE = 0.1031, $t(57) = -2.368$, $p = 0.0213$). The interaction effect reflects that individuals with a high task-related absolute modulation of GABA+ or Glx at the PRE measurement had a steeper motor-learning slope during the PRE measurement as

compared to individuals showing a high modulation in both NMs simultaneously or no modulation in either of the NMs. Moreover, moderate modulation of both NMs resulted in moderate motor-learning slopes (see Figure 6; see Appendix 5C for model details). Interestingly, for the interaction between the two metabolites, a data-driven tipping point for each NM was estimated, at which the relationship between the remaining metabolite modulation and motor learning changed the sign of the slope (see Figure 6B). For GABA_|MAXmod| values below or above 0.68, Glx_|MAXmod| had, respectively, a positive or negative influence on short-term motor-learning progress. Likewise, for Glx_|MAXmod| values below or above 1.00, GABA_|MAXmod| has a positive or negative association with short-term motor learning, respectively.

In contrast, long-term motor-learning progress was not associated with the absolute maximal NM modulation. More specifically, there was no relationship between the absolute maximal modulation during the PRE measurement and the subsequent 4-week motor learning for the learning progress on the simple ($PT\_BTTslope_{Lines}$; quadratic transformation applied) or the complex subtasks ($PT\_BTTslope_{Complex}$; quadratic transformation applied).

3.6.2 | No Relationship Between Directional Maximal Modulation at PRE Measurement and Motor Learning

The measure of the directional maximal modulation (MAXmod) within left PMd during the PRE measurement was neither predictive of short-term motor learning ($MR\_BTTslope_{allLines}$, after removal of one outlier with a highly negative learning slope, that is, the same data point as removed from |MAXmod| regression above), nor of simple ($PT\_BTTslope_{Lines}$) or complex long-term motor learning ($PT\_BTTslope_{Complex}$).

4 | Discussion

We investigated the role of GABA+ and Glx levels in human motor learning and revealed four main results. First, the 4-week training period on a complex bimanual coordination task (BTT) led to increases in performance that were significantly higher for participants enrolled in a complex as compared with a simple task training paradigm, and the complex subtasks continued to improve until the end of the training period. Second, baseline GABA+ levels in right SM1 and Glx levels in left PMd showed a positive association with the learning progress on the complex subtasks of the BTT, whereas baseline GABA+ levels in left PMd were negatively associated with long-term motor-learning progress on the simpler subtasks. There was no relationship between baseline NM levels and short-term motor learning. Third, left SM1 Glx levels at rest decreased over 4 weeks of motor training for the complex but not simple training group, whereas Glx levels in right SM1 increased during the first 2 weeks of motor training, independent of the training group. No change in resting GABA+ levels was reported in any of the VOIs. Likewise, for the left PMd, no changes in resting GABA+ or Glx levels were reported. Fourth, there was no task-related modulation in left PMd GABA+ or Glx levels at the group level. However, the task-related absolute maximal modulation of GABA+ and Glx levels in left PMd, assessed at the initial stage of the motor training

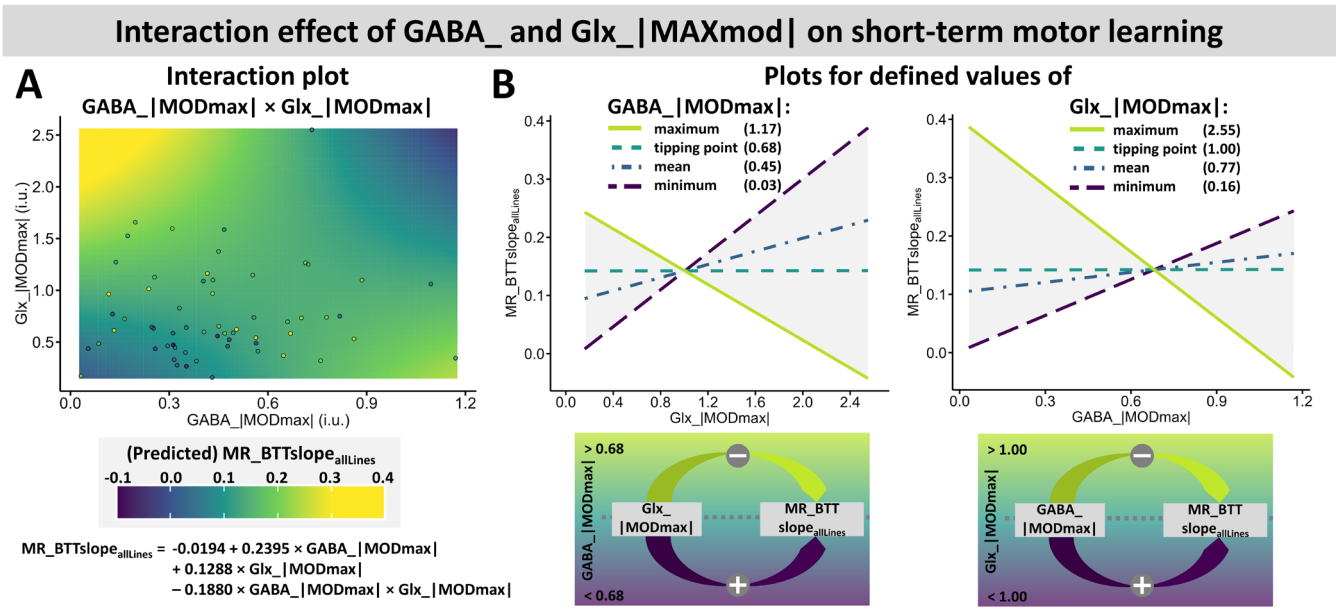

FIGURE 6 | Effect of absolute maximal modulation interaction between GABA+ and Glx on short-term motor learning during PRE measurement. (A) Interaction plot and formula with individual data points. High absolute maximal modulation (|MAXmod|) of either GABA+ or Glx, but not both simultaneously or neither, predicts better motor learning, creating a balance when both are moderately modulated. (B) *left*: Influence of Glx_|MAXmod| on short-term motor-learning progress ($MR\_BTTslope_{allLines}$) for defined values of GABA_|MAXmod|. *Right*: Influence of GABA_|MAXmod| on short-term motor-learning progress ($MR\_BTTslope_{allLines}$) for defined values of Glx_|MAXmod|. For both graphs, the minimum, mean, and maximum measured value for the neurometabolite absolute modulation were plotted. The "tipping point" value describes the value at which the influence of the neurometabolite on the *x*-axis on $MR\_BTTslope_{allLines}$ changes from a positive to a negative association or vice versa. Predictable gray areas are located between the minimal and maximal values that |MAXmod| assumed in the present study.

intervention, showed a relationship with short-term but not long-term motor-learning progress, indicating the importance of the ability to adapt or tune excitation and inhibition in function of the task requirements to facilitate motor learning.

4.1 | Ongoing Motor Learning Until the End of the Training Intervention

We implemented a 4-week bimanual motor training regimen designed to induce learning and consequent neuroplasticity in task-related brain regions, and to investigate the role of excitatory and inhibitory neurotransmitters during this process. The motor training led to ongoing performance improvements up to the end of the 4-week intervention period, equivalent to a total of approximately 11 h spent on the BTT. Notably, each subsequent pair of PTs up to the end of the intervention showed significant improvements for both training groups and on both subtask classifications, that is, the simpler straight-line tasks and the complex tasks. This finding contrasts with earlier studies (Beets et al. 2015; Solesio-Jofre et al. 2018) employing only straight-line subtasks of the BTT, which reported a performance plateau after 4 h of motor training. In contrast, in the current study, the relatively linear performance gains on the complex subtasks that were sustained until the end of the training period provide a solid behavioral foundation for examining neuroplastic changes associated with motor learning.

In contrast to the complex training group, the simple training group only practiced the iso-frequency (1:1 line) condition, a movement pattern that is part of the intrinsic motor repertoire (Wenderoth et al. 2005), and was only exposed to more difficult task variations during the PTs (~5 min each). Hence, we expected similar performance gains of both groups on the straight-line tasks, but smaller improvements in the simple group as compared with the complex training group for the complex subtasks of the BTT. In line with this hypothesis, the results indicated no group difference in the motor-learning progress on the straight-line tasks. However, for the complex subtasks of the BTT, the complex training group outperformed the simple training group from the third PT on (i.e., as soon as the actual training program started, because the first two PTs were acquired before the first training week). This indicates faster and more profound learning in the complex group as compared with the simple training group for the complex subtasks, which was also reflected in steeper learning slopes observed during the MRS acquisition (i.e., in-scanner learning progress when being exposed to 1:1, 1:3, and 3:1 lines) in the MID and POST sessions for the simple training group, as compared with the complex training group.

4.2 | Baseline NM Levels in Right SM1 and Left PMd Predict Long-Term Motor-Learning Progress

Investigating the relationship between baseline NM levels and subsequent short- and long-term motor-learning progress yielded no relationship between baseline GABA+ or Glx levels and short-term motor learning. In contrast, for long-term motor learning, significant associations with right SM1 GABA+ levels, as well as left PMd GABA+ and Glx levels, were observed. More specifically, left PMd GABA+ levels were negatively associated with long-term motor-learning progress on simpler subtasks, whereas the Glx levels in left PMd showed a positive association with the learning progress on complex subtasks of the BTT. Furthermore, baseline GABA+ levels in right SM1 also showed a positive relationship with long-term motor learning on the complex tasks.

4.2.1 | Baseline GABA+ Levels and Long-Term Motor Learning on the Simple Tasks

We found a significant association between lower GABA+ levels in left PMd and better long-term motor learning on the simple subtasks, and between higher GABA+ levels in right SM1 and better motor learning on the complex subtasks of the BTT. Although there is no previous evidence on the role of left PMd and right SM1 NM levels at baseline on the subsequent motor-learning progress, there have been various reports on the role of GABA and Glx baseline levels in left SM1 for motor learning. However, this prior evidence only concerns shorter periods of motor learning up to 5 days. For baseline GABA levels in left SM1, results of Chalavi et al. (2018) were in line with the current findings. More specifically, GABA levels in the left SM1 were not related to the learning progress of a 3-day BTT training, despite a negative association between GABA levels in left SM1 and initial performance (Chalavi et al. 2018). Similar results were reported for an SRTT, where baseline GABA levels in left SM1 were negatively correlated with initial performance, but showed no correlation with motor learning (Stagg et al. 2011). In contrast, other studies did show positive or negative associations between GABA levels in SM1 and motor learning. One study showed a positive association between baseline GABA levels in M1 (when no direct feedback was provided) or S1 (when direct visual feedback was given) and the initial learning progress on the BTT, but no association with the later learning progress over a 5-day BTT training (Li et al. 2024). Another study, however, reported a negative association between baseline GABA levels in left M1 and subsequent motor learning within a 40-min training session on a sequential reaction time task performed with the right hand (Kolasinski et al. 2019).

These findings are not surprising in light of the importance of GABA in motor learning. A single administration of baclofen, a $GABA_B$ receptor agonist which reduces neuronal excitability, has been shown to impair subsequent visuomotor learning (Johnstone et al. 2021) and neuroplastic LTP-like neuroplastic processes (McDonnell et al. 2007). Along the same lines, another study reported a decrease in GABA-related inhibition to facilitate practice-dependent plasticity in the motor cortex both neurophysiologically and behaviorally, whereas an increase in $GABA_A$-related inhibition via lorazepam administration depressed neuroplasticity (Ziemann et al. 2001). Hence, it could be hypothesized that lower GABA levels in motor-related brain regions at baseline may enable increased communication between neurons during motor practice. This would facilitate LTP-like neuroplasticity (Kim et al. 2014), which in turn can lead to alterations in synaptic communication (Castillo et al. 2011; Sanes and Donoghue 2000) and hence a better consolidation of motor skills (Harms et al. 2008). However, also higher baseline GABA levels have been linked to better motor learning (Li et al. 2024). One possible hypothesis for the underlying mechanism of these opposite results might be that higher baseline GABA levels could

provide a greater range for learning-related decreases in GABA, allowing more room for modulation and, consequently, better motor learning (King et al. 2020). Furthermore, the effect might be region-specific. For example, the current study yielded lower GABA+ levels in left PMd to be associated with better long-term motor learning. This decreased inhibition might be in line with the increased activation of PMd during complex bimanual motor tasks (Van Ruitenbeek et al. 2023).

4.2.2 | Baseline Glx Levels and Long-Term Motor Learning on the Complex Tasks

In addition, a positive association between higher Glx levels in left PMd and subsequent long-term motor learning on the complex subtasks was shown. Again, there is no prior evidence on the link between the PMd's NM levels and motor-learning progress, and only results for shorter motor-learning interventions are available. For the left SM1, however, previous work reported no relationship between baseline Glx or Glu levels and subsequent motor learning. More specifically, one study reported no link between baseline Glx levels in left M1 or S1 and initial or later learning progress (Li et al. 2024). Similarly, for an SRTT, no relationship between left M1 Glu (Kolasinski et al. 2019) or left SM1 Glx levels (Stagg et al. 2011) at baseline and learning progress within the same session was reported. In contrast, one study did report a positive relationship between baseline Glx levels in left SM1 and motor learning of an SRTT (Bell et al. 2023).

As the main excitatory neurotransmitter in the mammalian brain, the role of glutamatergic neurotransmission for motor learning is vital, because it underlies the synaptic plasticity processes necessary for acquiring and refining motor skills (Zhou and Danbolt 2014). This association has become clearer through animal research, in which the advantages of variable over constant practice were linked to a greater dependency on the glutamate receptors n-methyl-D-aspartate (NMDA) and more expression of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor has been shown (Apolinário-Souza et al. 2020). Furthermore, it has been repeatedly described that the induction of LTP- and LTD-like plasticity is dependent on the NMDA receptor (for review, see Lüscher and Malenka 2012). Besides this link between neuroplasticity and glutamatergic neurotransmission, also a positive association between brain activation (as measured with fMRI) and Glu levels has been shown (Maruyama et al. 2021), which might indicate the importance of Glu to effectively activate a brain region.

4.2.3 | Right Versus Left SM1

This study yielded no links between motor learning for the baseline NM levels in the left SM1, but a positive association between baseline GABA+ levels in right SM1 and complex motor learning. There are several possible explanations as to why the baseline NM levels in the right (nondominant) but not the left (dominant) SM1 were predictive of motor learning. First, in general, turning the BTT dial with the dominant hand might be easier compared with the nondominant hand, leading to greater motor learning effects in, and greater importance of, the right nondominant SM1. Behaviorally, this is supported by a BTT study that reported higher scores for straight-line tasks when the dominant hand had to move faster than the nondominant hand, as compared with the opposite condition (Sisti et al. 2011). Second, the encoding of right/left movements via the right/left dial rotations using the dominant hand might be more straightforward than the up/down encoding via the nondominant hand, and hence might have required more learning to cope with this less compatible visuomotor transformation, making the effect of baseline NM levels in the nondominant SM1 and especially GABA to shape motor output more influential.

4.2.4 | Left PMd

Lower GABA+ and higher Glx levels in left PMd at baseline were associated with better long-term motor learning on simple or complex tasks of the BTT, respectively. These findings can potentially be explained by fMRI findings on the role of left PMd in motor tasks and motor learning. More specifically, task-related premotor activity has been shown to increase with BTT complexity (Van Ruitenbeek et al. 2023). The observed higher excitatory Glx levels in left PMd might hence be a prerequisite for a better task-related activation of left PMd, in turn allowing for better movement planning and control during complex BTT trials. Furthermore, left PMd activation has been associated with better motion smoothness (Sosnik et al. 2014), an important feature for obtaining high scores on the BTT, especially when tracing straight lines. This might be linked to the observed correlation with lower baseline GABA levels, which might promote excitation and hence allow a better activation of the left PMd to accomplish smooth dial rotations. Overall, the current findings are in support of the diverse literature that underlines the PMd's central role in fine-tuning the motor output of M1 (Chouinard and Paus 2006; Dum and Strick 1991), planning of bimanual movements (Beets et al. 2015; Debaere et al. 2004; Fujiyama et al. 2016; Verstraelen et al. 2021), response selection (Chouinard and Paus 2006; Crammond and Kalaska 2000), and in learning a diversity of motor tasks (Hardwick et al. 2013).

4.3 | Changes in Resting Left and Right SM1 Glx Levels Associated With Motor Training

An interesting yet unresolved question is whether training does induce lasting changes in the baseline levels of NMs or whether such changes are only transient and short-lived. Here, changes in resting Glx levels of left and right SM1 were observed over a 4-week training paradigm. More specifically, left SM1 Glx levels decreased after 4 weeks of motor training in the complex but not the simple training group. In contrast, Glx levels in right SM1 increased during the first 2 weeks of motor training, independent of the training group. There were no changes in resting GABA+ levels in any of the VOIs or in the resting GABA+ or Glx levels of the left PMd.

Previous evidence on changes in resting NM levels with long-term motor-learning interventions is very scarce. Only in one study left SM1 neurotransmitter levels investigated over a longer period, that is, a 6-week juggling training intervention. A decrease in GABA was reported for a low-intensity (i.e., 15 min, 5×/week) but not high-intensity (i.e., 30 min, 5×/week) training

group (Sampaio-Baptista et al. 2015). This result is in contrast with the current study, where no changes in resting GABA+ levels were reported for any of the VOIs after 2 or 4 weeks of motor training. However, more in line with our findings, a study employing a shorter 3-day BTT training intervention reported no changes in left SM1 GABA levels (Chalavi et al. 2018). Changes in measurement timings, training intensity, and task requirements could have resulted in these discordant results. Unfortunately, neither of these studies (Chalavi et al. 2018; Sampaio-Baptista et al. 2015) investigated changes in Glu or Glx.

Of note is the opposite effect of the motor training intervention on Glx levels of the left and right SM1, with a decrease in Glx only in the complex training group after 4 weeks of training for the left SM1, and an increase in Glx for both groups after 2 weeks of training in the right SM1, with a partial (nonsignificant) renormalization after 4 weeks of motor training. Potentially, effects of lateralization and the frequency of hand use might play a role in this context. More specifically, the bimanual motor training might have led to an increase in excitation in the nondominant (right) SM1 because of the increased use and refinement of left hand control as compared with baseline. In contrast, the dominant (left) SM1 in control of the right hand is by default exposed to high dexterity demands. This might have resulted in a lack of changes in the simple training group, and a decrease in Glx in the complex training group in order to shift the laterality in favor of the nondominant hemisphere, a requirement that might be more prominent during complex rather than simple task training. However, to date, motor-learning studies reporting neurochemical levels in both SM1s simultaneously are lacking, and hence this hypothesis is speculative. Evidence from fMRI studies has shown partially dissimilar effects of 5 days of BTT training on brain activity of the right and left SM1. For example, one study reported a decreased activation of left M1 and right S1 during the planning phase of the BTT, whereas during the task execution phase of the BTT, bilateral S1 and M1 were less activated after the training intervention as compared with baseline (Beets et al. 2015). Furthermore, an increased intra-hemispheric connectivity within the motor network of the right but not left hemisphere has been shown after a 5-day BTT training intervention as compared with baseline (Solesio-Jofre et al. 2018). Taken together, bimanual motor training might lead to dissimilar effects in both hemispheres, potentially reinforced by slightly different task demands for the left versus the right hand (as discussed in the previous section) that might have led to the differential changes in resting Glx levels of the left and right SM1.

4.4 | No Group-Level Modulation of NM Levels in Left PMd During Task Execution, but Link Between Individual Modulatory Ability of Left PMd NMs and Short-Term Motor Learning

Lastly, this research tackled the question of whether there is a modulation of left PMd NM levels during task execution and how this relates to motor learning. First, we investigated the differences between resting or task-related acquisition blocks and the changes in this modulation with 4 weeks of motor learning. This yielded no modulation in GABA+ or Glx levels at the group level. However, at the level of the individual, the task-related absolute maximal modulation of GABA+ and Glx levels in left PMd during the PRE measurement session was associated with short-term but not long-term motor-learning progress. More specifically, there was an interaction effect of GABA+ and Glx absolute modulation on short-term motor learning during the PRE measurement that indicated the best learning progress for individuals with either a high GABA+ or a high Glx modulation, medium learning progress for individuals with simultaneous GABA+ and Glx modulation in a balanced manner, and least learning progress when neither of the NMs was modulated or both showed a strong modulation. This finding points toward the importance of the ability to adapt or tune excitation and inhibition in function of the task requirements to facilitate motor learning.

4.4.1 | Glx Modulations

A meta-analysis on task-related modulation of Glu/Glx yielded increased Glu and Glx levels in task-related brain regions during task execution for various task domains (Pasanta et al. 2022). For the motor domain, this analysis on SM1 data included studies investigating finger tapping (Schaller et al. 2014) and rhythmic hand clenching (Chen et al. 2017; Volovyk and Tal 2020). In the present study, we could not replicate these findings for the left PMd, which was more in line with a report of no change in SM1 Glu levels with motor learning (Bell et al. 2023). There are several possible explanations for this finding. First, the task employed in the present study has higher cognitive and coordinative demands than the tasks investigated in the previous reports, with hand clenching specifically requiring force rather than fine precision motor control (as required for the BTT). These different task requirements might have influenced the results. Second, although the meta-analysis revealed Glu/Glx changes across various brain areas including visual, cognitive, and learning-related task domains, there are no prior results on the premotor region. Third, MRS acquisition and quantification of Glu/Glx can be obtained using various approaches that influence the final quantification. In this study, a MEGA-PRESS sequence was used to acquire MRS spectra in order to study changes in GABA+ levels. Furthermore, the fitting algorithm included in the Gannet toolbox is specifically optimized for the quantification of GABA signals, with the Glx quantification rather being a byproduct, because neither the acquisition parameters nor the analysis is perfectly tailored to it. This also results in the pooling of Glu and glutamine signals into the Glx signal, which might conceal changes in Glu levels due to opposing changes in glutamine signals. At last, fMRS can be analyzed either blockwise (i.e., per acquisition block), which has limited temporal resolution, or in an event-related fashion, which can be more sensitive to transient changes in NMs (Koolschijn et al. 2023). The blockwise analysis employed in the current study has a coarse temporal resolution of 11 min per block, potentially concealing short-term neurotransmitter modulations. However, detecting small metabolites such as GABA at 3 T requires sufficient scanning time to achieve a sufficiently high SNR, limiting the feasibility of event-related designs at commonly available magnetic field strengths.

4.4.2 | GABA+ Modulations

For GABA, a meta-analysis (Pasanta et al. 2022) reported a nonsignificant tendency toward task-related decreases in GABA

levels. However, for the motor domain, only two studies (Chen et al. 2017; Kolasinski et al. 2019) examining GABA modulation were included in this meta-analysis. These studies reported a decrease in GABA levels after several minutes of continuous rhythmic bilateral hand clenching (Chen et al. 2017) and during motor learning on a unimanual SRTT (Kolasinski et al. 2019). Notably, both studies were conducted using a 7 T MR scanner and hence have a better spectral resolution than the 3 T results presented here. However, one study conducted at 3 T was also able to show GABA reductions with the learning of a unimanual serial force production task (Floyer-Lea et al. 2006). In contrast, other 3 and 7 T studies have reported no motor task-related GABA modulation for bimanual finger-tapping (Schaller et al. 2014), motor learning on a unimanual SRTT (Bell et al. 2023; Eisenstein et al. 2023, 2024), or during task transfer after 3 days of motor learning on a BTT (Rasooli et al. 2024). Comparison with the here-presented finding of no modulation in GABA+ levels during bimanual motor learning in the left PMd should, however, be done with care, as previous evidence is based on results from SM1, not PMd.

4.4.3 | Interaction Effect of Initial Absolute GABA+ and Glx Modulation on Short-Term Motor Learning

Although the present study yielded no modulation in GABA+ or Glx levels at the group level with motor learning, there was an interaction between the inter-individual amount of absolute GABA+ and Glx modulation before motor training and short-term motor learning during the PRE measurement. Several studies support the notion that the ability to modulate brain metabolites is essential for successful motor learning. One study employing anodal tDCS yielded that the ability to modulate (i.e., decrease) GABA levels in left M1 was associated with better motor learning on an SRTT (Stagg et al. 2011). Furthermore, greater decreases in the GABA/Glu ratio within a single session of SRTT training were correlated with higher performance gains (Maruyama et al. 2021). Additionally, higher Glu increases after SRTT motor learning were predictive of higher overnight offline learning gains (Eisenstein et al. 2024). Taken together, these results point toward the importance of the brain's flexibility to modulate NMs efficiently for better task performance and learning success, and more specifically shift the E-I balance in the direction of less inhibition and more excitation. Furthermore, the present results suggest that early NM flexibility in left PMd is particularly relevant for initial motor adaptation. It also appears that the process of motor learning, and its neural correlates are characterized by high inter-individual differences that might be too subtle or too diverse to be revealed at the group level.

5 | Conclusion

In this study, we aimed to address the gap in evidence regarding neurometabolic changes associated with long-term motor learning. We investigated the relationship between inhibitory and excitatory NMs and the motor-learning process. This study yielded that an initially more excitatory NM profile in left PMd, and increased inhibition in nondominant SM1 predicted long-term motor training success. Furthermore, after 2 weeks of motor training, the resting excitatory NM levels in the nondominant SM1 showed a transient increase regardless of training complexity, which partially renormalized after 4 weeks. For the dominant SM1; however, the excitatory NMs gradually decreased over the 4-week training period, but only for complex, but not simple, motor training. Lastly, NMs in left PMd showed no task-related modulation at the group level. However, the individual interplay between excitatory and inhibitory neurotransmitter modulation in left PMd during initial motor learning influenced short-term training success.

Author Contributions

M.H., K.C., and S.P.S. designed the study. M.H. performed the data acquisition with help from S.V.M. and K.C. M.H. analyzed the data. M.H. wrote the manuscript with input from all authors (S.V.M., S.B., S.S., R.A.E.E., S.P.S., and K.C.).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.