# The Cognitive Demand of Synchronizing to the Beat:

# An fNIRS and Gait Study

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# Table of Contents

**Abstract**

Similar motor regions of the brain are thought to be involved in external beat perception and internally generated rhythmic movement. Rhythmic auditory stimulation (RAS) therapy uses auditory cues such as music to treat gait impairments in neurological conditions such as Parkinson's disease. However, it remains poorly understood why populations varying in health, age, and beat perception ability exhibit different gait responses during walking with RAS. Moreover, little is known about the neural correlates of these populations exhibiting different gait responses during walking with RAS. We investigated differences in motor cortex activity and gait characteristics in young healthy adults across three conditions: silent walking; freely walking with music; synchronized walking to music, and across two groups: musicians and non-musicians. We hypothesized that requirements to synchronize to the beat of the music would increase activity in the supplementary motor area, premotor cortex, and primary motor cortex, and increase spatiotemporal gait variability. Participants walked on a pressure-sensitive walkway while functional near-infrared spectroscopy (fNIRS) recorded changes in oxygenated (HbO) and deoxygenated (HbR) hemoglobin as measures of cortical activity. Results revealed no significant differences activity within the motor regions of the brain during synchronization compared to free and silent walking. However, spatiotemporal gait parameters may interact differently with left primary motor cortex activity in musicians and non-musicians. Regardless of cortical activity, strides were longer, faster, and less variable when freely walking to music compared to silent and synchronized walking, with these changes more pronounced in musicians. These results indicate that free walking to music may be the optimal condition for gait outcomes in young healthy adults. Ultimately, more research is needed to understand the neural correlates of walking with RAS and how they may change depending on the population of interest.

## 1. Introduction

When walking down the street while listening to music, an individual will probably be cognisant of their favourite song and its beat pulsating in their ear. What they may be unaware of, however, is that their footsteps consistently produce their own beat as well. The rhythmic nature of walking suggests that beats are interpreted not only from external acoustical sources (such as music) but can also be internally generated by the brain (Nombela et al., 2013). External beat perception and internally generated rhythmic movement are also not mutually exclusive in function and can frequently interact through sensorimotor synchronization (hereafter synchronization): when movement is coordinated with an external beat or rhythm (Repp & Su, 2013). Rhythmic auditory stimulation (RAS) in gait rehabilitation research often involves music or a metronome which acts as an external cue. Parkinson's disease patients can synchronize their footsteps to this cue, providing a compensatory mechanism to improve what are normally debilitating gait impairments (Nombela et al., 2013; Thaut et al., 1996). However, person-to-person differences in temporal processing seen amongst healthy populations illuminate conditions which produce and amplify positive or detrimental gait changes from synchronization with RAS (Grahn & McAuley, 2009; Leow et al., 2014; Ready et al., 2019). Research has raised questions regarding the dynamics of RAS and how the requirement to synchronize footsteps to a beat increases cognitive demand and potentially impairs gait (Leow et al., 2018; Leow, Watson, et al., 2021; Ready et al., 2019, 2022). However, mobility limitations with functional magnetic resonance imaging (fMRI) have prevented correlations from being made between active changes in cortical activity (representative of cognitive demand) and changes in gait. Functional near-infrared spectroscopy (fNIRS) provides a means to obtain such correlation (Scarapicchia et al., 2017). This study assesses how synchronization demands affects motor regions of the brain and

gait characteristics simultaneously compared to when freely walking with RAS and silent baseline walking.

### 1.1 Factors Impacting Cognitive Demand and Gait Response

Synchronization, especially with gait, is naturally understood as a more difficult, and therefore more cognitively demanding task, compared to freely walking (Leow et al., 2018). Young, healthy participants who are good beat perceivers are the least affected by this increase in cognitive demand – frequently demonstrating maintenance of, or little detriment to gait stability parameters compared to free walking with RAS or silent walking (Ready et al., 2019, 2022). Poor beat perception reduces the ability to accommodate for the increase in cognitive demand – when asked to synchronize, poor beat perceivers exhibited larger impairments to gait stability, compared to free walking with RAS or silent walking (Ready et al., 2019, 2022). Using high-groove (how much the music makes one want to move) and familiar music has been observed to consistently reduce the cognitive demand of RAS and synchronization for strong and weak beat perceivers (Leow et al., 2014, 2015; Park et al., 2021; Ready et al., 2019). Yet, regardless of individual differences, groove or familiarity, multiple studies have concluded that healthy participants elicit longer, faster, and more stable strides when allowed to freely walk with RAS or walk in silence (Leow et al., 2018; Leow, Watson, et al., 2021; Ready et al., 2019).

Implementing RAS within pathologically poor beat perceivers, such as Parkinson's disease patients, reveals a completely different outcome in gait characteristics compared to healthy beat perceivers. In fact, most synchronization and RAS literature is grounded in the understanding that Parkinson's disease patients improve their stride length, velocity, and stability when they synchronize their walking to RAS (Ghai et al., 2018; Hove et al., 2012). Supposedly, RAS provides an external cue that Parkinson's disease patients can match their steps to, thereby

compensating for impairments in internal beat production, timing, and rhythmic movement (Nombela et al., 2013). However, the compensatory mechanisms have come under scrutiny recently as research suggests that beat perception ability, and consequentially, the ability to accommodate for increases in cognitive demand, also affects the efficacy of synchronization and RAS for gait rehabilitation in Parkinson's patients (Bella et al., 2017, 2018; Cochen De Cock et al., 2018). Nevertheless, the same increase in cognitive demand due to RAS and synchronization which is detrimental to healthy participants continues to improve gait in Parkinson's disease patients (Park, 2022). Such variety in gait outcomes from implementing synchronization and RAS across different populations reveals a need to understand the dynamics of the cognitive demand being imposed upon the neural circuitry involved in gait, beat perception and synchronization.

### 1.2 The Neural Mechanisms of Rhythmic Movement and Timing

In 2007, Grahn and Brett asked participants to perform a beat perception task while brain activity was monitored using an fMRI. Although participants were not moving, motor regions such as the pre-motor cortex (PMC), supplementary motor area (SMA) and basal ganglia (BG) were consistently activated. These regions are considered primary components of a motor-cortico-basal-ganglia-thalamo-cortical (mCBGT) circuit crucial for beat perception, synchronization, and internal beat production (Grahn & Rowe, 2009; Merchant et al., 2015; Proksch et al., 2020; Rao et al., 1997).

#### 1.2.1 Supplementary Motor Area in Beat Perception and Gait

To justify further research on the SMA and how it correlates with beat perception and gait in healthy individuals, it is often helpful to understand cases of joint SMA, beat perception and gait pathology. Because fMRI cannot be used while walking, the symptomology of

Parkinson's disease and stroke cases, along with brain stimulation methods, provide evidence that gait and beat perception processes are integrated into the same or similar mCBGT circuit (Konoike et al., 2015; Nombela et al., 2013; Rahimpour et al., 2022). People with Parkinson's frequently exhibit motor impairments in their gait (Mirelman et al., 2019) but also impairments in beat perception (Grahn & Brett, 2009). These symptoms have been attributed to abnormal hypo- and hyperactivation of the SMA when estimating time intervals, temporal processing, and tasks involving more cognitive effort such as synchronization and initiation of movement (Eckert et al., 2006; Elsinger et al., 2003; Konoike et al., 2015; Rahimpour et al., 2022). Recently, repetitive transcranial magnetic stimulation (rTMS) to the left pre-SMA was observed to widely improve Parkinson's disease symptoms, including gait-related impairments (Saricaoglu et al., 2022). In healthy individuals, using transcranial direct current stimulation (tDCS), researchers observed that increasing SMA excitability improved rhythm discrimination whereas decreasing SMA activity impaired rhythm discrimination (Leow, Rinchon, et al., 2021). Finally, a recent case study examined an individual with stroke-induced injury to their right SMA who displayed significant gait impairments, further emphasizing the SMA's role in rhythmic movement (Yada & Kawasaki, 2022). The SMA therefore plays an integral role in gait production and temporal processing, as elucidated by pathological cases and stimulation methods in healthy individuals.

### 1.2.2 Primary and Premotor Cortices in Beat Perception and Gait

The PMC is also implicated in rhythmic processing and gait through brain stimulation experiments, with the primary motor cortex (M1) often implicated in gait outcomes as well. In healthy participants, rTMS studies have demonstrated an important role for the dorsal PMC in integrating auditory processing of music and rhythm with motor production and synchronization processes (Giovannelli et al., 2014; Lega et al., 2016). Separate rTMS and tDCS studies have

observed joint stimulation of the PMC and M1 in Parkinson's disease patients to improve gait, namely though increased stability, and stride velocity (Kaski et al., 2014; Lomarev et al., 2006). Freezing of gait, a common cause of falling in Parkinson's disease, was also observed to decrease in frequency with anodal tDCS to the M1 in Parkinson's disease patients (Valentino et al., 2014). The PMC and M1 are therefore indicators of proper gait production, with the former also implicated in effective rhythmic-auditory processing and synchronization.

### 1.3 fMRI to fNIRS: Neuroimaging within Gait Research

#### 1.3.1 fMRI Limitations

The restraints of fMRI's during real-life tasks involving more global movement have been a caveat for decades and are prevalent in beat perception, synchronization, and gait research. Beat perception can occur within an fMRI, however, synchronization tasks are limited to peripheral movements such as finger tapping (Witt et al., 2008). Furthermore, research involving gait has been primarily behavioural, with gait characteristics assessed using different footstep tracking methods (Muro-de-la-Herran et al., 2014). fMRI studies researching gait are confined to participants imagining walking and, although it can be a reasonable representation of real walking (Hamacher et al., 2015), differences between real and imagined walking are unavoidable (la Fougère et al., 2010).

#### 1.3.2 The Utility of fNIRS

fNIRS sacrifices high spatial resolution to allow for incredible freedom of movement and functional activity recordings in real-life scenarios (Balardin et al., 2017). Source optodes shine infrared light at (760nm and 850nm) onto the scalp, penetrating two to three centimetres into the cortex, the light is then reabsorbed by detector optodes. The photons' path from the source to the detector creates an intracortical channel where relative changes in oxygenated

(HbO) and deoxygenated (HbR) hemoglobin can be calculated based on the absorption of the 760nm and 850nm light (Ferrari & Quaresima, 2012; Jöbsis, 1977) (*Figure 1*). Despite challenges with motion-induced physiological artifacts (Erdoğan et al., 2014; Obrig et al., 2000), fNIRS remains the best resource available for movement-based neuroimaging due to its affordability, safety profile, high temporal resolution and reasonable spatial resolution (Perrey, 2008).

### 1.3.3 fNIRS within Gait Research

Gait research implementing fNIRS has predominantly focused on motor cortices under different walking conditions involving obstructed paths, precision stepping and dual-task requirements (Bishnoi et al., 2021; Menant et al., 2020; Vitorio et al., 2017). fNIRS has also been used in conjunction with RAS but not during walking. For example, Curzel et al. (2021) had participants tap an e-drum at a particular beat and assessed the effects of short-term training with various cues to reduce beat speed variability. fNIRS results indicated that short-term behavioural improvements, represented by a decrease in drum tapping speed variability, correlated with a decrease in PMC and SMA activity. Remarkably, little research has yet to combine fNIRS with RAS and synchronization for gait rehabilitation. One study used fNIRS on young and old healthy participants and tested the effects of metronome RAS on gait characteristics (measured using an accelerometer attached to the waist) while walking on a treadmill (Vitorio et al., 2018). Older participants demonstrated activation in the PMC, SMA and primary motor cortex (M1) during synchronization to RAS, correlating with decreases in stride length and speed variability. Younger participants exhibited more stride length and speed variability as the PMC, SMA and M1 increased in activity. Vitorio et al. (2018) conclude that the increases in cortical activity observed in older participants were representative of compensatory

mechanisms allowing for increased temporal and spatial stability in gait. This dissociation in gait changes and cortical activity between young and old participants further emphasizes the lack of understanding regarding relationships between RAS-induced gait changes, cognitive demand, and cortical activity in different populations.

### 1.4 Present Study

To our knowledge, no study has yet combined fNIRS with specifically a pressure-sensitive walkway to simultaneously assess how gait parameters and cortical activity change between conditions of silent, free and synchronized walking to high-groove and high-familiarity musical RAS. We aim to identify how changes in the cognitive demand of each task are represented in SMA, PMC and M1 activity and the correlation it may have with gait response in young healthy participants. We also aim to provide an effective standard operating procedure for fNIRS that can be implemented in future gait research involving older participants and clinical populations such as people with Parkinson's disease. Firstly, we hypothesize that free walking with instrumental music will elicit faster and longer strides compared to synchronized walking. Secondly, instructions to synchronize will be associated with increased variability in stride length and velocity, indicative of increased cognitive demand. Thirdly, we hypothesize that SMA, PMC and M1 activity will increase as participants transition from the baseline silent walking to free walking, then to synchronized walking, representative of the increase in cognitive demand necessary for temporal processing and synchronization. Finally, proposed increases in SMA, PMC and M1 activity will positively correlate with detriments in stride length and stride velocity variability.

## 2. Methods

### 2.1 Participants

Thirteen young healthy adults (n = 13, 5 female, 8 male) with a mean age of 21.33 (SD = 0.78) were recruited from the University of Western Ontario. Eight participants considered themselves as musicians, holding a minimum of 5 years of formal musical training. Five participants responded as being non-musicians, with no formal musical training. Participants were required to walk for thirty minutes unassisted and have no neurological disorder, otherwise they were excluded. Written informed consent was gathered from all participants, with each compensated for their time accordingly. This study was approved by the University of Western Ontario's Health Sciences Research Ethics Board.

### 2.2 Stimuli and Apparatus

#### 2.2.1 Music Stimuli Selection

Before data collection began, music was gathered from the folders of musical stimuli present within the University of Western Ontario's gait lab used in previous experiments. Instrumental versions of six pop songs were selected for their high-groove and high-familiarity features. Stimuli were presented over loudspeakers for all trials with volume being controlled for.

#### 2.2.2 fNIRS Recording

The NIRSport 2 (NIRx Medical Technologies, Germany) fNIRS system was used in this study to measure the M1, PMC, SMA and pre-SMA motor regions of the brain. An 8x7 motor montage was used, creating 10 channels on the left hemisphere (source optode 1-4, detector optode 1-4) and 8 channels on the right hemisphere (source optode 5-8, detector optode 5-7). Detector 8, and hence two channels, were sacrificed to accommodate 8 short-distance detectors

at each source optode. Whereas the wiring for the primary source and detector optodes reside outside the cap, the short-distance detectors are on the inside of the cap with wiring that travels to the back of a participants head to connect to detector 8, which is the detector representing the short-distance channel signals *(Figure 2)*. Each participant had their head circumference measured before the in-person testing session to allow time to prepare the montage on the proper cap size. Measurements were taken from nasion to inion and left to right preauricular points to ensure the cap was properly oriented on the participant's head. Hair was then cleared away from each optode location before attaching the designated source or detector. Cable organizers and Velcro straps were used to organize wires and prevent tension that could move the optodes and reduce signal quality. An accelerometer probe was also attached to the cap near the participants forehead to track relative head movement during trials. Head caps and spring-tops were always sanitized between each participant.

The NIRSport 2 was connected wirelessly to a laptop (Windows 10) running the Aurora data collection software. Optimization of the signal quality across all source-detector channels was completed in Aurora before official data collection began. Optodes connected to poor signal quality channels (identified as red in Aurora) were re-adjusted with hair displaced from the optode location as best as possible. Moderate signal quality (identified as yellow in Aurora) was primarily avoided or resolved but if persistent, was accepted and testing proceeded. The NIRSport 2 recorded raw voltages (mV) at two wavelengths (760nm; 850nm) with a sample rate of 10 Hz. The raw voltages were streamed in real-time to Aurora and visualized as relative HbO and HbR concentrations using the Modified Beer-Lambert Law (mBLL) (Kocsis et al., 2006).

### 2.2.3 Gait Recording

Gait characteristics were tracked using a pressure sensitive Zeno™ walkway that was 16 feet long (4.88m) (*Figure 3*). Gait characteristics were actively recorded in the ProtoKinetics Movement Analysis Software on the PC within the gait lab as participants traversed the walkway.

### 2.2.4 fNIRS – Gait System Compatibility

Two devices were used to collect the data for this study: a PC within the gait lab used the PKMAS to record and analyze gait data from the Zeno™ walkway; a laptop running the fNIRS data collection software Aurora collected the raw data being recorded by the NIRSport 2. The fNIRS laptop network settings were modified to allow for a physical ethernet connection to the local internet network and a wireless connection to the NIRSport 2 simultaneously. The ethernet connection ensured the fNIRS laptop was on the same network as the gait PC (also with an ethernet connection). This permitted triggers to be sent wirelessly from MATLAB on the gait PC to Aurora on the fNIRS laptop via the Lab Streaming Layer (LSL) trigger protocol.

LSL triggers were used to mark the beginning and end of each sixty second resting baseline, silent baseline walk and musical trial within the fNIRS data. For the baseline trials marked by audible beeps, initiating the beep manually in MATLAB would automatically send an LSL trigger to Aurora on the fNIRS laptop and begin the trial. Sixty seconds later, an automatic beep would signal the end of the baseline trial and another LSL trigger would be sent. To initiate a walking trial, the “Start Walk” button would be clicked within PKMAS on the gait PC, this initiated the Zeno™ walkway to signal it is actively by sending a 5V transistor-transistor logic (TTL) signal back to the gait PC. The MATLAB script would read this signal and start playing the musical stimuli automatically while simultaneously sending an LSL trigger to Aurora on the fNIRS laptop.

Walking trials were stopped manually: when the sixty seconds was completed, any key would be pressed in MATLAB to stop the music and send another LSL trigger. “End Walk” would then be clicked within PKMAS to stop the Zeno™ walkway from recording and prepare for the next walk trial for that participant.

### 2.3 Procedure

#### 2.3.1 Experimental Paradigm

#### 2.3.2 Starting Baseline Paradigm (*Figure 4*)

Before walking, participants were asked to stand at the line of tape (*Figure 3*) before the gait mat and not move for sixty seconds. This was intended to gather a resting baseline recording of cortical activity with fNIRS.

For all walking trials, participants were instructed to walk up and down the gait mat, taking large U-turns at each end to avoid pivoting and disrupting their stride. At the end of each trial, participants were asked to stop wherever they were until instructed to return to the start position. Participants were then asked to perform a silent baseline walk in which they walked on the gait mat for sixty seconds. This allowed for the baseline gait parameters such as cadence to be gathered and for fNIRS to record cortical activity during movement but void of any musical stimuli. If participants were walking incorrectly, further instruction and clarification was given after the silent baseline walk before proceeding to further trials.

The sixty second trials for both the resting baseline and silent baseline walk were marked by audible beeps to inform the participant of the beginning and end of these trials.

#### 2.3.3 Cadence adjustment

The silent baseline walk was then analyzed to determine the baseline cadence of the participant. This cadence value was then inputted into a MATLAB script which adjusted the

instrumental music stimuli to have the same cadence (for music beats/min.) as the participant (steps/min.).

### 2.3.4 Experimental Paradigm (*Figure 4*)

Participants performed twelve trials of walking with the six musical stimuli: six trials of free walking and six trials of synchronized walking. For all music trials, participants were instructed to begin walking when the music commenced and continue until the music stopped (each music clip lasted sixty seconds). Odd numbered participants performed the six free walking trials first, then six synchronized trials. The even numbered participants counterbalanced the order of the walking conditions by performing the synchronized trials first and the free walking trials second. The order of the six musical stimuli was randomized for both the free and synchronized conditions for all participants.

When tasked with free walking, participants were instructed to "walk normally, as if you were walking down the sidewalk." When tasked with synchronized walking, participants were instructed to "synchronize your footsteps to the beat of the music." For the participants who performed the free walking condition second, care was taken to avoid instructing participants to "not walk on the beat" as to prevent them from attempting to walk on the off beat of the music.

### 2.3.5 Ending Baseline Paradigm (*Figure 4*)

After completing the twelve musical trials, participants completed another silent baseline walk lasting sixty seconds, followed by another resting baseline recording lasting sixty seconds. These final baseline trials were also marked by audible beeps to inform participants of the beginning and end of the trials. Across baseline and experimental paradigms, there were fourteen walking trials in total, with a resting baseline recording at the beginning and the end. All trials lasted 60 seconds.

### 2.4 Data Processing and Analysis

Gait data and fNIRS data were both analyzed using normalized versions of the dependent variables: *Beta* for relative HbO and HbR concentrations; NPC for each gait parameter. A *Beta* or NPC equal to zero signified no change from silent baseline walking (resting baseline for *Figure 12*) to the experimental conditions of free and synchronized walking (all walking for *Figure 12*). P-values equal to and below .05 resulting from paired, single t-tests and Pearson's R correlational tests were considered statistically significant.

#### 2.4.1 Gait Processing and Analysis

All fourteen walking trials from all 13 participants were processed in PKMAS. Synchronization ability was assessed with cadence (steps/min.), where a cadence similar to the cadence of the music (beats/min.) indicated relative synchronization to the beat of the music. Temporal gait parameters were stride time (sec.) and stride velocity (cm/sec.). Stride time represents the time between two footsteps of the same foot. Stride velocity indicates distance covered per unit time. Stride length was the primary spatial gait parameter, representing the distance between two footsteps of the same foot. Stability gait parameters were double limb support time (sec.) and stride width (cm.). Double support time represents the percentage of time with both feet simultaneously on the ground. Stride width indicates the lateral distance between the centre of one foot to the line of progression formed by footsteps of the opposite foot. Variability parameters were examined using the coefficient of variation (CV) where increases in stride time, stride length and stride velocity CV were indicative of more variability within those respective parameters. Refer to Leow et al. (2021) for further explanation of gait parameters.

Parameters were exported in a .txt file for each trial of every participant. The mean parameters for the free walking trials and synchronized walking trials across all participants were

then collated into a table. A normalized percent change (NPC) for each mean parameter during the free walking and synchronized walking across all participants was acquired to compare the proportion of change from baseline:

$$\boldsymbol{Normalized}\ \%\ \boldsymbol{Change} = \left(\frac{(\boldsymbol{Free\ or\ Sync\ Mean\ Parameter}) - (\boldsymbol{Baseline\ Walk\ Mean\ Parameter})}{\boldsymbol{Baseline\ Walk\ Mean\ Parameter}}\right) \times \mathbf{100}\%$$

Two-sample t-tests were completed in R for each normalized gait parameter between free and synchronized walking conditions. Single sample t-test were completed in R for each normalized gait parameter during either the free or synchronized walking condition to assess significance from baseline silent walking. These same t-tests were completed between and within musician and non-musician groups across all gait parameters and walking conditions.

### 2.4.2 fNIRS Processing and Analysis

fNIRS data was processed using the NIRS Toolbox and Homer 3 programs within MATLAB. Data was analyzed using the ordinary least squares (OLS) method for solving the general linear model (GLM) (Dans et al., 2021). First, using NIRS Toolbox, we converted raw voltage signals for each source-detector channel to optical density at 760nm and 850nm wavelengths. Spline interpolation was then performed on the optical density in Homer 3 to remove baseline shifts and slow drift, two common motion artifacts (Brigadoi et al., 2014). A wavelet filter was then passed over the data using NIRS Toolbox. Wavelet filters are effective at removing the sharp motion-induced spikes within the data that spline interpolation misses (Dans et al., 2021). The wavelet filter interquartile range (IQR) was set for each individual participant (IQR range: 0.2 – 0.5). Higher IQRs set a higher threshold of amplitude needed for a spike in the data to be removed as a motion artifact but allow for smaller motion-induced spikes to be missed. Contrarily, low IQRs set a low threshold of amplitude, ensuring no motion-induced spikes are missed, however, lower IQRs risk removing data that is accurately representative of

cortical activity. Setting a unique IQR for each participant is therefore important to accommodate person-to-person differences in signal quality. We then down sampled our sampling rate from 10 Hz to 4 Hz, an approach often used to avoid violating the GLM (Pinti et al., 2019). This down-sampled 4 Hz data, with motion artifacts removed, was then converted to relative HbO and HbR concentrations using the mBLL (Kocsis et al., 2006). A partial pathlength factor of 0.1 was used, indicating approximately 10% of the data retrieved from each source-detector channel (see *Figure 1*: *Left*) is representative of cortical activity.

The Neurosynth database (https://neurosynth.org/analyses/terms/), in conjunction with the fOLD toolbox (Zimeo Morais et al., 2018), determined the precise location of the regions of interest.

*Neurosynth Parameters*

| Region | Threshold | x | y | z |
|---|---|---|---|---|
| Pre-Supplementary Motor Area (term 'pre sma') | 5 | 0 | 10 | 52 |
| Supplementary Motor Area (term 'supplementary motor') | 10 | 0 | -4 | 56 |
| Premotor Cortex (term 'premotor') | 8 | -2 | -8 | 54 |
| Primary Motor Cortex (term 'primary motor') | 9 | -36 | -22 | 52 |

Within the fOLD toolbox, Brodmann's Brain Atlas was used with the label 'pre-motor and supplementary motor cortex' selected at 55% specificity. This revealed the sources and detectors on the 8 x 7 motor montage (*Figure 2*) which would produce data representing, with 55% specificity, pre-motor and supplementary motor cortex activity. These regions of interest, with their associated source-detector channels, were then aligned onto the Colin 27 brain atlas in NIRS Toolbox for visualization (*Figure 5*).

First-level statistical analysis using the OLS GLM was used to model the relationship between the actual fNIRS data measured and the predicted model at the individual level. The predicted model pertains to the conditions outlined within the experimental design (all baseline

and experimental conditions outlined in *Figure 4*). Motion and physiological artifacts were also regressed as factors of no interest by regressing the short-distance channels.

Second-level statistical analysis using the Mixed Effects Model and Fixed Effects (FFX) gathered results from the first-level statistical analysis and aggregated them across all participants. This allowed for group-level effects to be extrapolated from the initial individual effects observed in the first-level analysis. Group-level contrasts were then performed between the desired conditions, namely 'All Walking' vs 'Resting Baseline', 'Free Walking' vs 'Silent Baseline Walking' and 'Synchronized Walking' vs 'Silent Baseline Walking'.

## 3. Results

### 3.1 Gait Results

#### 3.1.1 Free Walking Compared to Synchronized Walking Across All Participants

We took the NPC from silent baseline walking to free walking and compared them to the NPC from silent baseline walking to synchronized walking for each mean gait parameter. Three gait parameters differed significantly between free walking and synchronized walking conditions across participants: Cadence, Double Support and Stride Time (*Figure 6*). Cadence was higher during free walking ($M$ = 4.03, $SE$ = 0.238) compared to synchronized walking ($M$ = 0.637, $SE$ = 0.142), $t(20) = 3.39$, $p < .05$. Double support time was lower during free walking ($M$ = -5.58, $SE$ = 0.353) compared to synchronized walking ($M$ = -1.77, $SE$ = 1.16), $t(24) = -2.20$, $p < .05$. Stride time was lower during free walking ($M$ = -3.86, $SE$ = 0.225) compared to synchronized walking ($M$ = -0.435, $SE$ = 0.145), $t(21) = -3.54$, $p < .05$.

We then compared the NPC from silent baseline walking to free walking to the NPC from silent baseline walking to synchronized walking for the three variability parameters (*Figure 7*). Stride length variability was the only parameter to differ significantly between free walking

($M$ = -10.2, $SE$ = 7.54) and synchronized walking ($M$ = 35.4, $SE$ = 16.6), becoming more variable during synchronized walking compared to free walking, $t(17) = -2.50$, $p < .05$. Stride velocity increased from free walking ($M$ = -15.4, $SE$ = 6.38) to synchronized walking ($M$ = 13.5, $SE$ = 13.7) but did not reach significance ($p = .07$).

### 3.1.2 Between Group Comparison by Gait Parameter and Walking Condition

Comparisons for each gait parameter between musicians and non-musicians for free walking (*Figure 8*, *Figure 9*) and synchronized walking (*Figure 10*, *Figure 11*) was also completed. No significance differences were found between musician and non-musician groups for any gait parameter during either free walking or synchronized walking. One-sampled t-tests, however, revealed that cadence (*Figure 8.A*), double-support time (*Figure 8.B*), stride length (*Figure 8.C*), stride time (*Figure 8.D*), stride velocity (*Figure 8.E*) and stride velocity variability (*Figure 9.C*) were significantly different from silent baseline walking within the musician group during free walking ($p < .05$). Stride width (*Figure 8.F*) was also significantly different from silent baseline walking within the non-musician group during free walking ($p < .05$). No gait parameters differed significantly from silent baseline walking during synchronized walking in either group.

### 3.1.3 Within Group Comparison of Walking Condition by Gait Parameter

Within group comparisons of free walking compared to synchronized walking were also completed. The musician group exhibited a significant decrease in cadence when going from free walking ($M$ = 5.17, $SE$ = 1.12) to synchronized walking ($M$ = 0.296, $SE$ = 0.388), $t(9) = 4.11$, $p < .05$. Stride time corresponded with this finding, increasing significantly from free walking ($M$ = -4.95, $SE$ = 1.05) to synchronized walking ($M$ = -0.137, $SE$ = 0.452), $t(9) = -4.20$, $p < .05$. Stride length variability also increased from free walking ($M$ = -14.9, $SE$ = 6.73) to synchronized

walking ($M$ = 21.1, $SE$ = 16.0) in the musician group but did not reach significance (p = .07). Non-musicians only exhibited a significant increase in stride width from free walking ($M$ = -6.78, $SE$ = 2.40) to synchronized walking ($M$ = 7.27, $SE$ = 5.11), $t(6) = -2.49$, $p < .05$).

### 3.2 fNIRS Results

#### 3.2.1 Comparing All Walking Conditions to Resting Baseline

We compared the mean effect of walking on relative HbO and HbR concentrations to the relative HbO and HbR concentrations gathered during the resting baseline condition across all participants. Three regions of interest displayed HbR measures which differed significantly from resting baseline to walking conditions: left dorsal PMC (*Figure 12.A*), pre-SMA (*Figure 12.D*) and SMA (*Figure 12.G*). HbR concentrations were lower in the left dorsal PMC during walking conditions compared to the resting baseline, $t(36) = -2.75$, $p < .05$. The pre-SMA also displayed lower HbR concentrations during walking conditions compared to resting baseline, $t(36) = -2.29$, $p < .05$. Furthermore, the SMA displayed lower HbR concentrations during walking conditions compared to resting baseline as well, $t(36) = -2.45$, $p < .05$. The right M1 was the only region of interest which displayed an HbO measure which differed significantly from resting baseline to walking conditions (*Figure 12.F*). HbO concentrations were lower in the right M1 during walking conditions compared to resting baseline, $t(36) = -2.25$, $p < .05$.

#### 3.2.2 Comparing Free and Synchronized Walking to Silent Walking

We compared the relative HbO and HbR concentrations resulting from the mean effect of free walking and synchronized walking (separate HbO/HbR readings for both conditions) to the relative HbO and HbR concentrations gathered during silent baseline walking (*Figure 13.1* and *Figure 13.2*). No significant differences in relative HbO and HbR concentrations were found between free walking and synchronized walking. Only the *beta* values for HbR concentrations

representing the pre-SMA and SMA during synchronized walking approached significance when compared to silent baseline walking (pre-SMA HbR: $p < 0.07$, SMA HbR: $p < 0.08$). One-sampled t-tests revealed only the HbR readings for the left dorsal-PMC during free and synchronized walking to be significantly different from silent baseline walking (*Figure 13.2.B*) ($p < .05$).

### 3.2.3 Between Group Comparison by Motor Region and Walking Condition

No significant differences in activation for any motor region were found between musicians and non-musicians when comparing activation during either free walking or synchronized walking.

### 3.2.4 Within Group Comparison of Walking Condition by Motor Region

No significant differences in activation for any motor region were found between free and synchronized walking conditions when comparing activation within either the musician group or non-musician group.

## 3.3 fNIRS and Gait Correlational Results

Across all correlational matrices completed, few significant correlations were discovered between regions of interest (*Figure 5*) and gait parameters across walking conditions. Furthermore, most significant correlations found were in only one of either the HbO reading or the HbR reading for that region of interest. For example, the left dorsal PMC was the most common region significantly correlated with a gait parameter, however, if determined to be significant in the HbO reading, the companion HbR reading was often found to be far from anti-correlated. This produced uncertainty as to which reading was more indicative of genuine activation trends within that region. Despite this, we found the left M1 to be most reliable in producing anti-correlations between the HbO and HbR readings. Significant correlations with

cadence, stride time, stride length and stride velocity parameters across walking conditions and participant groups were observed with left M1 activity. Notably, within SMA HbO readings, significant correlations were observed with stride time variability and stride velocity variability (*Figure 23*, *Figure 25*), however, anti-correlated HbR readings were absent.

### 3.3.1 Left Primary Motor Cortex

#### 3.3.1.1 Cadence

A significant positive correlation was observed between an increase in cadence and left M1 activity (HbO) in non-musicians during free walking (*Figure 15; left*), $r(3) = 0.875$, $p = .05$. Therefore, when non-musician participants increased their cadence (steps/min.) during free walking and therefore walked faster, this corresponded with an increase in left M1 activity.

#### 3.3.1.2 Stride Time

A significant negative correlation was observed between an increase in stride time and left M1 activity (HbO) in non-musicians during free walking (*Figure 17; left*), $r(3) = -0.900$, $p < .05$. This suggests that as non-musicians decreased the time between each stride (an indicator of faster cadence) during free walking, they walked faster. This change corresponded with an increase in left M1 activity.

#### 3.3.1.3 Stride Length

A significant negative correlation was observed between an increase in stride length and left M1 activity (HbO) in musicians during free walking (*Figure 19; left*), $r(6) = -0.864$, $p < .05$. Musicians, therefore, increased their stride length when walking freely compared to silent baseline walking, however, this corresponded with a decrease in left M1 activity.

#### 3.3.1.4 Stride Velocity

A significant positive correlation was observed between an increase in stride velocity and left M1 activity (HbO) in non-musicians during free walking (*Figure 21; left*), $r(3) = 0.900$, $p < .05$. Non-musicians therefore covered more distance per unit time when they were free walking with music compared to silent walking and this corresponded with an increase in left M1 activity.

A significant negative correlation was also observed between an increase in stride velocity and left M1 activity (HbR) in non-musicians during synchronized walking (*Figure 21; right*), $r(3) = -0.906$, $p < .05$. Therefore, non-musicians also covered more distance per unit time during synchronized walking to music compared to silent walking and this corresponded with a decrease in HbR, representative of increased left M1 activity.

### 3.3.2 Supplementary Motor Area

#### 3.3.2.1 Stride Time Variability

A significant negative correlation was observed between an increase in stride time variability and SMA activity (HbO) in non-musicians during synchronized walking (*Figure 27*), $r(3) = -0.942$, $p < .05$. Therefore, non-musicians whose time between ipsilateral footsteps became more variable during synchronized walking to music compared to silent walking, corresponded to decreases in SMA activity.

#### 3.3.2.2 Stride Velocity Variability

A significant negative correlation was observed between an increase in stride velocity variability and SMA activity (HbO) in non-musicians during synchronized walking (*Figure 23*), $r(3) = -0.897$, $p < .05$. Therefore, more variable distance covered per unit time exhibited by non-

musicians during synchronized walking to music compared to silent walking corresponded with decreases in SMA activity.

## 4. Discussion

In this study we examined how spatiotemporal gait parameters and activity in motor regions of the brain of young healthy adults are influenced by free walking and synchronized walking to instrumental music. We also examined how changes in gait parameters due to the walking condition may also be correlated to changes in activation within these motor regions. Moreover, we assessed how these changes in gait parameters and cortical activity may be differentiated in musicians and non-musicians. Overall, spatiotemporal gait outcomes were influenced when walking to music freely, with this effect being driven primarily by musicians. Specifically, strides became longer, faster, and less variable when freely walking to music compared to silent walking, with these changes more pronounced in musicians. Instructions to synchronize saw stride width increase significantly from silent walking; compared to free walking, strides were less stable, with stride lengths becoming more variable. Motor regions of the brain did not differ in activity from silent walking during either free or synchronized walking; activity also did not differ from free to synchronized walking. In non-musicians, increases in stride velocity were associated with more activation in the left M1 during free and synchronized walking; increased cadence correlated with more left M1 activity during free walking. During synchronized walking, non-musicians with more variable stride velocity and stride time exhibited less activation in the SMA but with prudent caveats. In musicians, increases in stride length were associated with less activation in the left M1 during free walking.

### 4.1 Free Walking improves gait parameters compared to silent and synchronized walking

#### 4.1.1 Across All Participants

Free walking to instrumental music elicited longer and faster strides then synchronized and silent walking. These findings support our first hypothesis and are supported by a previous study indicating longer and faster strides during free walking (Leow et al., 2018). Interestingly, Leow et al. (2018) saw prominent decreases in stride velocity and stride length during synchronized walking compared to silent walking whereas our findings show these parameters remained above silent baseline levels, although without significance (*Figure 6*). One reason for our findings not supporting the broader literature may be because we did not increase the cadence of our music (beats/min.) but instead matched it to the baseline cadence of our participants (steps/min.). The cadence of music stimuli are often increased by ten to twenty percent to prevent spontaneous synchronization during free walking and amplify gait changes during synchronized walking (Leow et al., 2015, 2018; Ready et al., 2022). We avoided this to ensure that potential changes in cortical activity observed during free and synchronized walking trials were indicative of increased movement or cognitive demand *not* induced by music with a higher cadence than that of the participant's silent baseline walk.

Our study also found that instructions to synchronized increased stride length, stride velocity and stride time variability compared to baseline as hypothesized, although only stride length reached significance (*Figure 7*). These increases in spatiotemporal variability support previous findings stating that increased gait variability is indicative of increased cognitive demand (Leow et al., 2014, 2015, 2018, 2021; Ready et al., 2022). However, contrary to the studies which observed either no change or increases in variability parameters from baseline to free walking, we found free walking to significantly decrease stride velocity variability

compared to silent walking (Leow et al., 2018; Leow, Watson, et al., 2021; Ready et al., 2022). Stride time and stride length variability trended in the same direction but did not reach significance.

### 4.1.2 Musicians compared to Non-Musicians

We then dissected our mean gait results to assess potentially different trends found within musicians and non-musicians. Compared to silent baseline walking, musicians exhibited significantly longer and faster strides during free walking, whereas non-musician were elevated above baseline but lacked significance (*Figure 8*). These results contradict previous studies which show stride length and velocity decreasing in strong and weak beat perceivers (Leow, Watson, et al., 2021) or stride length and velocity increasing only in weak beat perceivers (Ready et al., 2019). Interestingly, stride width trends in our participants support previous research indicating that free walking was significantly more stable in weak beat perceivers compared to baseline, whereas strong beat perceiver trended towards having wider strides compared to baseline (*Figure 8*) (Ready et al., 2019). However, when instructed to synchronize, strides in non-musicians became significantly wider compared to free walking and trended significantly above baseline levels (*Figure 10*). Although this trend was observed in weak beat perceivers in Ready et al. (2019), examining their strong beat perceivers saw stride width decrease when instructed to synchronize, whereas our musicians increased their width more, although not significantly.

The decrease in variability parameters observed across participants during free walking was also driven primarily by musicians, with stride velocity variability being significantly lower compared to silent walking (*Figure 9*). Contrarily, the increase in stride length and velocity variability seen during synchronized walking was supposedly driven by non-musicians, although

they did not differ significantly from baseline or musicians (*Figure 11*). Non-musicians driving increases in stride variability during synchronized walking is supported by previous literature (Leow et al., 2014; Leow, Watson, et al., 2021). However, the notion that variability improvements during free walking exist and are driven by musicians is a novel finding that is unsupported by the relevant preceding studies (Leow et al., 2018; Leow, Watson, et al., 2021; Ready et al., 2022). It is important to note that although we equivalate musicianship with strong beat perception ability and non-musicianship with weak beat perception ability, beat perception is not entirely correlated with musical ability. Therefore, 'strong beat perceivers' mentioned in previous studies does not ensure that these individuals were also musicians and therefore must be considered as a confound within these comparisons.

**4.2 Motor regions of the brain do not change in activity across walking conditions**

Contrary to our third hypothesis, activity in motor regions of the brain did not change significantly from silent walking to either free or synchronized walking, nor did any changes occur from free walking to synchronized walking. Only the HbR reading of the left dorsal PMC was observed to increase significantly during free and synchronized walking when compared to silent walking (*Figure 13.2; B*). Assuming this reading is accurate, it would suggest the presence of music during walking conditions correlated with a decrease in left PMC activity, however, corresponding increases in the HbO reading suggest otherwise (*Figure 13.2; right*). Only Vitorio et al. (2018) have utilized fNIRS to observe cortical activity in young healthy adults during walking with metronome RAS. Similar to our findings, they did not observe any significant differences between silent walking and synchronized walking in HbO readings of the M1, PMC or SMA.

No significant differences in cortical activity were found between musicians and non-musicians during any walking conditions, nor did any changes occur between walking conditions when comparing within the respective groups. No studies to this date have implemented fNIRS to assess differences in cortical activity between musicians and non-musicians during walking with RAS.

**4.3 The SMA and Left M1 correlate with gait parameters by walking condition and group**

Across all participants and across the seven motor regions assessed, our study found no significant correlations between motor activation and gait parameters. Notably, Vitorio et al. (2018) observed that increased stride length variability correlated with increased activity in the left M1 during synchronized walking compared to silent walking. We also found this positive correlational trend between the stride length variability and left M1 activity during synchronized walking, albeit without significance.

When divided into musician and non-musician groups, a plethora of significant correlations were discovered between various gait parameters and motor regions in either the HbO or HbR recordings. However, only the left M1 produced significant correlations with gait parameters while maintaining relative anticorrelations between HbO and HbR readings, an important criterion for validity purposes. In non-musicians, temporal gait parameters were correlated with left M1 activity during free walking. Specifically, increases in left M1 activity were correlated with increased cadence (*Figure 15*), decreased stride time (*Figure 17*) and increased velocity (*Figure 21*) in free walking when compared to silent walking. These increases suggest non-musicians began walking quicker during the free walking condition, and this increase in movement most likely corresponded with elevated activity in the M1. In musicians, stride length was correlated to left M1 activity during free walking. Namely, longer strides in

musicians corresponded with decreased left M1 activity when compared to silent walking (*Figure 19*).

Interestingly, neutral trends in stride length (*Figure 18*) and velocity (*Figure 20*) when correlated to left M1 activity across all participants are lost when musicians and non-musicians are differentiated. Non-musicians exhibited trends which suggest increased stride length (*Figure 19*) and velocity (*Figure 21*) correlate with increased activity in the left M1. Contrarily, musicians exhibited trends indicating that increased stride length (*Figure 19*) and velocity (*Figure 21*) correlate with decreased activity in the left M1. Although these trends predominantly lack significance, they suggest potentially different mechanisms of musical processing in musicians and non-musicians, resulting in different spatial and temporal gait outcomes.

Finally, although anticorrelation between HbO and HbR was not prevalent, non-musicians exhibited significant correlations between stride time (*Figure 25*) and stride velocity (*Figure 23*) variability and SMA activity. Contrary to our fourth hypothesis, synchronized walking revealed more variable stride velocity and stride time correlated with decreased SMA activity in non-musicians. This may indicate that recruitment of the SMA is required to reduce temporal variability during walking, at least in non-musicians.

**4.3 Limitations**

Several limitations apply throughout this study. Most prominently, sample size was approximately half of what was originally desired. This lack of statistical power made the removal of outliers increasingly difficult as different participants had profound effects on trends observed, especially in cortical activity and gait correlations. For example, participant 5 was a heavy outlier in HbO readings within the SMA (*Figure 23*, *Figure 25*) and the significant

negative correlations found with stride time and velocity variability can be attributed to this individual.

The experimental paradigm also produced limitations. Walking can often produce motion artifacts such as baseline drifts, which can occur from optode movement on the scalp and cause HbO readings to gradually fall over time (Dans et al., 2021). Implementing consistent periods of resting baseline with no movement or external stimuli between experimental trials can effectively counteract these baseline drifts. Although resting baseline was taken at the beginning of our testing, this was not a sufficient reference of baseline cortical activity throughout the experiment to then account for baseline shifts. Resting periods were implemented between each walking trial, however, they were not equivalent to resting baseline as participants were not instructed to remain motionless during this period and verbal instruction was often given. Motion artifacts are also likely the reason for decreases in activation from resting baseline to walking conditions in regions such as the right M1 (*Figure 12*). This decrease certainly contradicts what activation the M1 ought to be exhibiting during walking and cultivates uncertainty regarding the validity of activation trends in other motor regions during walking tasks.

Further limitations are prevalent in fNIRS when attempting to assign recordings in specific source-detector channels to regions of interest. Unlike fMRI, investigating brain regions using fNIRS is entirely dependent on the accurate placement of optodes on the scalp to form signal channels which pass through regions of interest (*Figure 1*). Often, a source-detector channel may be representative of multiple regions of interest, resulting in estimated proportions for a channel being indicative of a specific ROI (*Figure 5*). Therefore, although regions such as the pre-SMA, SMA and PMC often exhibit different activation, our study may have found them to be similar in activity because similar channels were used to infer their activity. Finally, SMA

activity is intrinsically difficult to assess with fNIRS due to its proximity with the longitudinal fissure and increased volumes of cerebrospinal fluid. Cerebrospinal fluid can significantly impact the propagation of infrared light and therefore creates more uncertainty when interpreting an fNIRS recording of the SMA (Okada & Delpy, 2003).

**4.4 Future Directions**

Future research should seek to build upon this study by expanding the participant demographic under investigation, exploring the effects of different RAS stimuli, and ameliorating experimental paradigms for fNIRS and gait integration. The beat alignment test (BAT) is commonly used to determine beat perception ability (Müllensiefen et al., 2014) and should be implemented alongside the collection of musical training data to improve distinctions between musical and non-musical groups. Research in young and older healthy adults suggests opposing correlations in cortical activity and gait variability during synchronization (Vitorio et al., 2018), with differences in gait outcomes also influenced by the groove and familiarity of the music and beat perception ability (Ready et al., 2022). Future studies should begin to examine the neural correlates of these different variables with fNIRS to elucidate conditions which may produce optimal gait outcomes in different healthy populations. Musical ability seems to impact gait outcomes with RAS in Parkinson's patients as well (Cochen De Cock et al., 2018), indicating a need to understand how neural correlates and gait outcomes found in healthy population may change in populations with impaired gait. It may also be beneficial for future gait studies implementing fNIRS to include the prefrontal cortex in motor montages as it has been associated with cognitive demand during dual task walking in healthy and sick populations (Vitorio et al., 2017). Finally, studies incorporating fNIRS within gait protocols should ensure

that adequate resting baseline periods are implemented before, between and after walking trials to combat inevitable motion artifacts and improve analysis.

### 4.5 Conclusion

Currently, gait research is often completed in different populations under different walking conditions, where the cognitive demand of a walking task is attributed to changes in gait outcomes. fNIRS provides the means to examine cortical activity in real time as participants walk under different experimental conditions. In the context of RAS, fNIRS provides insight into how external musical cues may alter motor regions of the brain and correspond with changes in gait outcomes within different populations such as musicians and non-musicians. To the best of our knowledge, we have completed the first study examining cortical activity in musicians and non-musicians during free and synchronized walking to musical RAS on a pressure sensitive walkway. The present findings suggest gait parameters in young healthy adults may benefit from free walking with instrumental music, with a greater effect in musicians. Musicians were also seen to have longer and faster strides which correlated with decreased left M1 activity, whereas non-musicians exhibited increased left M1 activity in correlation with longer and faster strides. We speculate that these results suggest different neural trends may exist in musicians and non-musicians even when exhibiting similar gait outcomes. This study lays groundwork for future research to improve and strengthen protocols which integrate fNIRS and gait recording. Ultimately, this may expand to clinical populations such as people with Parkinson's disease and give insight into the neural correlates of gait impairments and the circumstances under which RAS may be most beneficial.

## 5. Acknowledgements

I wish to thank Riya Sidhu, Kristi Von Handorf, Androu Abdalmalak, Kevin Stubbs, Ethan McNaughton, Chantal Rochon and Dr. Jessica Grahn for their respective contributions and support throughout the entirety of this project. This study was supported by Natural Sciences and Engineering Research Council of Canada (NSERC).

## 6. Figures

### 6.1 fNIRS Fundamentals

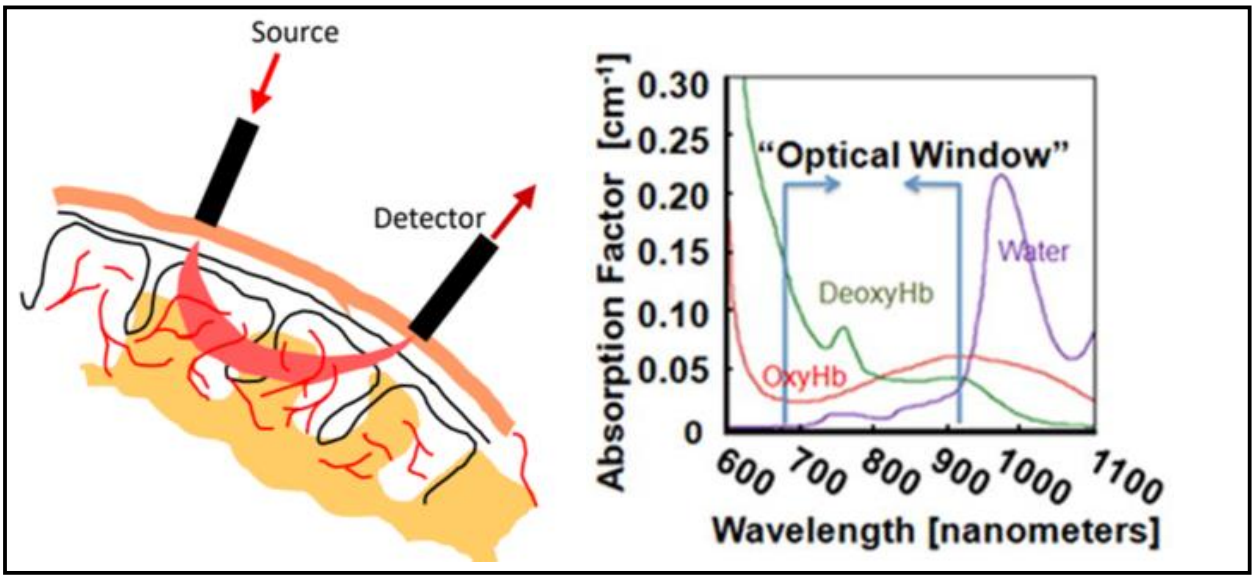


*Figure 1.* Left: Red banana-shaped photon channel formed between the light source and light detector penetrates 2-3cm of cortical tissue. Right: Preferential DeoxyHb (deoxygenated hemoglobin) and OxyHb (oxygenated hemoglobin) absorption spectra of near-infrared light. (Left) Retrieved from NIRx Medical Technologies; (Right) Retrieved from Karim et al. (2012)

## 6.2 8x7 Motor Montage with Short-Distance Detectors

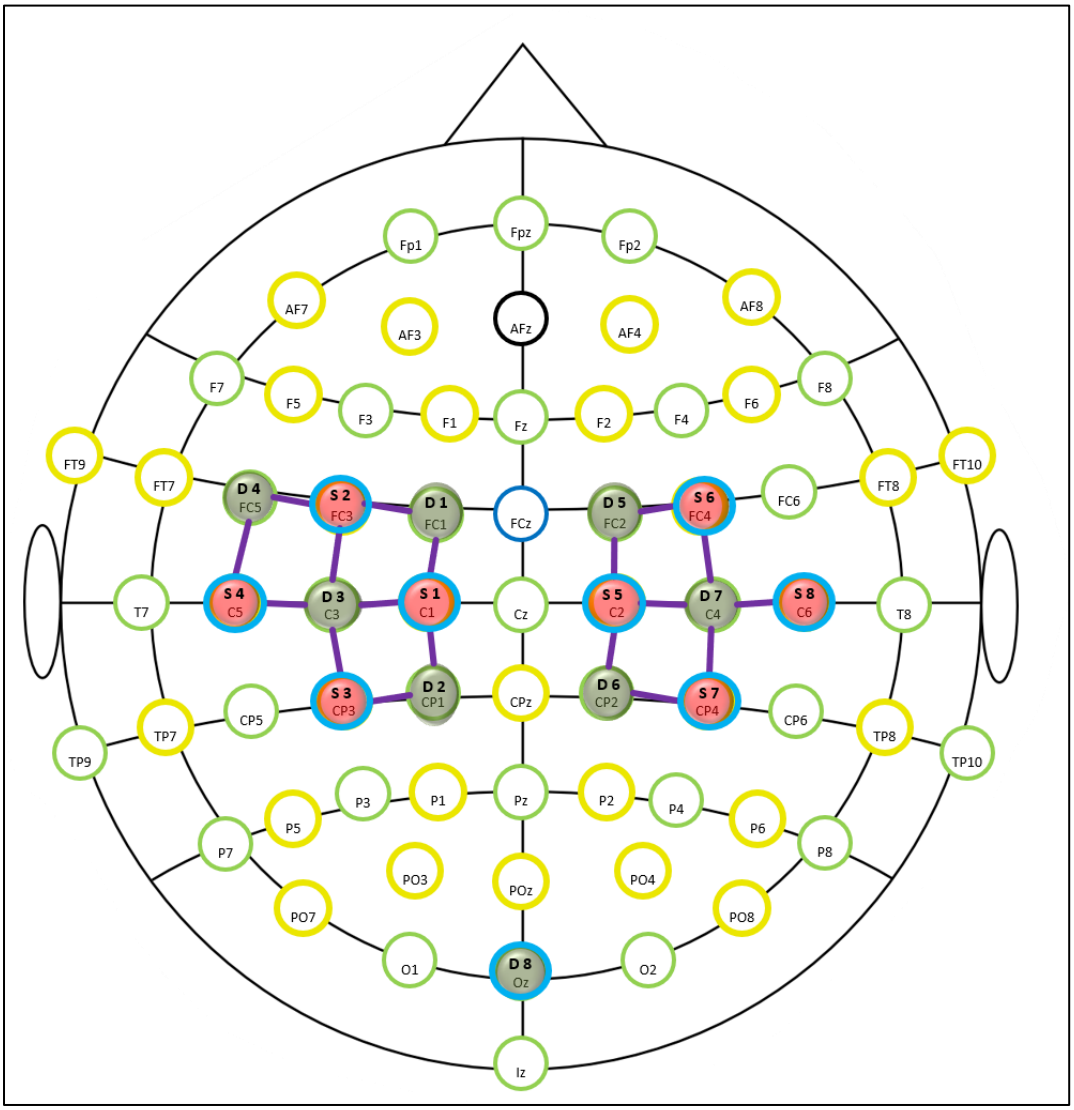


*Figure 2.* 10-20 system of electrode placement used to describe location of fNIRS source (red circles labeled S1 – S8) and detector (green circles labeled D1 – D8) optodes. Blue rings around each source optode represent a short-distance channel. All 8 short-distance channel connect back into one wire which is then connected to D8 near the back of the participant's head. Purple lines represent the signal channels created between each source and detector. Interoptode distance ranged from 35mm to 40mm; short-distance channels were 8mm in diameter (diameter of blue ring).

### 6.3 Pressure Sensitive Walkway Layout

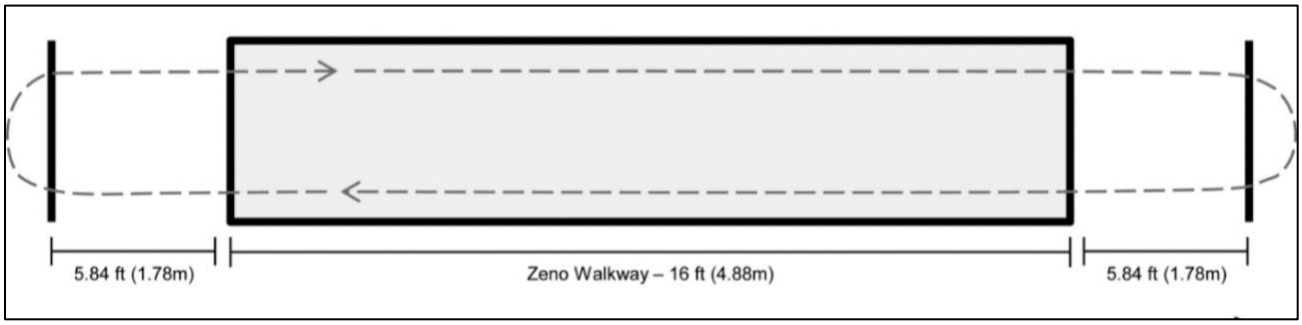


*Figure 3.* Participants walked back and forth on a 16-foot (4.88m) pressure sensitive walkway from Zeno™. Participants began at the 'starting position' indicated by a line of tape (vertical black line on left). Dashed oval with arrows shows walking path up and down the walkway. Six gait parameters were tracked for each trial: cadence (steps/min), stride time (sec), stride length (cm), stride width (cm), stride velocity (sec) and double limb support time (sec).

## 6.4 Experimental Paradigm

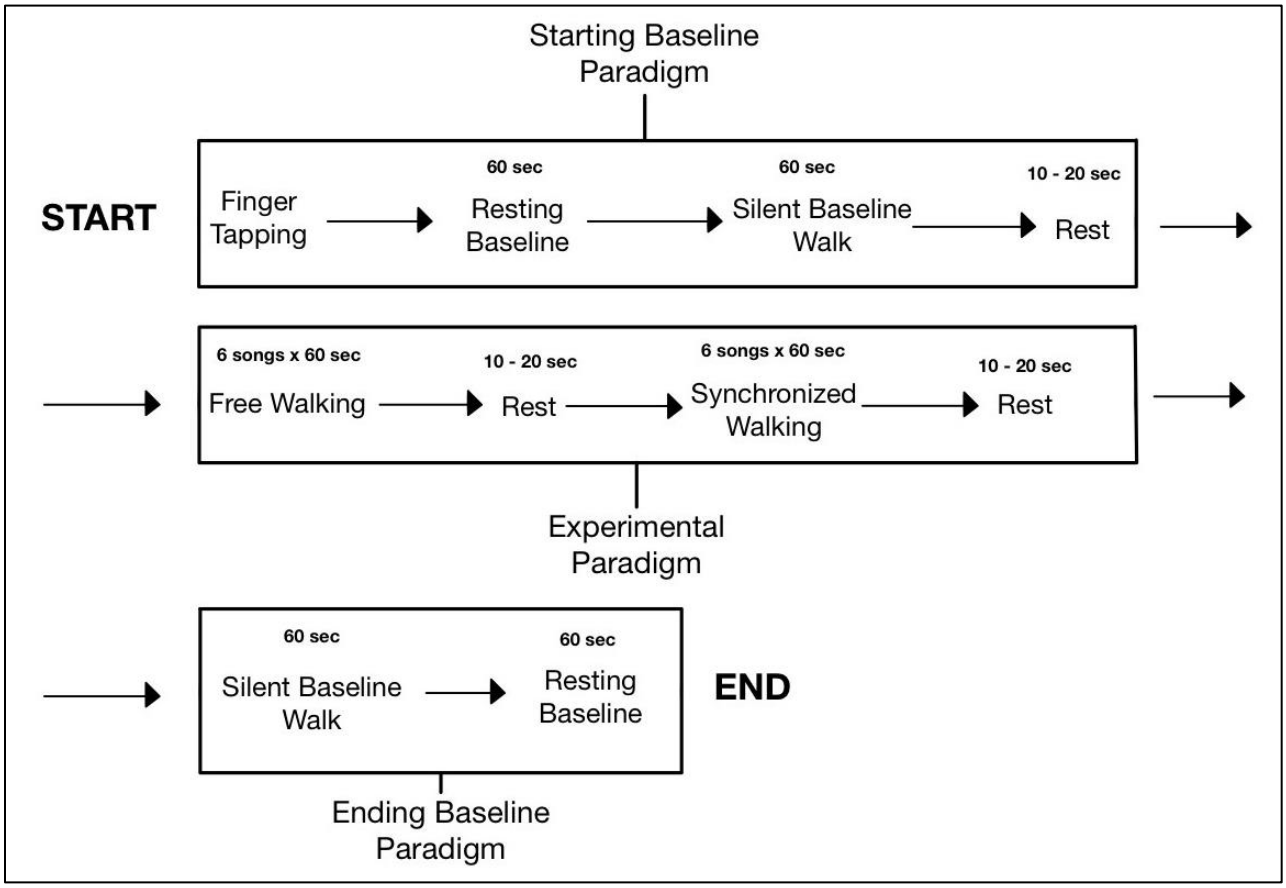


*Figure 4.* Testing day began with the 'Starting Baseline Paradigm' then progressed to 'Experimental Paradigm' and then concluded with the 'Ending Baseline Paradigm'. Extra time between each paradigm which was used for giving instructions to participants and is not visualized. All 'Free Walking' and 'Synchronized Walking' trials were separated by 10-20 second rest periods. Odd-numbered participants performed all 'Free Walking' trials first, even-numbered participants performed all 'Synchronized Walking' trials first. All procedures from 'START' to 'END' occurred during fNIRS recording.

## 6.5 Regions of Interest by Optode Channel

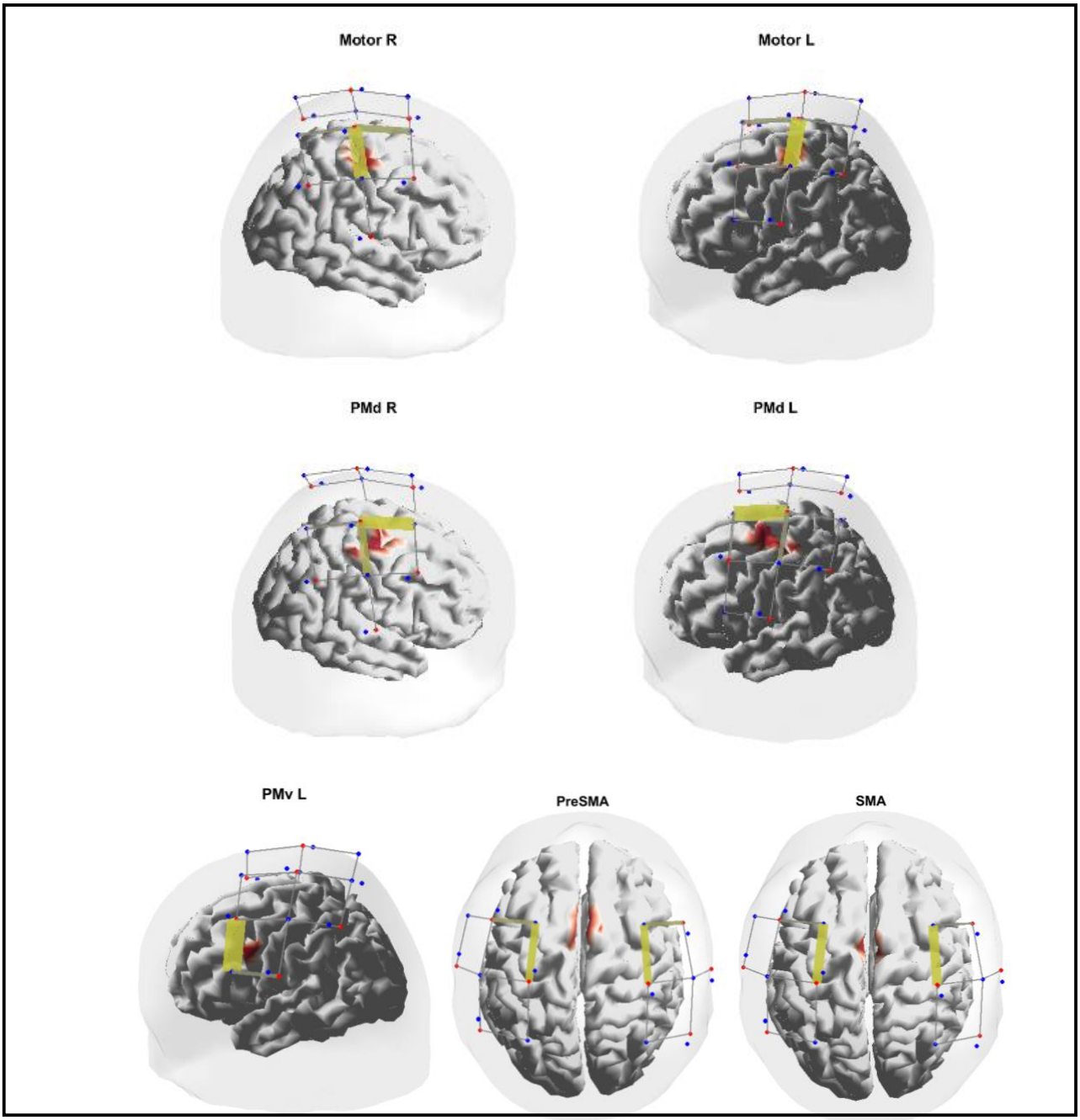


*Figure 5.* Thicker yellow lines on a channel suggest a higher probability the data recorded in that channel is representative of that region of interest (highlighted in red on cortex). R: Right, L: Left, Motor: Primary Motor Cortex (M1), PMd: Dorsal Premotor Cortex, PMv: Ventral Premotor Cortex, SMA: Supplementary Motor Area, PreSMA: Pre Supplementary Motor Area.

## 6.6 Mean Gait Parameters for Free and Synchronized Walking

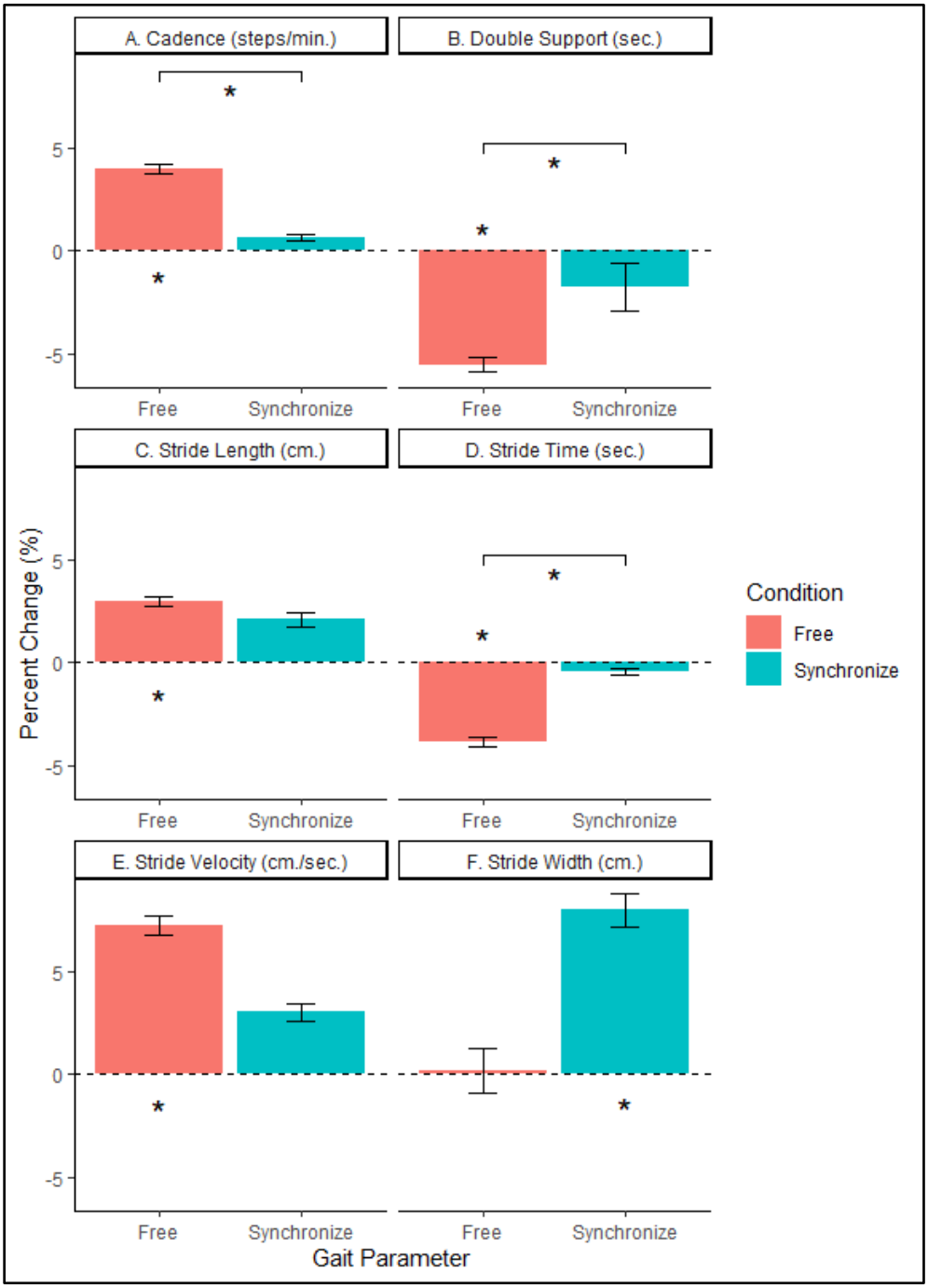


*Figure 6.* The mean normalized percent change from silent baseline walking for each gait parameter of interest is displayed for free walking (red) and synchronized walking (turquoise) conditions across participants. Error bars are the standard error of the mean, $n = 13$. Asterisks '*' represent significant difference in normalized percent change between free walking and synchronized walking and/or silent baseline walking for each gait parameter ($p < .05$).

### 6.7 Variability Gait Parameters for Free and Synchronized Walking

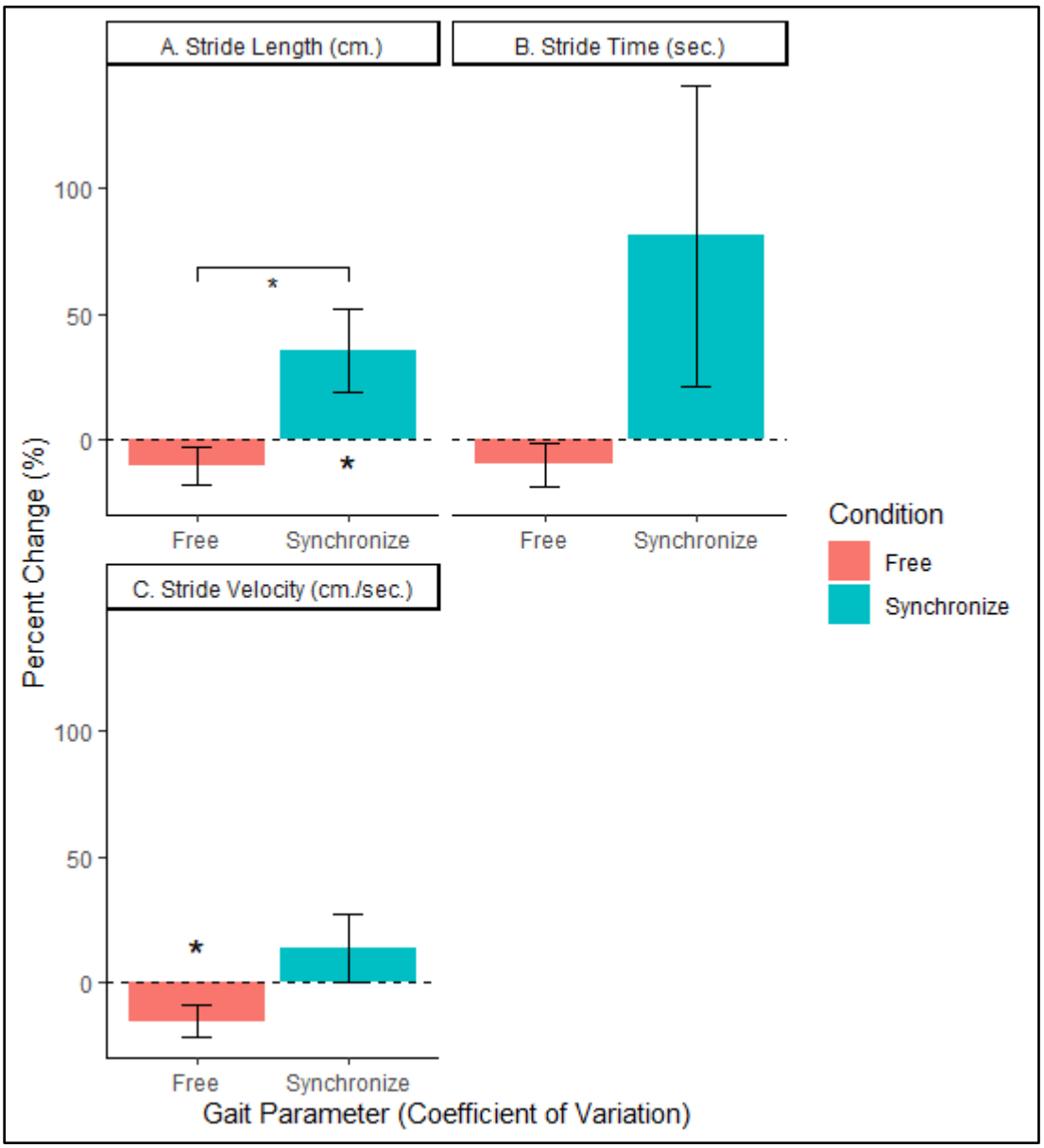


*Figure 7.* The normalized percent change from silent baseline walking for each gait variability parameter is displayed for free (red) and synchronized (turquoise) walking conditions. Error bars are the standard error of the mean, $n = 13$. Asterisks '*' represent significant difference in normalized percent change between free walking and synchronized walking and/or silent baseline walking for each gait parameter ($p < .05$).

### 6.8 Mean Gait Parameters during Free Walking: Musicians vs non-Musicians

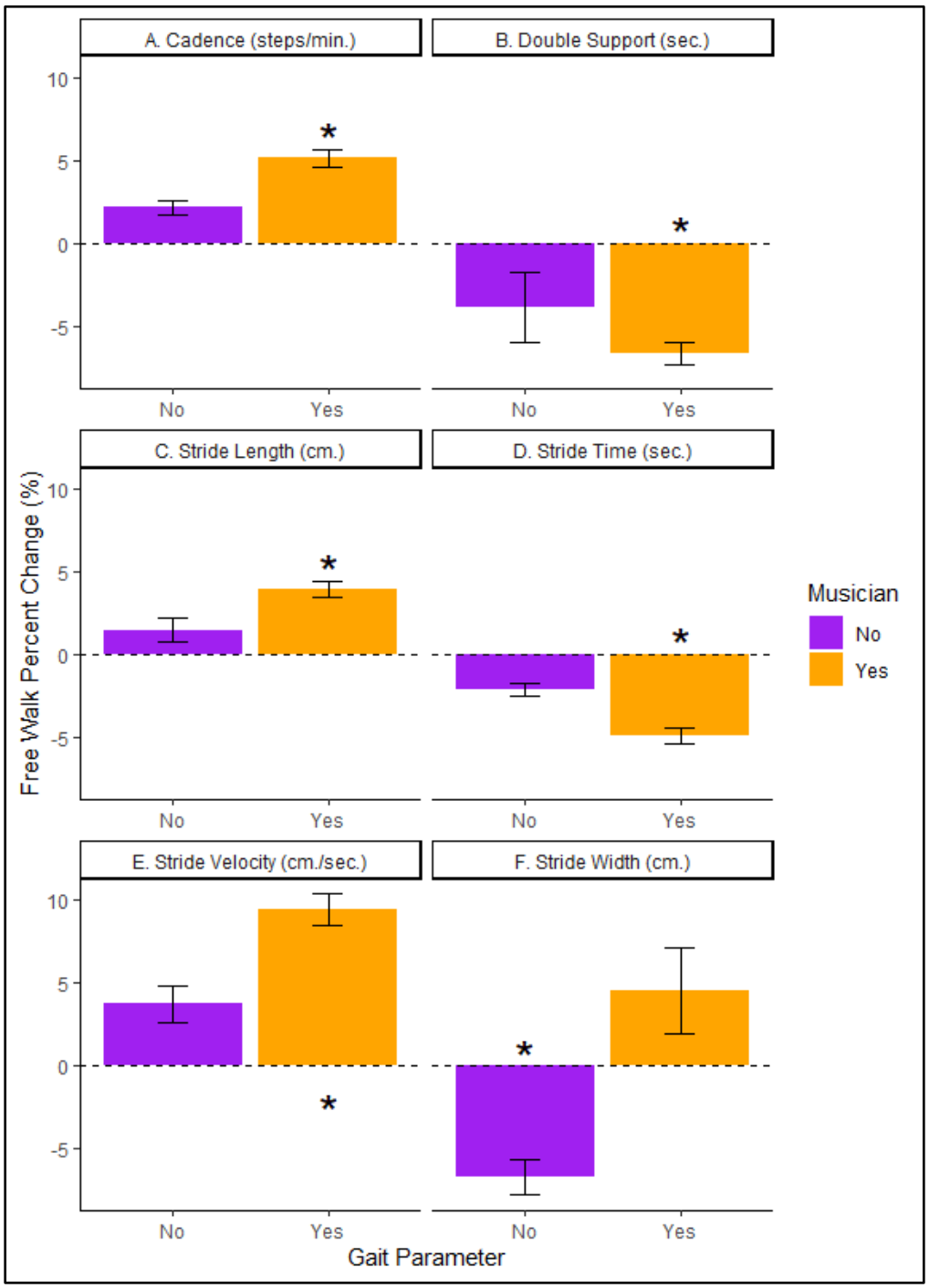


*Figure 8.* The normalized percent change from silent baseline walking to **free walking** for each mean gait parameter is displayed for musician group (orange) and non-musician group (purple). Error bars are the standard error of the mean. Musicians, $n = 8$; non-musicians, $n = 5$. Asterisks '*' indicate significant difference from silent baseline walking for that parameter and group ($p < .05$).

### 6.9 Variability Gait Parameters during Free Walking: Musician vs non-Musician

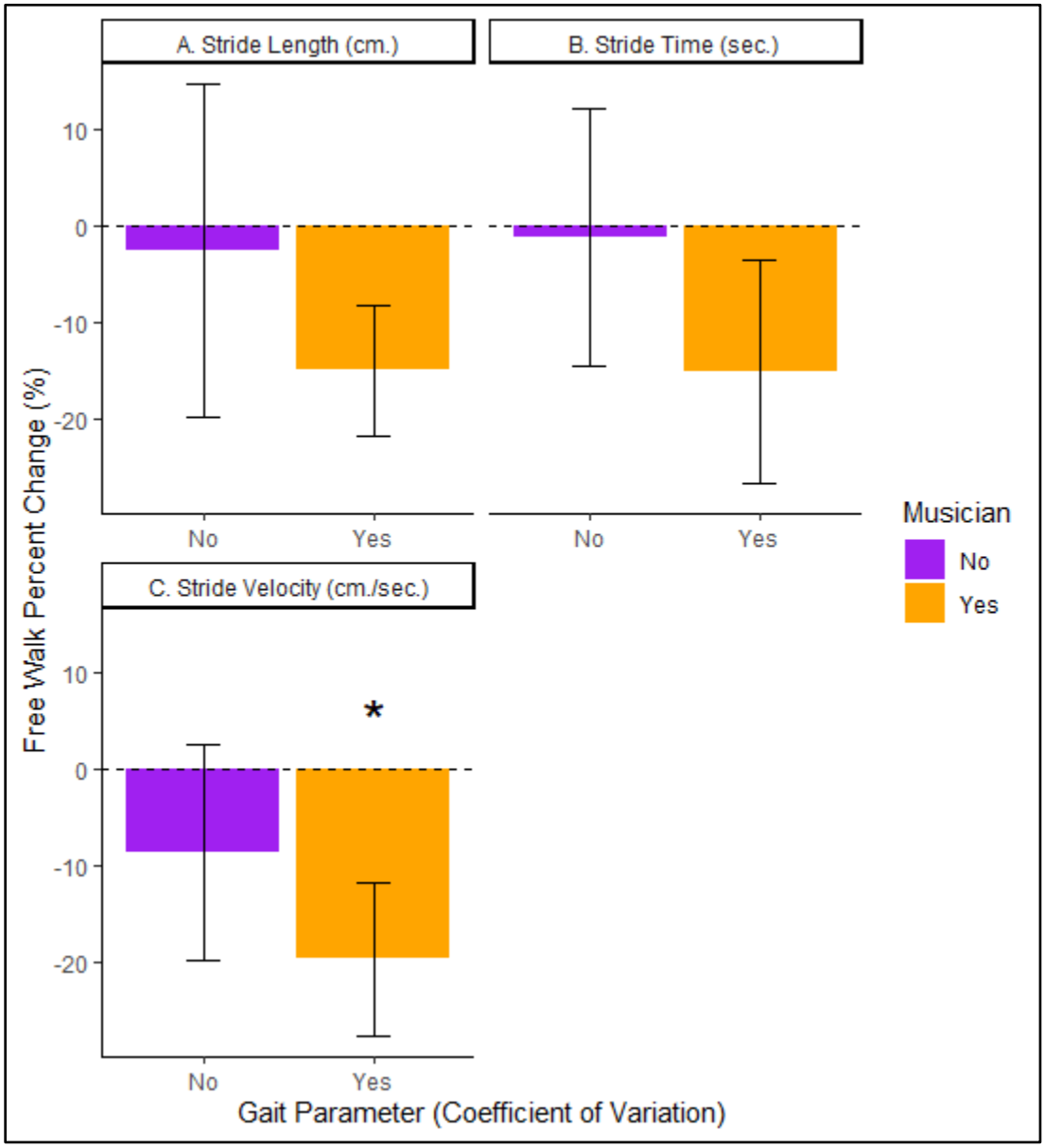


*Figure 9.* The normalized percent change from silent baseline walking to **free walking** for each variability gait parameter is displayed for musician group (orange) and non-musician group (purple). Error bars are the standard error of the mean. Musicians, $n$ = 8; non-musicians, $n$ = 5. Asterisks '*' indicate significant difference from silent baseline walking for that parameter and group ($p < .05$).

### 6.10 Mean Gait Parameters during Synchronized Walking: Musicians vs non-Musicians

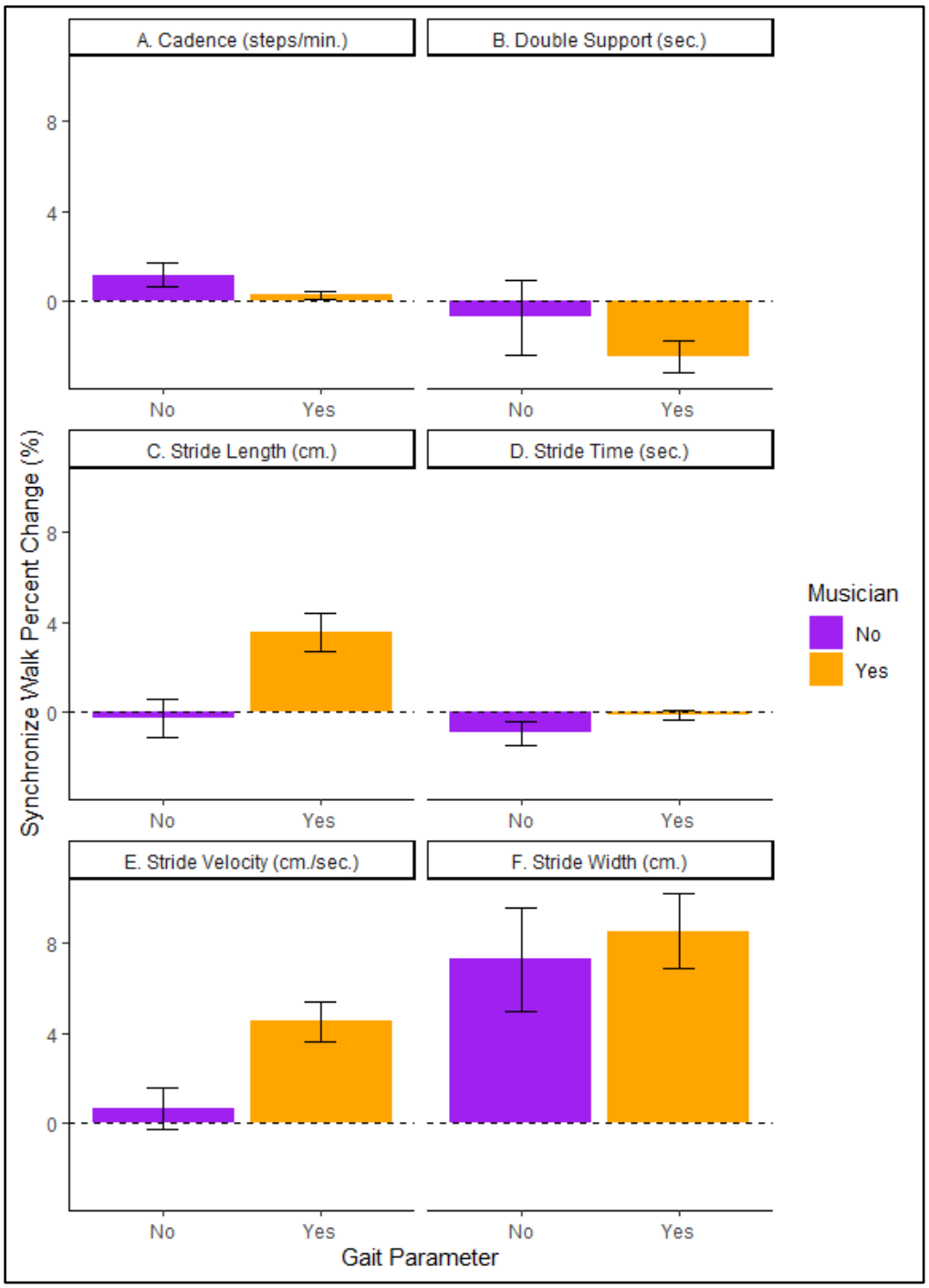


*Figure 10*. The normalized percent change from silent baseline walking to **synchronized walking** for each mean gait parameter is displayed for the musician group (orange) and non-musician group (purple). Error bars are the standard error of the mean. Musicians, $n$ = 8; non-musicians, $n$ = 5.

### 6.11 Variability Gait Parameters during Synchronized Walking: Musicians vs non-Musicians

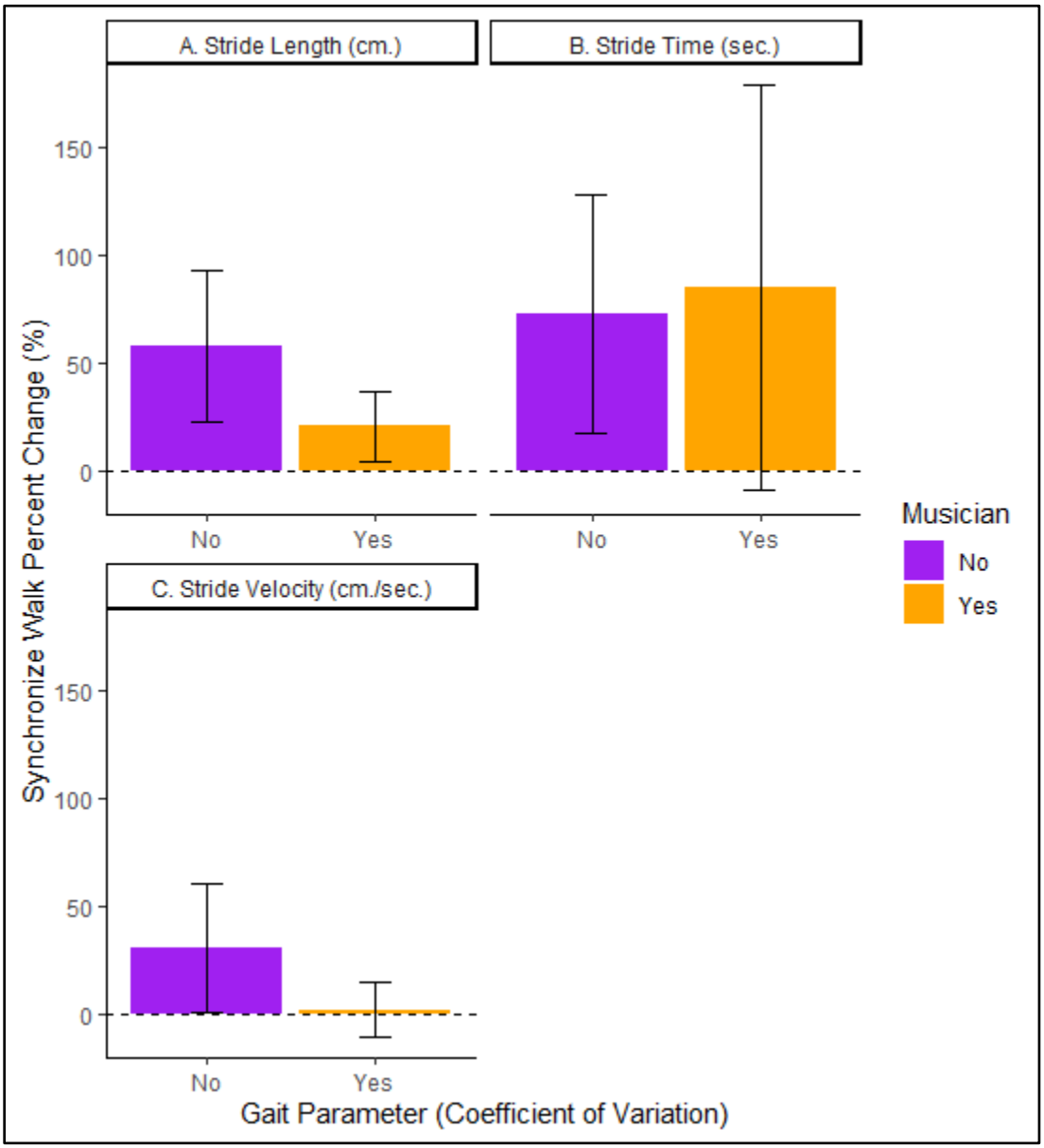


*Figure 11*. The normalized percent change from silent baseline walking to **synchronized walking** for each variability gait parameter is displayed for musician group (orange) and non-musician group (purple). Error bars are the standard error of the mean. Musicians, $n$ = 8; non-musicians, $n$ = 5.

## 6.12 Mean Change in Hemoglobin from Rest to Walking

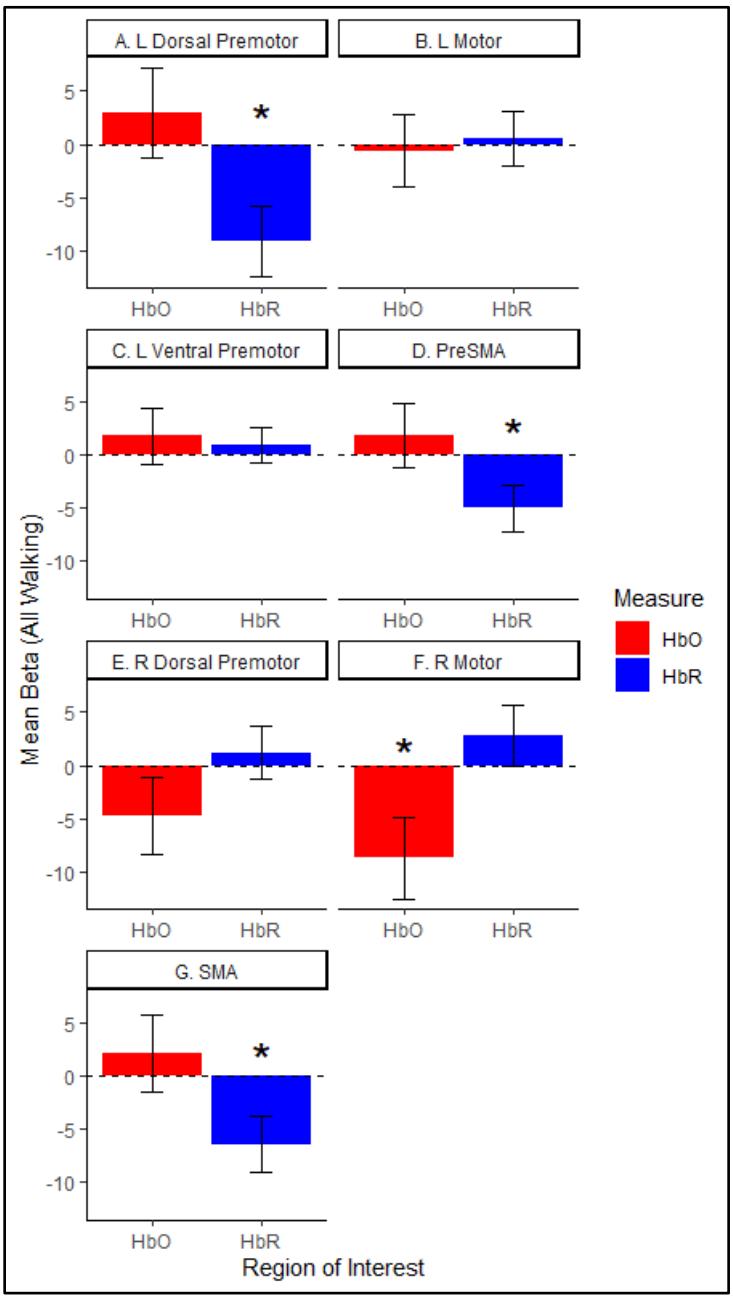


*Figure 12.* The mean change in *beta* magnitude (multiplied by a factor of 100 to improve visualization) across participants from resting baseline (*Beta* = 0) to all walking conditions is displayed for each region of interest and its respective HbO (red) and HbR (blue) reading. Error bars are the standard error of the mean, $n = 13$. Asterisks '*' represent a significant difference in either HbO or HbR concentration at a specific region of interest between resting baseline and all walking conditions ($p < .05$). R: Right. L: Left. Motor: Primary Motor Cortex (M1), PMd: Dorsal Premotor Cortex, PMv: Ventral Premotor Cortex, SMA: Supplementary Motor Area, PreSMA: Pre Supplementary Motor Area.

### 6.13.1 Mean Change in Activity from Silent Walking to Free and Synchronized Walking

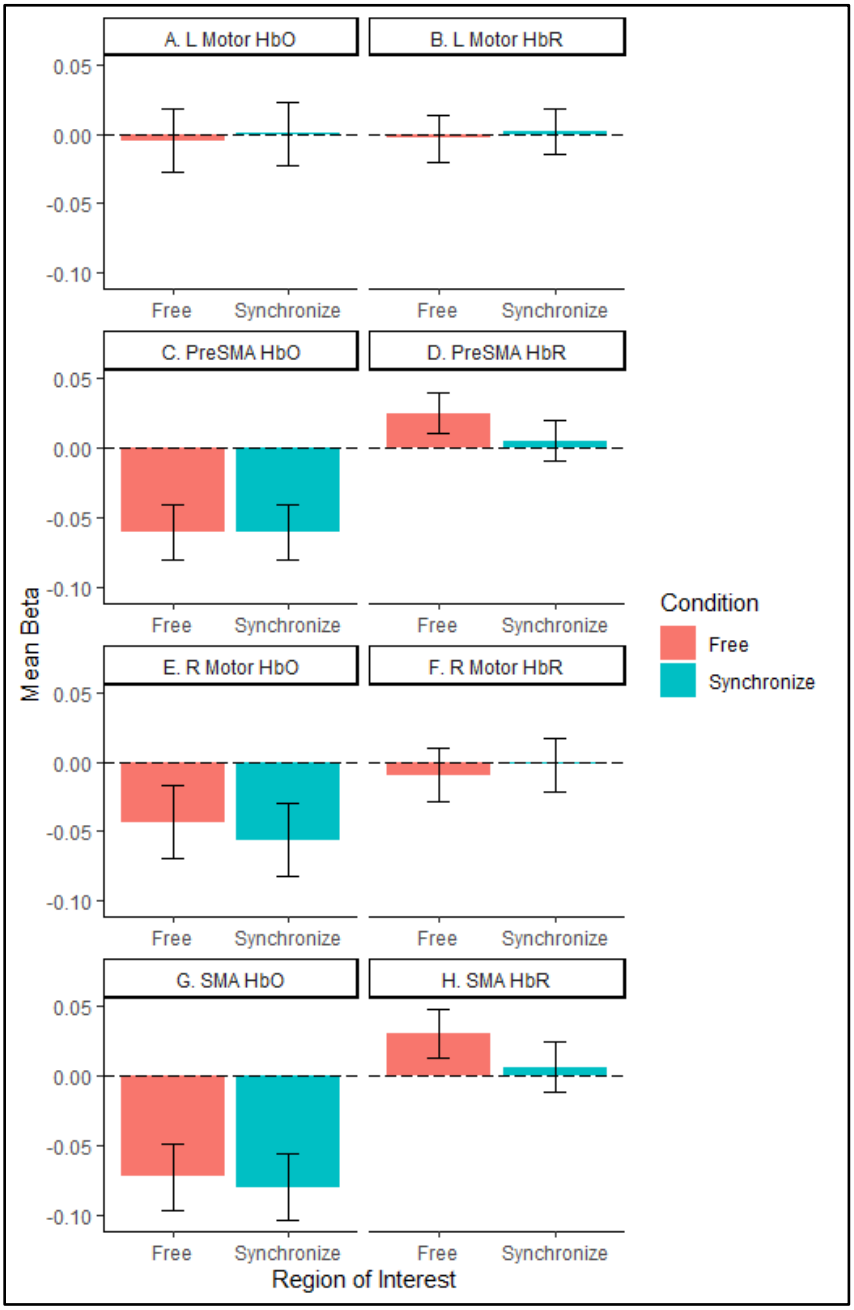


*Figure 13.1*. The mean change in *beta* magnitude across participants from silent baseline walking (*Beta* = 0) to either free walking (red) or synchronized walking (turquoise) for a particular region of interest and its respective hemoglobin reading (either HbO or HbR). Error bars are the standard error of the mean, *n* = 13. R: Right, L: Left, Motor: Primary Motor Cortex (M1), SMA: Supplementary Motor Area, PreSMA: Pre Supplementary Motor Area.

### 6.13.2 Mean Change in Activity from Silent Walking to Free and Synchronized Walking

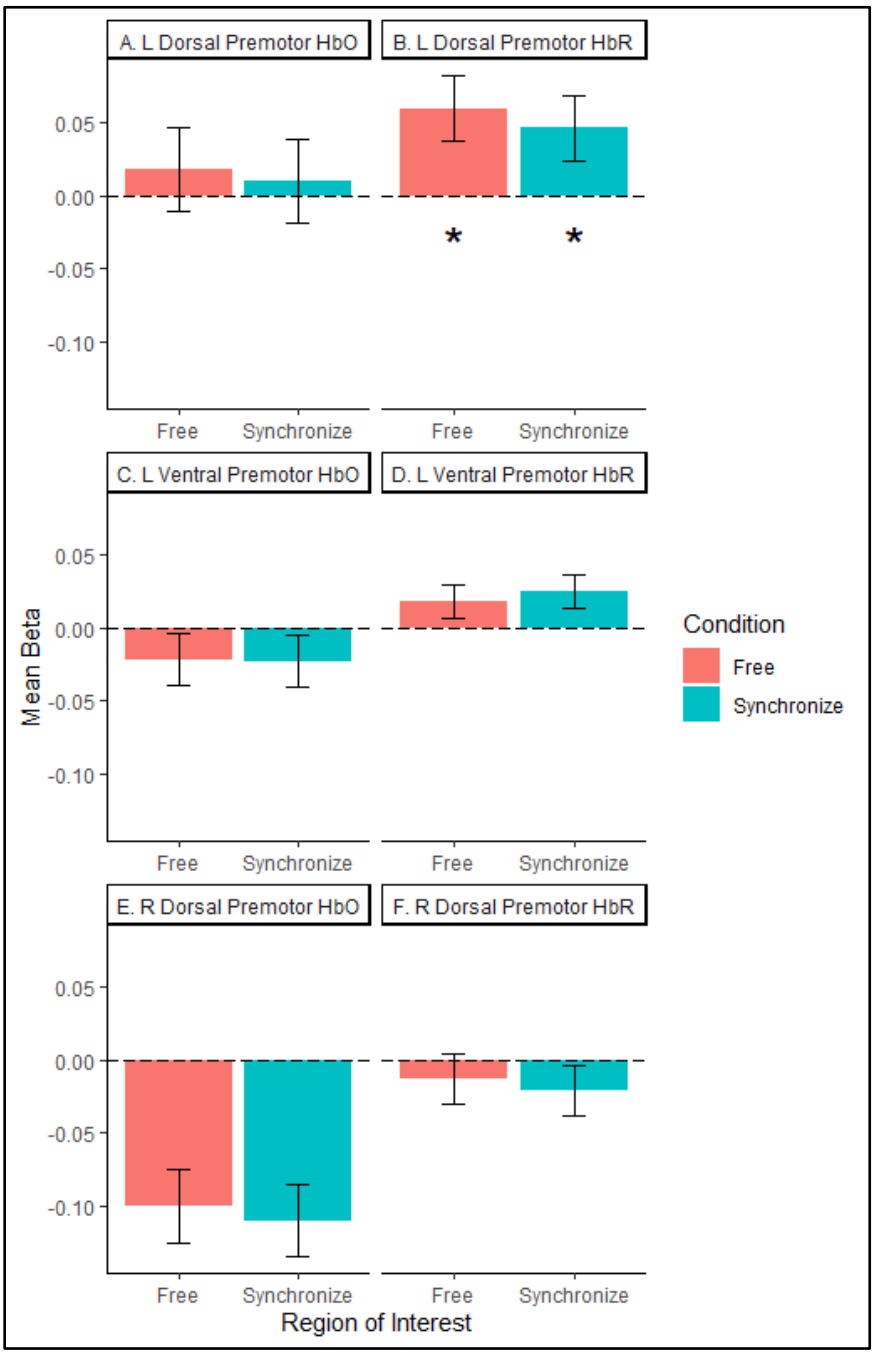


*Figure 13.2.* The mean change in *beta* magnitude across participants from silent baseline walking (*Beta* = 0) to either free walking (red) or synchronized walking (turquoise) for a particular region of interest and its respective hemoglobin reading (either HbO or HbR). Error bars are the standard error of the mean, *n* = 13. Asterisks '*' represents a significant difference in either HbO (left column) or HbR (right column) from silent baseline walking for a particular region of interest and walking condition. R: Right. L: Left. Premotor: Premotor Cortex.

### 6.14 Cadence vs Left Primary Motor Cortex Activity: All Participants

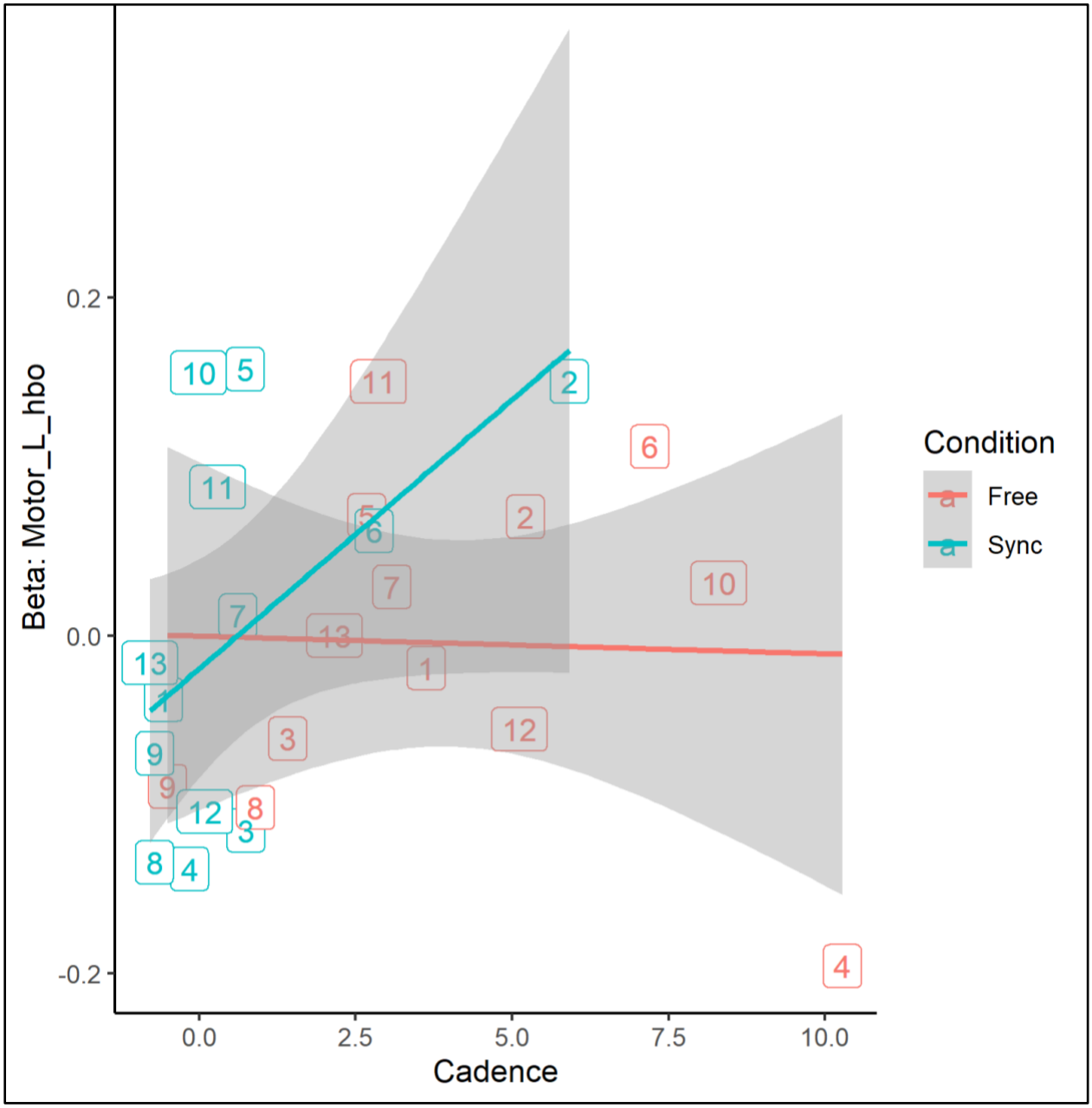


*Figure 14.* Correlation between left primary motor cortex activity and cadence during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. Cadence = 0 suggests no normalized percent change in cadence from silent baseline walking. Numbers indicate participant, *n* = 13. Motor_L_hbo: HbO reading of Left Primary Motor Cortex.

### 6.15 Cadence vs Left Primary Motor Cortex Activity: Musicians vs non-Musicians

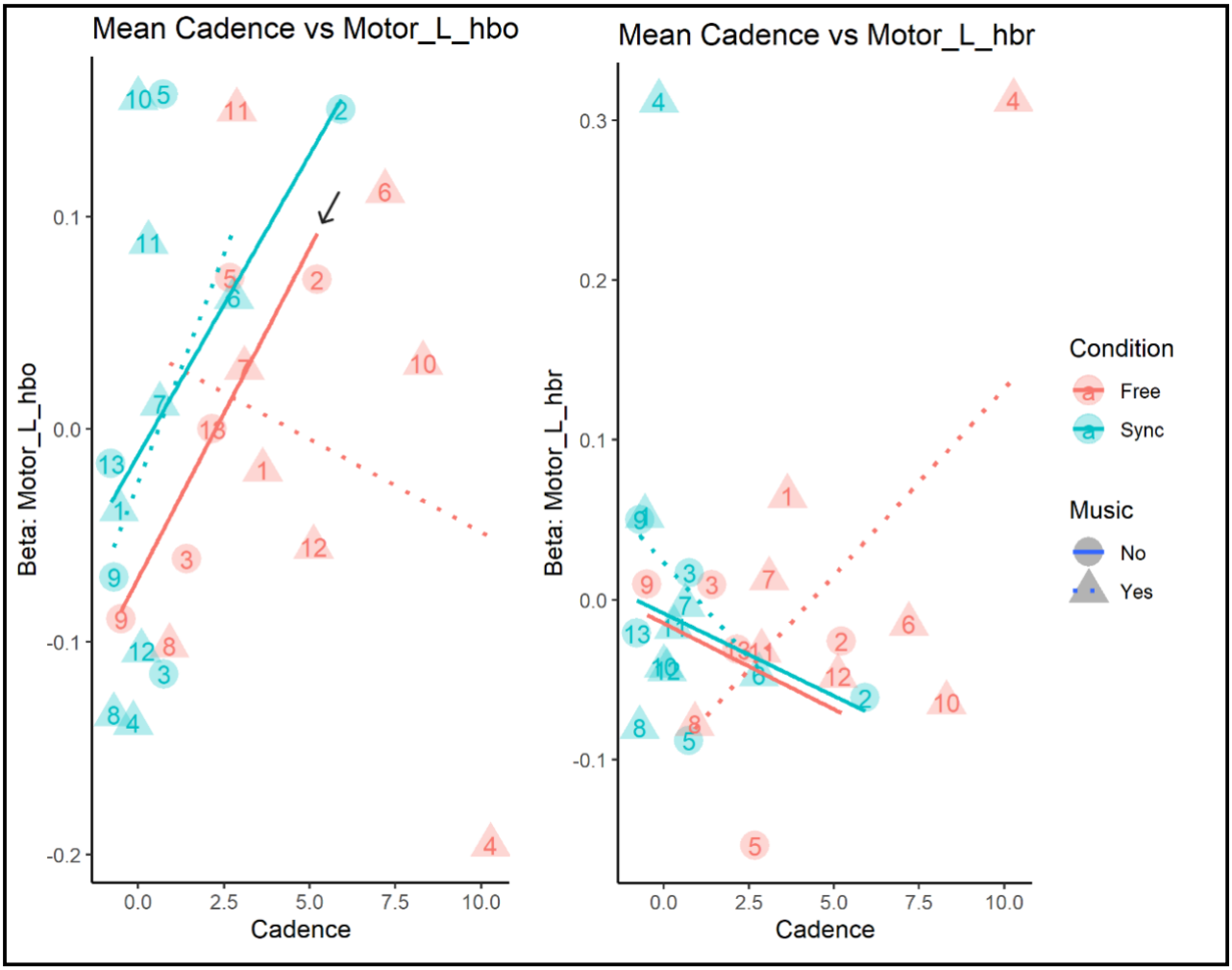


*Figure 15.* Correlation between left primary motor cortex activity and cadence during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. Cadence = 0 suggests no normalized percent change in cadence from silent baseline walking. Numbers indicate participant, $n$ = 13. Left plot shows HbO reading, right plot shows HbR reading. Significant correlation between Motor_L_hbo and cadence for non-musicians during free walking indicated by black arrow on left plot ($r$ = 0.88, $p$ = .05). Motor_L_hbo: HbO reading of Left Primary Motor Cortex.

**6.16 Mean Stride Time vs Left Primary Motor Cortex: All Participants**

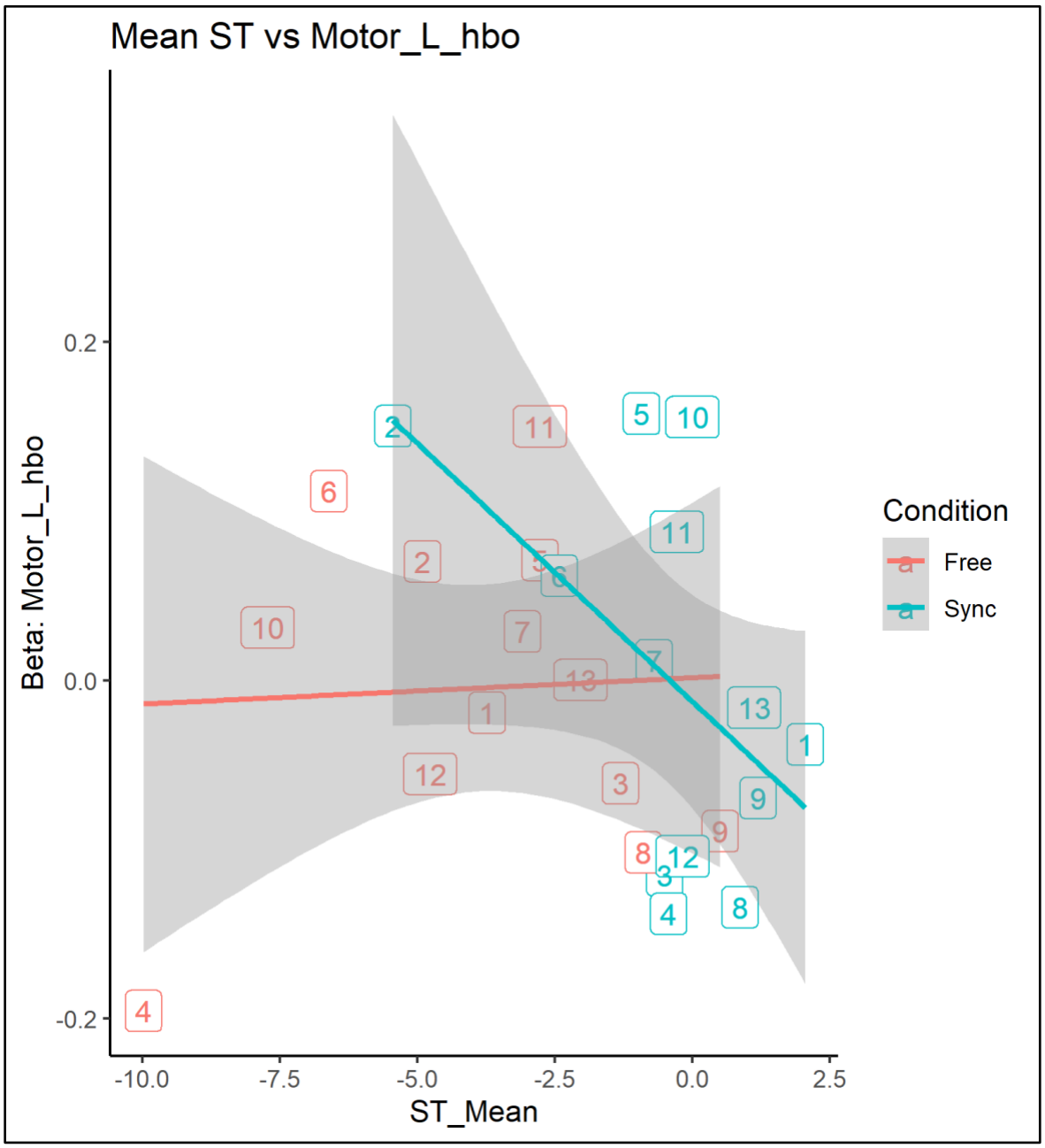


*Figure 16*. Correlation between left primary motor cortex activity and stride time during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. ST_Mean = 0 suggests no normalized percent change in stride time from silent baseline walking. Numbers indicate participant, *n* = 13. Motor_L_hbo: HbO reading of Left Primary Motor Cortex. ST_Mean: Mean Stride time.

### 6.17 Mean Stride Time vs Left Primary Motor Cortex: Musicians vs non-Musicians

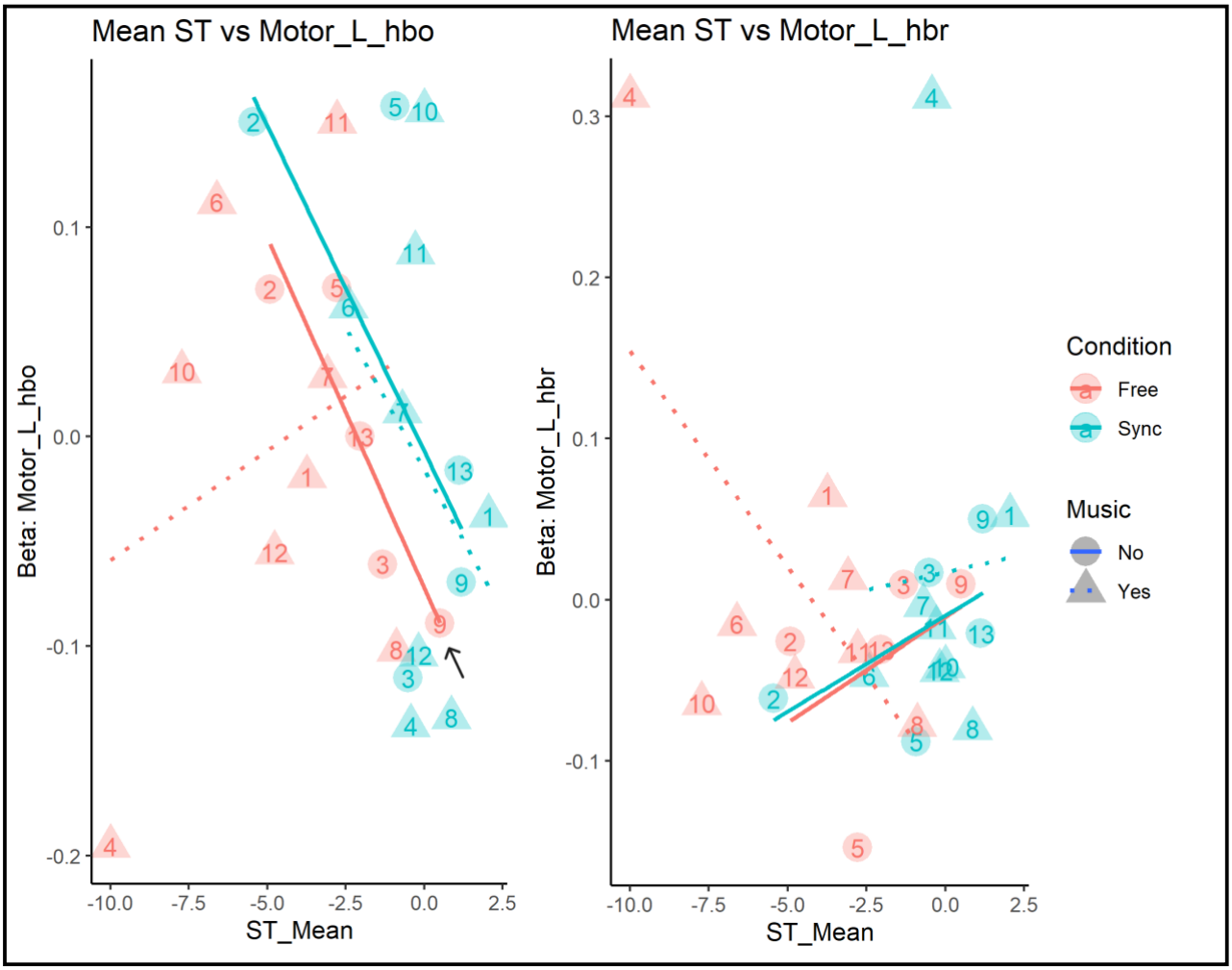


*Figure 17.* Correlation between left primary motor cortex activity and stride time during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. ST_Mean = 0 suggests no normalized percent change in stride time from silent baseline walking. Numbers indicate participant, $n$ = 13. Left plot shows HbO reading, right plot shows HbR reading. Significant correlation between a Motor_L_hbo and stride time for non-musicians during free walking indicated by black arrow on left plot ($r$ = -0.90, $p$ < .05). Motor_L_hbo: HbO reading of Left Primary Motor Cortex. ST_Mean: Mean Stride time.

**6.18 Mean Stride Length vs Left Primary Motor Cortex: All Participants**

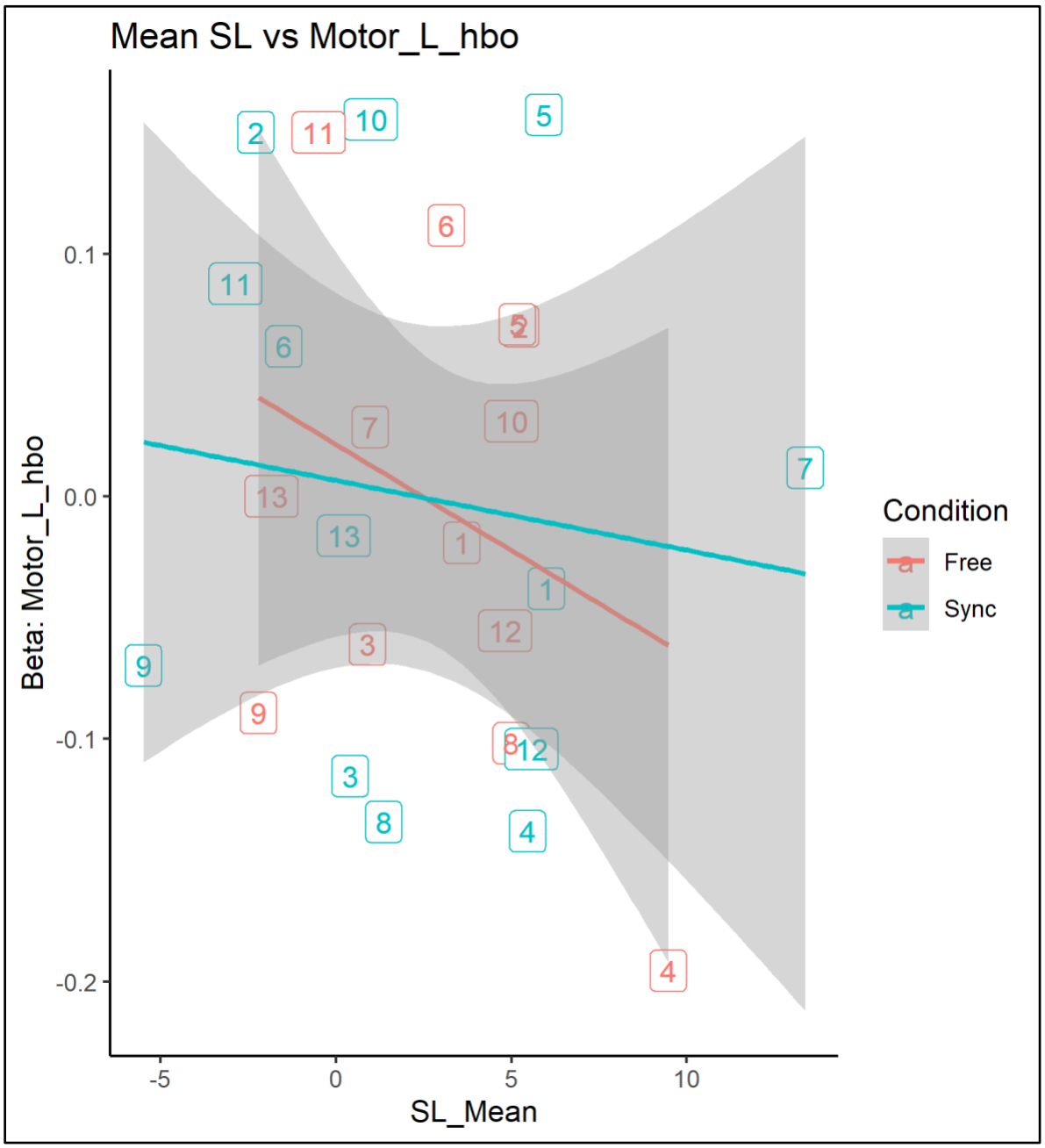


*Figure 18*. Correlation between left primary motor cortex activity and stride length during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SL_Mean = 0 suggests no normalized percent change in stride length from silent baseline walking. Numbers indicate participant, *n* = 13. Motor_L_hbo: HbO reading of Left Primary Motor Cortex. SL_Mean: Mean Stride length.

### 6.19 Mean Stride Length vs Left Primary Motor Cortex: Musicians vs non-Musicians

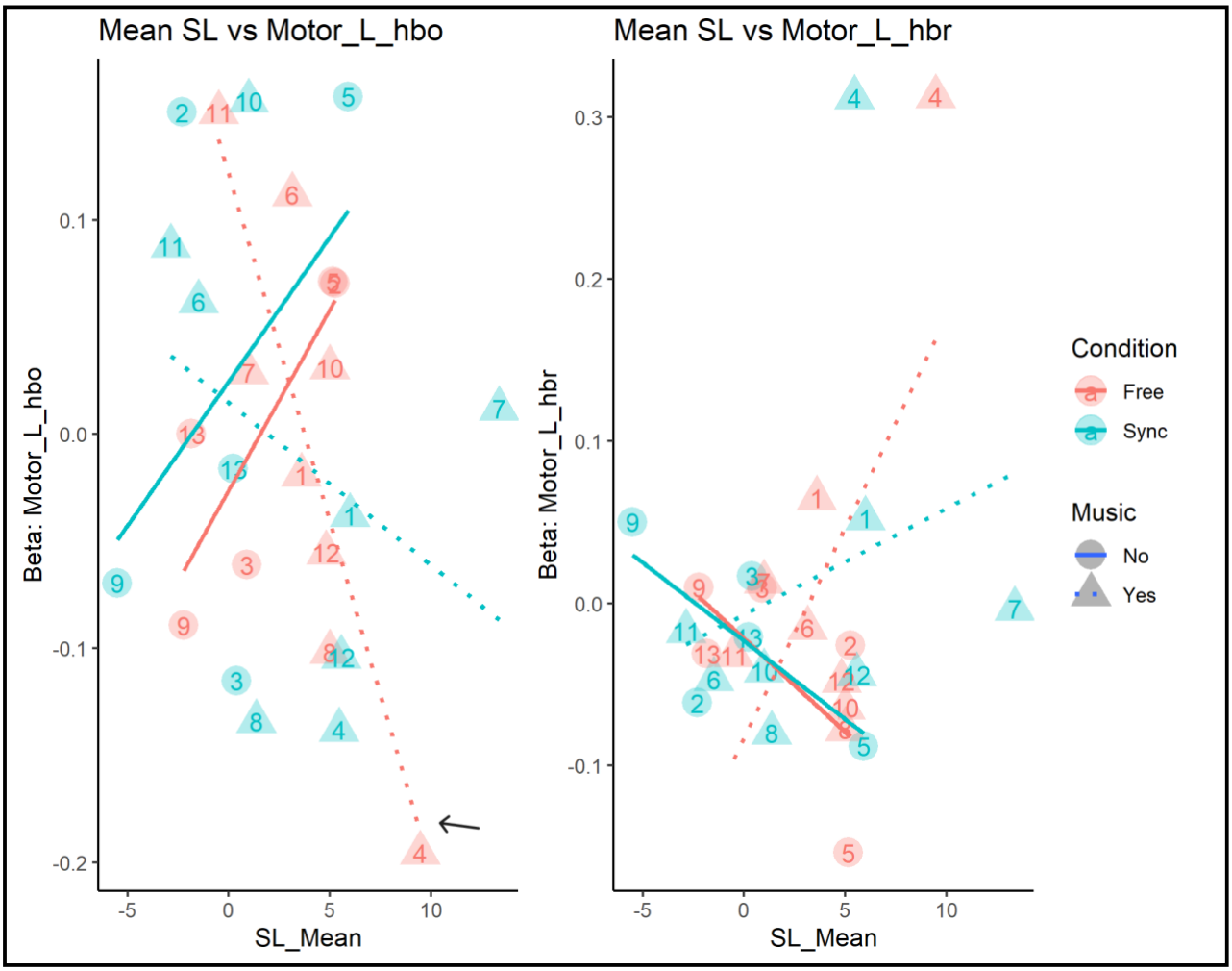


*Figure 19.* Correlation between left primary motor cortex activity and stride length during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SL_Mean = 0 suggests no normalized percent change in stride length from silent baseline walking. Numbers indicate participant, $n$ = 13. Left plot shows HbO reading, right plot shows HbR reading. Significant correlation between a Motor_L_hbo and stride length for musicians during free walking indicated by black arrow on left plot ($r$ = -0.86, $p$ < .05). Motor_L_hbo: HbO reading of Left Primary Motor Cortex. SL_Mean: Mean Stride length.

**6.20 Mean Stride Velocity vs Left Primary Motor Cortex: All Participants**

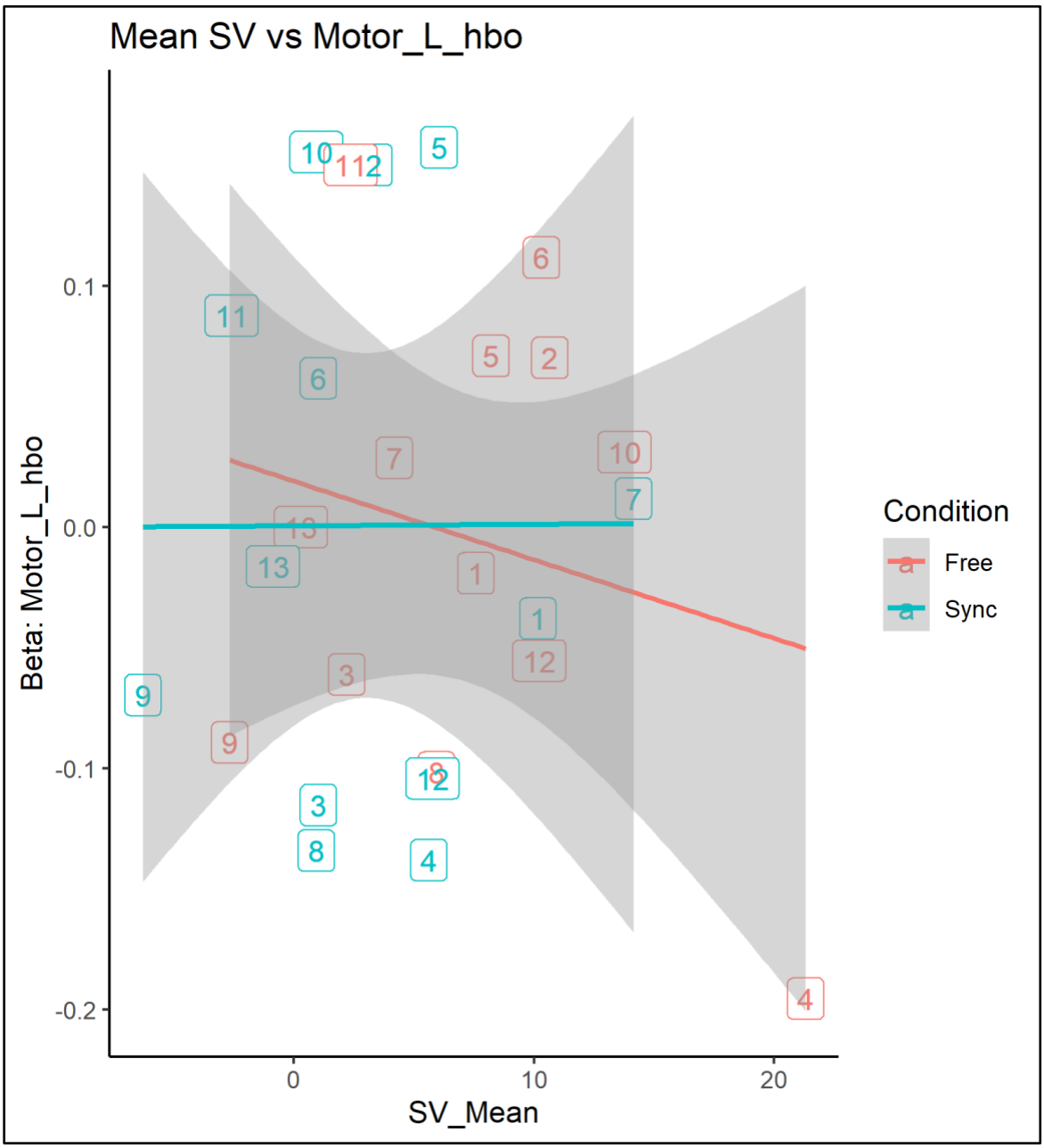


*Figure 20*. Correlation between left primary motor cortex activity and stride velocity during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SV_Mean = 0 suggests no normalized percent change in stride velocity from silent baseline walking. Numbers indicate participant, *n* = 13. Motor_L_hbo: HbO reading of Left Primary Motor Cortex. SV_Mean: Mean Stride velocity.

**6.21 Mean Stride Velocity vs Left Primary Motor Cortex: Musicians vs non-Musicians**

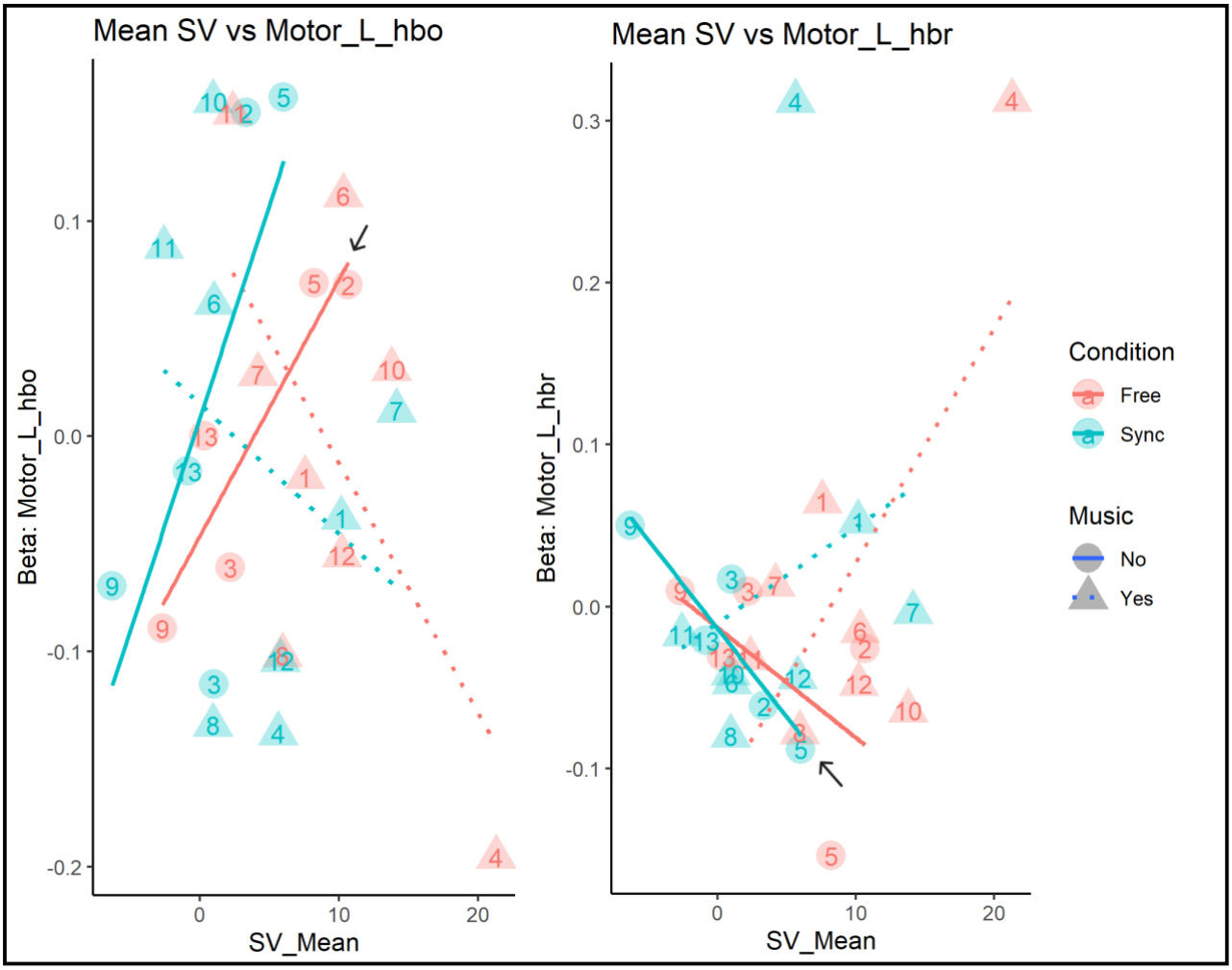


*Figure 21*. Correlation between left primary motor cortex activity and stride velocity during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SV_Mean = 0 suggests no normalized percent change in stride velocity from silent baseline walking. Numbers indicate participant, $n$ = 13. Left plot shows HbO reading, right plot shows HbR reading. Significant correlation between Motor_L_hbo reading and stride velocity for non-musicians during free walking indicated by black arrow on left plot ($r$ = 0.90, $p$ < .05). Significant correlation between Motor_L_hbr reading and stride velocity for non-musicians during synchronized walking indicated by black arrow on right plot ($r$ = -0.91, $p$ < .05). Motor_L_hbo: HbO reading of Left Primary Motor Cortex. SV_Mean: Mean Stride velocity.

**6.22 Stride Velocity Variability vs Supplementary Motor Area: All Participants**

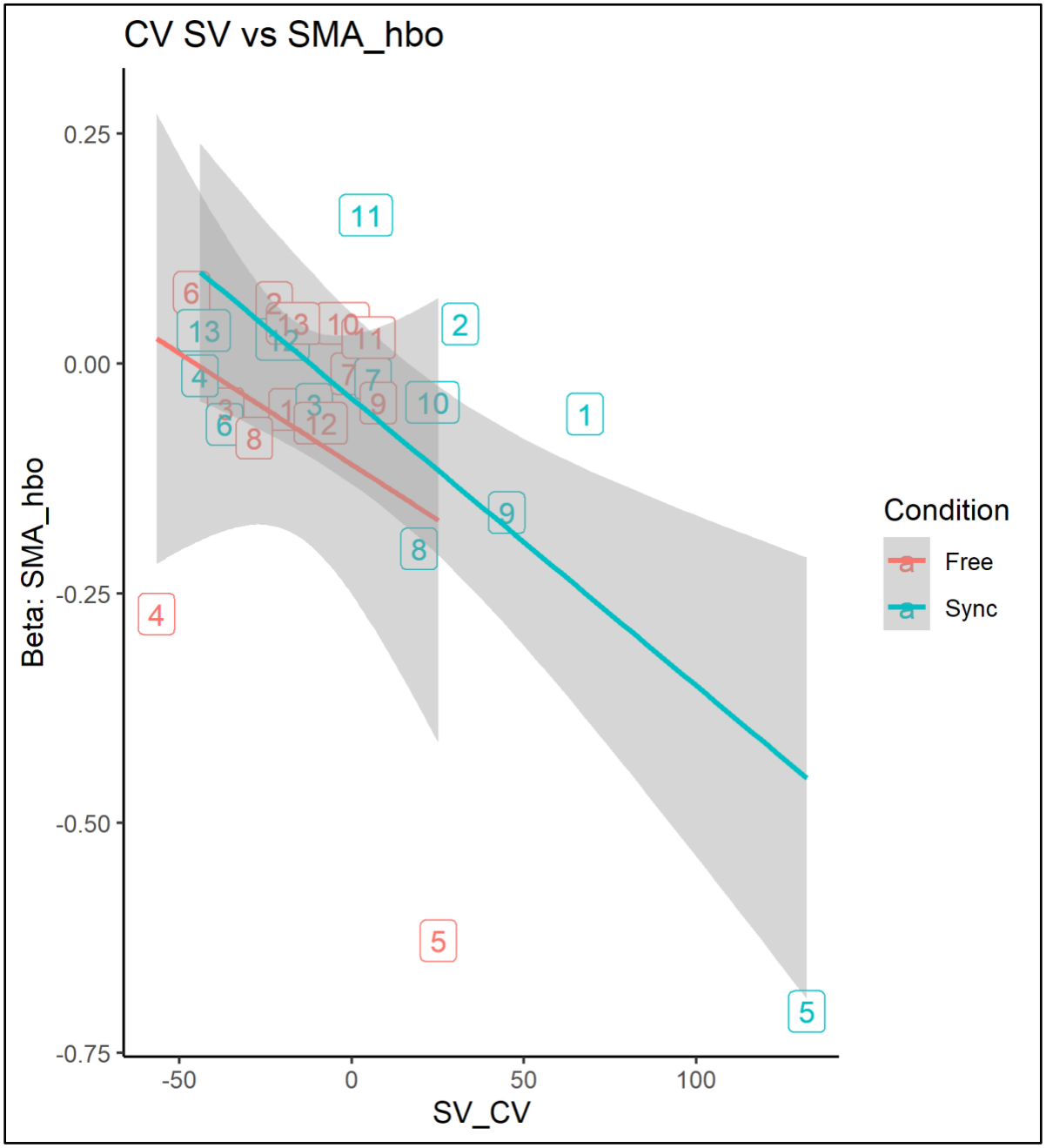


*Figure 22*. Correlation between supplementary motor area activity and stride velocity variability during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SV_CV = 0 suggests no normalized percent change in stride length variability from silent baseline walking. Numbers indicate participant, *n* = 13. SMA_hbo: HbO reading of Supplementary Motor Area. SV_CV: Stride Velocity coefficient of variation, indicative of variability.

**6.23 Stride Velocity Variability vs Supplementary Motor Area: Musicians vs non-Musicians**

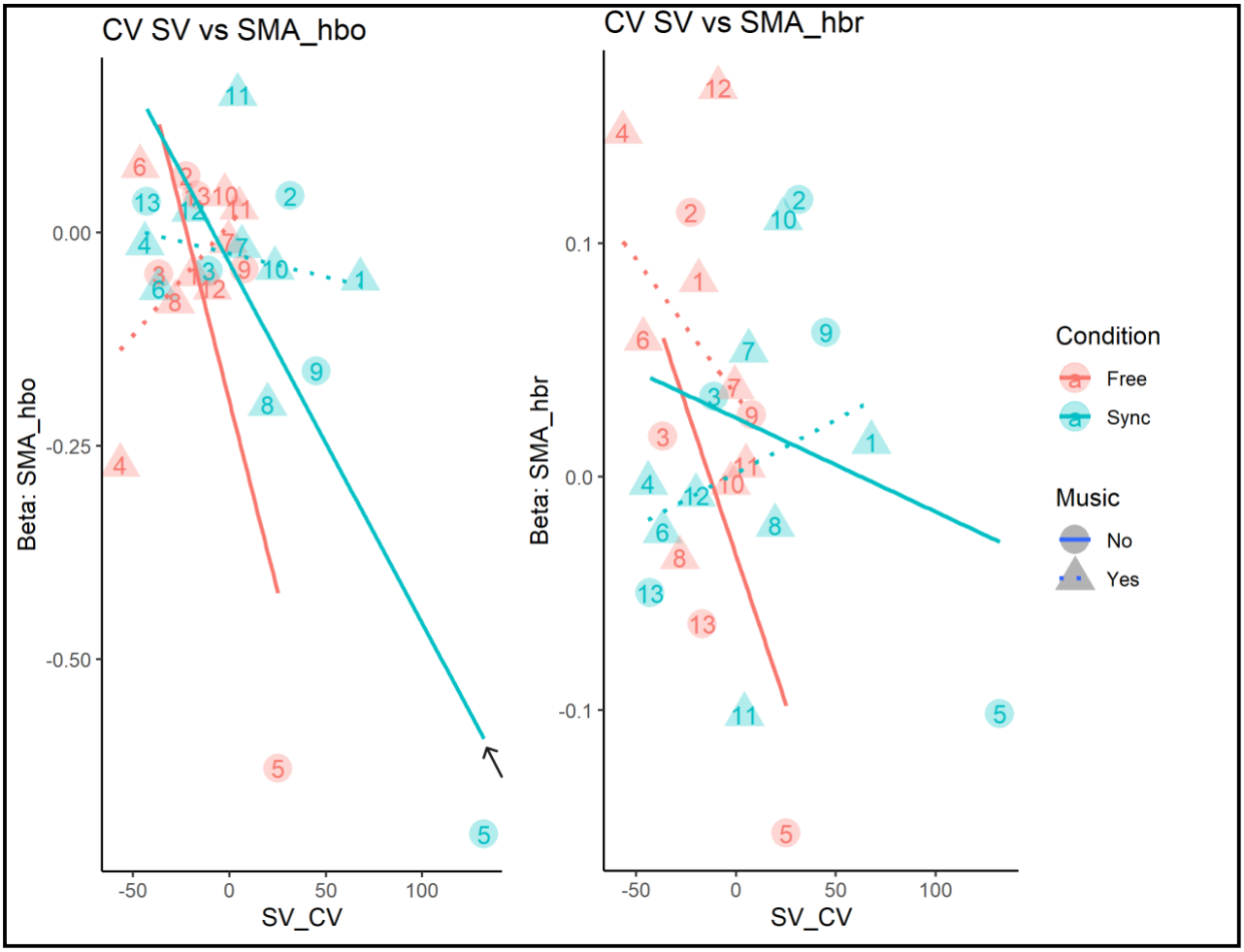


*Figure 23*. Correlation between supplementary motor area activity and stride velocity variability during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. SV_CV = 0 suggests no normalized percent change in stride velocity variability from silent baseline walking. Numbers indicate participant, $n$ = 13. Significant correlation between SMA_hbo reading and stride velocity variability for non-musicians during synchronized walking indicated by black arrow on left plot ($r$ = -0.90, $p$ < .05). Left plot shows HbO reading, right plot shows HbR reading. SMA_hbo: HbO reading of Supplementary Motor Area. SV_CV: Stride Velocity coefficient of variation, indicative of variability.

**6.24 Stride Time Variability vs Supplementary Motor Area: All Participants**

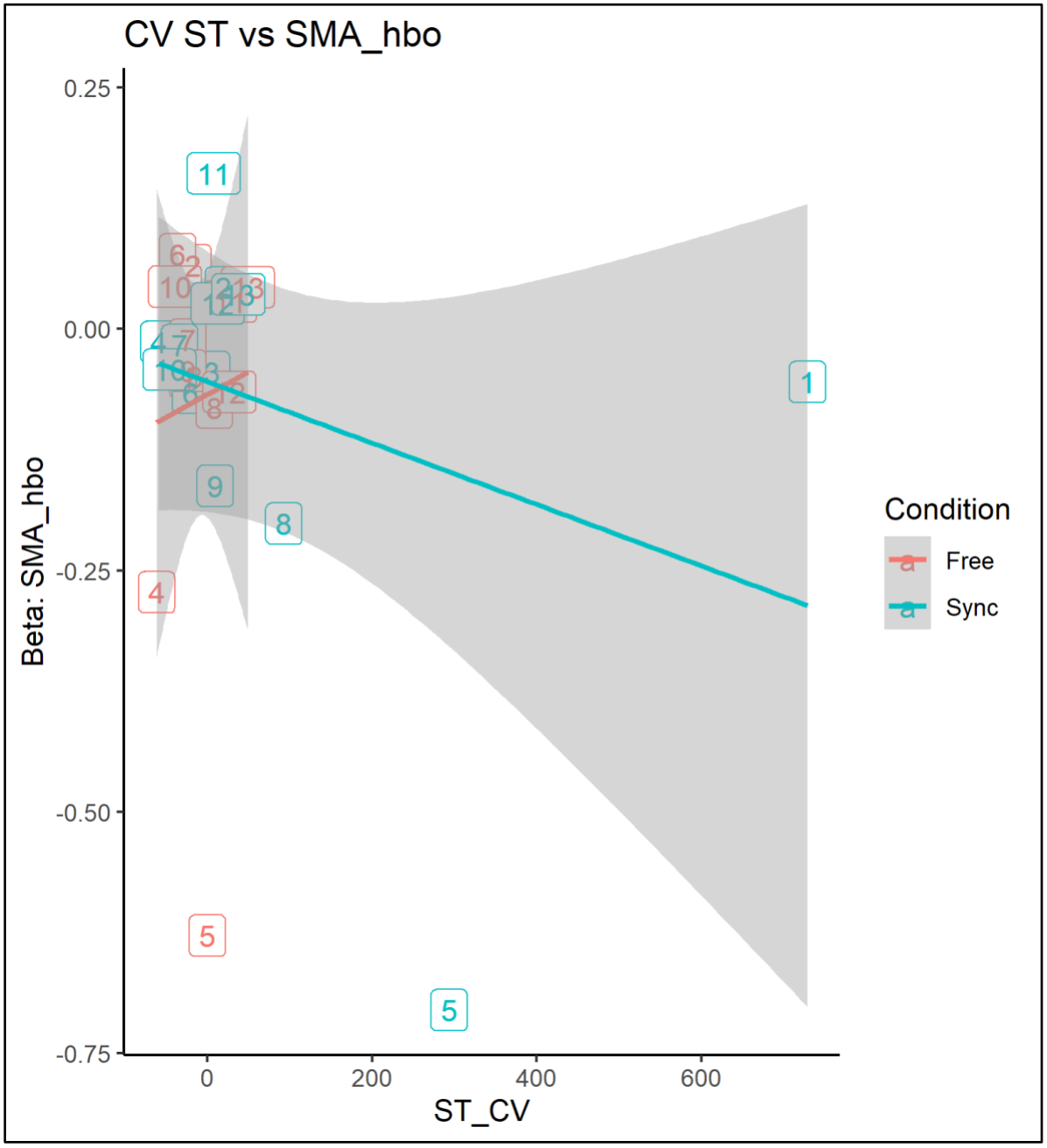


*Figure 24*. Correlation between supplementary motor area activity and stride time variability during free walking (red) and synchronized walking (turquoise). Solid lines show correlational trends for either the free walking (red solid line) or synchronized walking (turquoise solid line). Beta = 0 suggests no change in cortical activity from silent baseline walking. ST_CV = 0 suggests no normalized percent change in stride time variability from silent baseline walking. Numbers indicate participant, *n* = 13. SMA_hbo: HbO reading of Supplementary Motor Area. ST_CV: Stride Time coefficient of variation, indicative of variability.

**6.25 Stride Time Variability vs Supplementary Motor Area: Musicians vs non-Musicians**

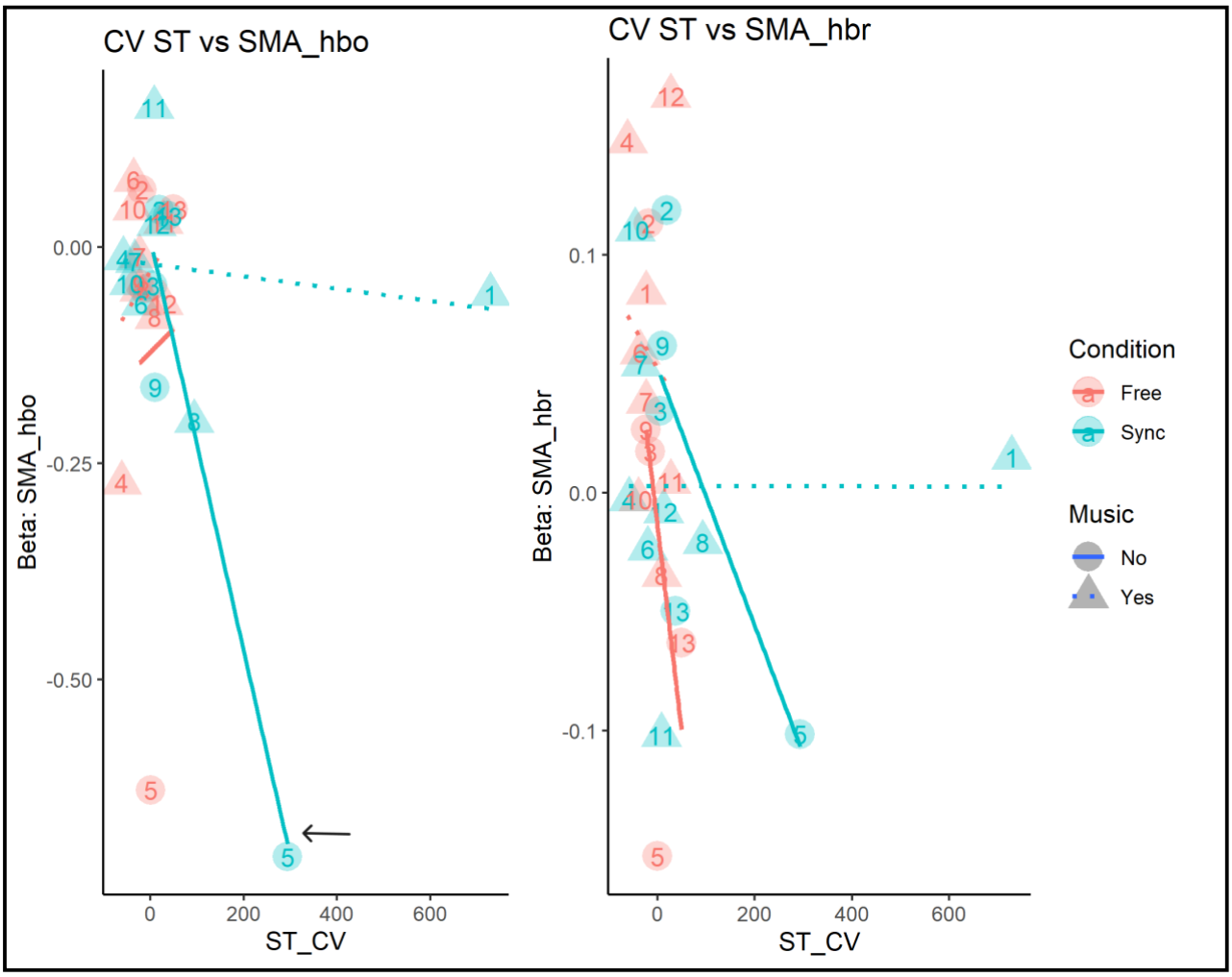


*Figure 25*. Correlation between supplementary motor area activity and stride time variability during free walking (red) and synchronized walking (turquoise) between musicians (triangles) and non-musicians (circles). Dotted lines show correlational trends for musicians for free walking (red dotted line) and synchronized walking (turquoise dotted line). Beta = 0 suggests no change in cortical activity from silent baseline walking. ST_CV = 0 suggests no normalized percent change in stride time variability from silent baseline walking. Numbers indicate participant, $n$ = 13. Significant correlation between SMA_hbo reading and stride time variability for non-musicians during synchronized walking indicated by black arrow on left plot ($r$ = -0.94, $p$ < .05). Left plot shows HbO reading, right plot shows HbR reading. SMA_hbo: HbO reading of Supplementary Motor Area. ST_CV: Stride Time coefficient of variation, indicative of variability.

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