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Title: Cortical excitability after pediatric mild traumatic brain injury

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Keywords: mild traumatic brain injury; concussion; pediatrics; transcranial magnetic stimulation; cortical silent period; long interval intracortical inhibition

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Abstract: Introduction: Mild traumatic brain injury (mTBI) outcomes are variable, and 10-15% may suffer from prolonged symptoms beyond 3 months that impair the child's return to normal activities. Neurophysiological mechanisms of mTBI are incompletely understood, particularly in children, but alterations in cortical excitability have been proposed to underlie post-concussion syndrome. Improved understanding is required to advance interventions and improve outcomes.
Objective/Hypothesis: To determine if cortical excitability is altered in children with mTBI, and its association with clinical symptoms.
Methods: This was a cross-sectional controlled cohort study. School-aged children (8-18 years) with mTBI were compared to healthy controls. Cortical excitability was measured using multiple TMS paradigms in children with (symptomatic) and without (recovered) persistent symptoms one-month post-injury. Primary outcome was the cortical silent period (cSP), a potential neurophysiological biomarker of GABAergic inhibition. Secondary outcomes included additional TMS neurophysiology, safety and tolerability. Associations between neurophysiology parameters and clinical symptoms were evaluated.
Results: Fifty-three children with mTBI (55% male; mean age 14.1 SD: 2.4 years; 35 symptomatic and 27 asymptomatic participants) and 28 controls (46% male; mean age 14.3 SD: 3.1 years) were enrolled. cSP duration was similar between groups ($F(2, 73)=0.55$, $p=0.582$). Log10 Long interval intracortical inhibition (LICI) was reduced in symptomatic participants compared to healthy controls ($F(2, 59)=3.83$, $p=0.027$). Procedures were well tolerated with no serious adverse events.
Conclusions: TMS measures of cortical excitability are altered at one month in children with mTBI. Long interval cortical inhibition is decreased in children who remain symptomatic at one month post-injury.

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Keywords: Transcranial magnetic stimulus, mild traumatic brain injury, pediatrics, cortical silent period, long interval intracortical inhibition

Introduction

Mild traumatic brain injury (mTBI) is a significant public health concern as it is both common, occurring in 350-799 per 100,000 per year(1–4), and 11-31% of children mTBIs have symptoms which last longer than 1 month: defined as post-concussion syndrome (PCS)(5,6). PCS is a constellation of physical, emotional, and cognitive symptoms following mTBI(7) that significantly impacts the quality of life of the child and family(8). The mechanisms underlying the pathophysiology of PCS are poorly understood(9–11), which significantly impedes the development of better diagnostic tools and treatments.

Traumatic brain injury results in dysregulated neurotransmitter release, altered receptor expression, and injury to interneurons and microcircuits, potentially leading to disruption in the functional balance between cortical excitation and inhibition. This is supported by both murine models of TBI(12,13), and adult human research(14–17). Initially, TBI results in an uncontrolled glutamate release and a disruption of ionic balance across neuronal membranes, the extent of which is dependent on the severity of the injury(18,19). Subsequent alterations in receptor expression occur, such as early changes in n-methyl-d-aspartate (NMDA) receptor subunit composition (20) and later shifts in γ-aminobutyric acid (GABA) subtype receptor subunits ratios(21,22).

Cortical excitation and inhibition can be interrogated *in vivo* in humans using transcranial magnetic stimulation (TMS)(23,24). Using TMS methodologies, cortical inhibition has been found to be increased both acutely(25) and chronically in adult athletes recovering from mTBI

(e.g., increased cortical silent period (cSP)(26,27) and long interval intracortical inhibition (LICI)(27,28)). Whether such alterations in cortical inhibition occur in children, who have shorter cSP(29), different physiological responses to injury, and different recovery profiles(30,31), is unknown. Nor is it known how these physiological changes relate to clinical symptoms.

We explored cortical excitability following mTBI in children and its relationship with clinical symptoms to better understand mechanisms of symptom persistence and the variability in subject recovery. Specifically, we asked whether children with early versus late recovery differed in their neurophysiological parameters of cortical excitation and inhibition when compared to healthy controls of similar age and sex.

Methods

This prospective controlled cohort study was performed as part of PLAY GAME, a randomized controlled trial of melatonin for the treatment of PCS following childhood mTBI(32) (https://clinicaltrials.gov/ct2/show/NCT01874847). This study was approved by the University of Calgary Conjoint Health Research Ethics Board (REB13-0372).

Participants:

Children and adolescents (ages 8 to 18 years) presenting to the Alberta Children's Hospital with an mTBI were eligible. Mild TBI was defined as an impact to the head or body with a Glasgow Coma Score of 13-15 resulting in at least one of the following: an observed loss of

consciousness less than 30 minutes, or at least one acute symptom suggesting neurological dysfunction attributable to the injury (e.g., headache, confusion, vomiting, amnesia, balance problems)(8,33). Concussion was considered part of the mTBI spectrum(34). Exclusion criteria were: suspected child abuse; alcohol or drug use at the time of injury; inability to complete questionnaires; significant past medical or psychiatric history requiring medication; contraindications to TMS(35); previous mTBI within 3 months or failure to recover from a previous mTBI; and/or use of neuroactive drugs. Untreated Attention Deficit Disorders (ADHD) or mild learning disorders were not excluded. Typically developing children (ages 8 to 18 years) were eligible if they satisfied exclusion criteria and had no history of TBI (healthy controls).

Children with mTBI were identified from a tertiary care pediatric Emergency Department (n=761) and eligible children with mTBI were contacted by telephone at 4 weeks post-injury (n=294). The recruitment process is shown in Figure 1. Parental consent and participant assent were obtained. The Post-Concussion Symptom Inventory (PCSI) was used to document symptoms. Participants who had clinically recovered were selected to be similar in age and sex to the symptomatic group. Controls were recruited from friends or siblings of the mTBI participants. Outcome was assessed at 4-6 weeks post-injury before enrolment into the treatment trial.

Figure 1: Participant recruitment flow

A flow chart of the recruitment of participants through each step in screening and final samples. Analysed participants are those whose thresholds permitted at least one TMS paradigm to be performed

Clinical Outcome measures

Post-concussion symptom inventory: This age-appropriate, standardized questionnaire provides ratings for 26 symptoms (Guttman scale: 0 to 6) and an overall rating of post-concussive symptoms(5,36). It has 4 factor derived-domains: somatic, cognitive, affective, and sleep. Participants were asked to retrospectively report pre-injury symptoms at enrolment (baseline), and were considered *symptomatic* if they had an increase of two in two or more symptoms compared to baseline and a score greater than 0 to "Have you felt different from before your injury?" (score: 0 to 4) (5,32), or *recovered* if there was no increase in symptoms and a score of 0 to the "feeling different" question.

CNS Vital Signs: This is a computerized neuropsychological test battery with adequate test-retest reliability(37) and is a validated measure of cognitive skills in children with TBI(38). The neurocognition index (NCI) is a summary score of the 5 domain scores: composite memory, psychomotor speed, reaction time, complex attention, and cognitive flexibility. All domain scores are normalized (mean: 100, SD: 15). The NCI was used to provide an overall estimate of cognitive function. As children may have an abnormal response to injury or illness, effort during cognitive testing was assessed using the test of memory malingering (TOMM)(39). Children were excluded from regression analyses if they scored less than 45 on the test and re-test TOMM.

Transcranial magnetic stimulation protocol Participants and parents were first informed about TMS. Once comfortably seated, participants watched a movie of their choice during the TMS

session. Ag/AgCl EMG electrodes (Kendall; Chicopee, MA, USA, 1.5cm radius) were used to record surface EMG from first dorsal interosseous (FDI) muscles of both hands with a wrist ground band. EMG signals were amplified by 1000 and band-pass filtered from 20 to 2000 Hz and then digitized at a rate of 5000 Hz using CED 1401 hardware and Signal 6.0 software (Cambridge Electronic Design, Cambridge, UK). Using a Magstim BiStim 200 Transcranial Magnetic Stimulator (Magstim Company Limited, Carmarthenshire), stimuli were applied using an Alpha Branding Iron Range (70mm internal diameter) under image-guided neuronavigation (Brainsight2, Rogue Research Inc., Montreal) to define the FDI hotspot in the dominant motor cortex. The hotspot is the point where stimulation over the primary motor cortex produced the largest contralateral motor evoked potentials (MEPs). MEPs were recorded in Signal 4.0.6 (Cambridge Electronic Design Limited, Cambridge, England). Voluntary contraction was measured using an EMG oscilloscope (GwINSTEK GDS-1022, 25MHz, 250M Sa/s, Good Will Instrument Co, New Taipei City, Taiwan).

Single pulse paradigms

Rest motor threshold (RMT) was defined as the lowest stimulus intensity eliciting an MEP response of 50µV (the 50µV RMT) in 5 out of 10 consecutive trials. Suprathreshold test stimuli (TS) were defined by the 1000µV (1mV) motor threshold. Active motor threshold (AMT) was the lowest stimulus intensity eliciting 200µV during isometric FDI contraction at 20% maximum voluntary effort. Stimulus response curves (SRC) were generated using pseudorandomized stimulus intensities of 10% intervals between 100-150% of the 50µV RMT (rest) and AMT (active).

Cortical silent period (cSP) was the primary outcome based on previous adult mTBI studies(40). Fifteen suprathreshold stimuli were applied (separated by 3s) to the dominant FDI hotspot during contralateral hand contraction at 20% of maximal effort(41). The silent period was defined as the onset of disrupted EMG waveform after the MEP to the point where EMG activity exceeds 25% of the rectified pre-stimulus EMG. Ipsilateral silent period (iSP) was measured in the dominant FDI during 50% maximal contraction in the hand ipsilateral to stimulation (non-dominant hand).

Paired-pulse paradigms

Paired pulse TMS was used to evaluate cortical excitatory and inhibitory cortical circuitry. Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) stimulations were randomized. Here, a conditioning stimulus set to 90% of the 50µV RMT preceded a suprathreshold conditioning test stimulus of 120% of the 50µV RMT. The inter-stimulus interval was 2ms for SICI and 10ms for ICF. Ten conditioning-test stimuli pairs were applied for SICI and ICF and pseudorandomized with 10 unconditioned test stimuli. Long interval intracortical inhibition (LICI) was investigated with both the conditioning and test stimuli set to the 1000µV RMT, separated by 100ms. Ten conditioning-test stimuli pairs and 10 test stimuli alone were applied in pseudorandom order.

TMS Analysis

Data were processed using Matlab (MATLAB and Statistics Toolbox Release 2014b, The MathWorks, Inc., Natick, Massachusetts, United States) by an assessor blinded to group. The duration of cSP was defined as the period between the onset of the disrupted waveform after

the MEP and the point where the EMG activity returned to 25% of rectified background activity. iSP durations were defined as the onset of EMG disruption after the stimulation to point where the EMG activity returned to 25% of rectified background activity.

In the paired-pulse paradigms, peak-to-peak MEP amplitudes were calculated for each stimulus, then sorted into conditioned or unconditioned. The means of each state were calculated (unconditioned test stimulus amplitudes below 100μV and their corresponding conditioned states were removed, as they likely reflect issues with the neuronavigation goggles shifting). Paired pulse paradigms for each participant are expressed as a ratio of the mean conditioned response amplitude divided by their mean unconditioned response amplitude.

Safety and tolerability

At the end of each session, participants completed the pediatric TMS tolerability questionnaire, documenting and quantifying all potential adverse events (headache, nausea, dizziness, and neck pain) and ranking their TMS experience against 7 other common childhood experiences(42).

Statistical analyses

Analyses were performed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Graphs were created in Sigmaplot 13.0 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com). The sample size was estimated as 24 per group using the cSP data from Miller et al.(43). Normality was tested using Shapiro-Wilks analyses. RMT at 50μV, AMT, rest SRC area under the curve, rest ICF ratio, LICI ratio were

transformed to a normal distribution using a $\log_{10}$ transformation. Group differences (CSP, iSP, SICI, ICF, and LICI) were assessed using analysis of variance (controls, recovered and symptomatic). Mixed models ANOVAs were used to compare between group differences for repeated measures paradigms and a Greenhouse-Geisser correction was applied where sphericity could not be assumed following Mauchly's test (MT, SRC, cSP). Tukey's post-hoc tests were used to correct for multiple comparisons between groups. Differences in group proportions were compared using chi squared tests. Exploratory analyses of the potential influence of gender, age, previous concussion, ADHD, PCSI score, and NCI on the outcome measures (cSP, SICI, LICI, iSP and ICF) were performed. Significant correlating factors ($p<0.1$) on univariate analysis were included in exploratory regression models to analyze the relationship between clinical symptoms (post-injury PCSI score, NCI), cSP and LICI, and mTBI whilst controlling for the potential effects of age, sex, ADHD(44) (including inattentive subtypes) and previous mTBI.

Results

Thirty-five symptomatic, 27 recovered, and 28 healthy control participants were enrolled. Groups were similar in age (overall mean age 14.16, SD 2.69 years), sex (42 males), handedness (77 right-handed), ADHD (n=3), and learning support requirements (n=5), see Table 1. A similar proportion of symptomatic and recovered participants had previous concussions, 22% ($\chi^2(4)=2.01$, $p=0.366$). Pre-injury PCS symptoms did not differ between groups ($H(2)=0.19$, $p = 0.909$). Injury characteristics are shown in Table 2 and were similar between groups. As

expected, the median post-injury PCSI score was higher in the symptomatic group: 35 (range: 6-122), compared to the recovered group: 3 (range: 0-26), $H(2)=4.81$, $p<0.001$.

Neurophysiology

TMS was well-tolerated with minimal adverse effects reported (see Table 3). Individual TMS paradigms were excluded if they could not be performed due to the participant's threshold. Thirteen participants had thresholds too high to complete rest SRCs, (3 control, 2 recovered, and 8 symptomatic). Test stimuli could not be evoked in one additional recovered participant. Two control, 1 recovered, and 2 symptomatic participants had thresholds too high to perform ICF and SICI.

Table 1: Pre-injury clinical and demographic details

Table 2: ***Injury characteristics and symptom scores in children with mTBI***

Table 3: Tolerability of TMS with subjective sensations

Table 4: Single pulse TMS paradigm data

The results of the single pulse paradigms are shown in Table 4, demonstrating that motor thresholds were similar between groups. Groups show no group X stimulation intensity interaction in rest ($F(4.52, 167.14)=1.09$, $p=0.368$)) or active SRCs ($F(4.48, 183.84)=1.36$,

p=0.244)), see Figure 2. Similarly, during active SRC, there was no group X stimulation interaction for cSP (F(4.53, 179.03)=0.58, p=0.702). cSP was dependent on the strength of the stimulation (F(2.27, 179.03)=419.58, p<0.01, see Figure 3) but did not differ between groups with increasing stimulus intensity (F(2, 79)=0.28, p=0.753). With the more commonly used practice or using 1000μV RMT, there also were no group differences (F(2, 73)=0.55, p=0.582). iSP was also similar between groups (F(2,70)=0.12, p=0.890) (Figure 4).

Figure 2: Rest and active stimulus response curves (SRCs) are shown for healthy controls, symptomatic and recovered groups.
(A) Line graph shows line graphs of resting stimulus response curve (SRC) amplitude for healthy controls, recovered, and symptomatic. (B) shows line graphs of the active SRC response amplitudes for the healthy controls, recovered, and symptomatic groups.

Figure 3: Cortical silent period paradigms.
(A) Boxplot of the cortical silent period (cSP) duration in milliseconds showed no differences between healthy controls and mTBI groups. (B) Line graph shows the mean and standard deviation of the $\log_{10}$ cSP with increased stimulation intensity during active stimulus response curve trials for healthy controls, recovered, and symptomatic groups with increasing stimulation intensity (no group X stimulus intensity interaction with healthy controls, F(4.53, 179.03)=0.58, p=0.702).
Boxplots show the group median as a black horizontal line inside the box. The top edge of the box is the third quartile, and the bottom of the box is the first quartile, with the group mean in the middle of the box. The box's whiskers denote the ends of the inner fence, or normal range of data. To calculate the inner fence, 1.5 times the interquartile range is subtracted or added to the first or third quartile, respectively. Outliers are shown as points.

Figure 4: Ipsilateral silent period
Boxplot of the ipsilateral silent period (iSP) were similar between healthy controls, recovered, and symptomatic groups, F(2,70)=0.12, p=0.890.

ICF (F(2, 56)=1.81, p=0.174) was similar between groups (Figure 5 and Table 5). SICI (Figure 6) was similar across groups (F(2, 56)=1.04, p=0.359). LICI differed between groups, see Figure 6 (F(2, 59)=3.83, p=0.027). Post-hoc analysis using Tukey's correction revealed that the symptomatic group demonstrated less $\log_{10}$ LICI effect compared to controls (p=0.027). Reverse

transformed LICI means for control, recovered and symptomatic were 0.31 (SD: 0.38), 0.44 (SD: 0.74), and 0.58 (SD:0.60), respectively.

Table 5: Paired pulse paradigms

Figure 5: Intracortical facilitation
Intracortical facilitation (ICF) ratio of conditioned stimulus amplitude over the test stimulus amplitude, separated by 10ms. Values above 1 (black line) are considered facilitation, while below 1 indicate inhibition. There were no group differences ($F(2, 56)=1.81$, $p=0.174$).

Figure 6: Inhibitory paired pulse paradigms
(A) Short interval intracortical inhibition (SICI): the ratio of conditioned stimulus to test stimulus at 2ms inter-stimulus intervals. Values above 1 (black line) are considered facilitation, while below 1 indicate inhibition. There are no differences between groups ($F(2, 56)=1.04$, $p=0.359$). (B) Log_{10} long interval intracortical inhibition (LICI): the log_{10} of the ratio of conditioned stimulus to test stimulus alone when inter-stimulus interval is set to 100ms. Values above 0 are considered facilitation, while below 0 indicate inhibition. There was a difference between groups in omnibus ANOVA tests ($F(2, 59)=3.83$, $p=0.027$), which post-hoc analyses revealed to be between healthy controls and PCS participants ($p=0.004$)

The influence of covariates

The correlation coefficient matrix is shown in Table 6. The presence of mTBI, ADHD, and LICI were included in a regression model to predict the PCSI score. The model was significant, predicting 26.2% (adjusted) of the variance ($F(3, 59)=8.34$, $p< 0.001$). The variables that significantly contributed to the model were ADHD (Beta=0.354, p=0.002), and mTBI (Beta=0.292, p=0.012). LICI was not predictive of symptoms (Beta=0.194, p=0.094). Factors influencing LICI were further explored in a regression model including TBI, gender, number of previous mTBIs, PCSI score, and the interaction effect between gender and PCSI score. The overall model was significant ($F(5, 61)=3.269$, $p<0.012$) and explained 16% of the variance.

When controlling for the significant interaction between gender and PCSI score (Beta = -.874, p = 0.041), LICI was predicted by gender (Beta 0.339, p=0.016) and PCSI score (Beta 1.071, p=0.012).

Table 6: Correlation matrix

Discussion

This is the first study to investigate cortical excitation-inhibition balance using TMS in children with different recovery patterns after an mTBI. We are also the first to demonstrate that TMS is well tolerated by children after an mTBI, and that any adverse events reported were mild to moderate and were not different between groups. This is similar to children with ADHD who also tolerate TMS (42), and who share a similar predisposition to injury as children with mTBI(45).

In our study, the motor thresholds and SRCs were similar between groups, which is consistent with the previous literature(27,46,47). We also evaluated different measures of synaptic excitability, using silent periods and the MEPS of paired-pulse paradigms. ICF, a measure of net facilitation mediated via NMDA glutamate (excitatory) receptors, was similar between groups. SICI is a measure of net inhibition: the short-lasting inhibitory component of SICI is mediated by GABAa receptor activity. Contrary to our hypothesis of mTBI-induced increased local cortical inhibition underlying PCS, cSP durations (a GABAb receptor-mediated inhibition dependent effect) and SICI did not differ between control and mTBI groups regardless of recovery status. However LICI, which reflects long-lasting inhibition(23,24,48), was decreased in the

symptomatic mTBI group when compared to healthy controls, suggesting a decrease in $GABA_b$ receptor-mediated cortical inhibition.

Our findings of normal cSP duration and decreased LICI following mTBI is in contrast to results from the majority of adult studies, which have reported increased inhibition (cSP) after mTBI(25–27), although two small adult studies have also reported a normal cSP (16,46). It is unlikely that our observations are due to differences in TMS protocols as we used previously described standard practices and methods(49). And, the cSP durations in our control group were similar to reference data for children(29) and were correlated with other measures of cortical excitability (e.g., LICI and ICF).

Several factors can affect cortical excitability after TBI including age, time since the injury, severity of injury, ADHD, use of medications, and repeated mTBI(23,26,28,29,50–52). A comparison between Miller et al.'s study and ours allows us to consider the effect of age and population on cortical excitability after mTBI(25). Miller et al. found a prolonged cSP that was evident 72 hours after the mTBI that persisted at 2 months(25), whereas we found no difference in the cSP duration. Our cohort was very similar to Miller et al.'s cohort, including similar methods of eliciting cSP and a common analysis time point of 1 month post-injury(25). Other than age (mean 14.1 vs. 20.8 years, respectively) and population (paediatric emergency department patients vs. adult concussion clinic, respectively), the cohorts were similar in sex (53% vs. 47%) and mechanism of injury (sport-related mTBI: 73% (11 of 15) participants in Miller's study compared to 60% in our study). Age and sex are significant predictors of

symptom persistence after a concussion, controlling for these factors between groups allowed us to examine the effect of mTBI and recovery on cortical excitability(53–56). Notably, cSP duration was not correlated with age within our cohort perhaps because the age range (8 to 18 years) was not large enough to detect this. So, although children do have greater variability in their cSP durations (29) which could have decreased the power of our study (29,51), it is likely that age is a significant contributor to the differences in cSP duration observed between the two studies.

Chistayakov et al. found that injury severity may influence cortical excitability(17). They report a cohort of adult participants who were admitted to hospital with mTBI. Similar to our study, participants with “minor head injury” (GCS 15, n=10) did not show an increase in cSP duration at two weeks post-injury whereas those participants with “mild head injury” (GCS 13-14, n=22) and moderate head injury (GCS 9-12, n=6) did show increased cSP durations(17). Although this suggests that increases in cSP may be more likely in more severe injuries, it is also possible that this effect could be explained by the high proportion of diphenylhydantoin anticonvulsant medication use in the mild and moderate TBI groups (19 of 22 cases)(17,57). A strength of our study was that we excluded any children treated with psychoactive or anticonvulsant medications.

Other studies that have found prolonged cSP focussed primarily on sports-related concussions, but do not define the severity of injury(16,25,27). However, those studies show a strong effect of repeated concussions and sub-concussive events (events that resemble the mechanics of a

concussive event but do not result in symptoms) on cortical excitability. Tremblay et al.(26) and De Beaumont et al.(27) found increased cSP in adult Canadian athletes with multiple sport-related concussions examined more than 9 months post-injury. In our study, although the number of previous mTBIs was correlated with LICI on univariate analysis, it was not an independent predictor of LICI when TBI, PCSI score, and gender were taken into account. Increases in cortical inhibition in athletes with multiple concussive and sub-concussive events may take years to develop and reach detectable levels. Therefore, the effect of multiple concussions on cortical excitability in children over time is worthy of future study.

In contrast to our cSP results, there was evidence of decreased inhibition i.e. reduced LICI responses in children who remained symptomatic at one-month post injury, compared to healthy controls. LICI was modified by sex (more pronounced inhibition in females than males) and the severity of PCS symptoms. Although cSP and LICI are both considered to reflect GABAb receptor-mediated inhibition, LICI is thought to measure activity in different aspects of the inhibitory interneuronal circuit than cSP(58). Previous reports of LICI alterations after TBI are varied, reporting a range of LICI responses between increased(26–28) and normal(16), to decreased(59). For example, while Powers et al.(46) did not find differences between mTBI at one month post-injury and control subjects, most of the other TMS studies were performed at time points quite remote from the injury and in the setting of multiple mTBIs making it difficult to directly compare with our data. Therefore, although it is possible that inhibitory cortical interneuronal circuits may be preferentially affected in pediatric mTBI, our finding of decreased LICI and its relationship to PCS symptoms needs to be replicated in future studies.

We are the first to study iSP in mTBI. iSP is thought to be a measure of inhibition of the contralateral motor cortex via excitatory transcallosal pathways and is often prolonged in severe TBI(60). These transcallosal tracts are of particular interest as they are susceptible to injury in TBI(61,62) and we have previously demonstrated altered interhemispheric connectivity in persistent post-concussion syndrome following mTBI in children(63). The normal values of iSP after mTBI in our study suggests either no dysfunction in the transcallosal tracts or a compensated contralateral response. Future studies investigating iSP in the presence and absence of transcallosal injury could provide some insight about compensatory intracortical mechanisms following TBI.

The ICF paradigm is thought to reflect glutamatergic NMDA-mediated activity(23,64,65), which animal models have found to be dysregulated within hours of the injury, recovering by 24 hours(12). In our study we found no differences in ICF between groups, which is in keeping with other studies of mild, moderate, and multiple TBIs(66,67). These studies were performed longer after the injury than in our study, which may indicate that the normalization of NMDA receptor-mediated facilitation that is believed to underlie ICF(65) occurs by one month after injury in children.

Our study has several limitations. Firstly, only post-injury measures of cortical excitability were obtained. It is possible that cortical excitability may be different pre-injury in children at risk of mTBI, especially in females with higher pre-injury PCSI scores. Secondly, our study may be

underpowered to detect group differences given the increased variability of TMS parameters in children and given the smaller number of participants with LICI measurements. Thirdly, TMS is an indirect measure of cortical physiology. TMS paradigms were applied to a focal region of the cortex, which is used as a generalisation of the whole cortex. It is possible that cortical excitability varies in different regions of the brain especially after injury and that such generalization is incorrect. The sensitivity of TMS in mTBI could potentially be increased by correlating cortical excitability with the presence or absence of microstructural injury. Wewe did not exclude children with a history of attentional problems in order to increase the generalizability of our results to the group of children who sustain mTBI. However, this could also have increased the variability of our cortical excitability observations. Lastly, although our TMS biomarkers are likely to reflect alterations in cortical neurophysiology at the cellular level, it should be noted that the preclinical and neuropharmacological studies suggesting these associations are not well established in the developing brain.

In summary, children are likely to differ from adults in their cortical excitation-inhibition balance following mTBI. Most TMS parameters of cortical excitation and inhibition are normal by one month post-mTBI. Long-lasting intracortical inhibition, however, is decreased in children who remain symptomatic which suggests a potential vulnerability of select inhibitory interneurons. Further research using sensitive TMS paradigms is required to validate these findings, and examine how cortical excitability changes over time and its relationship with cognitive and behavioural function.

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Cortical excitability after pediatric mild traumatic brain injury

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Introduction: Mild traumatic brain injury (mTBI) outcomes are variable, and 10-15% may suffer from prolonged symptoms beyond 3 months that impair the child's return to normal activities. Neurophysiological mechanisms of mTBI are incompletely understood, particularly in children, but alterations in cortical excitability have been proposed to underlie post-concussion syndrome. Improved understanding is required to advance interventions and improve outcomes.

Objective/Hypothesis: To determine if cortical excitability is altered in children with mTBI, and its association with clinical symptoms.

Methods: This was a cross-sectional controlled cohort study. School-aged children (8-18 years) with mTBI were compared to healthy controls. Cortical excitability was measured using multiple TMS paradigms in children with (symptomatic) and without (recovered) persistent symptoms one-month post-injury. Primary outcome was the cortical silent period (cSP), a potential neurophysiological biomarker of GABAergic inhibition. Secondary outcomes included additional TMS neurophysiology, safety and tolerability. Associations between neurophysiology parameters and clinical symptoms were evaluated.

Results: Fifty-three children with mTBI (55% male; mean age 14.1 SD: 2.4 years; 35 symptomatic and 27 asymptomatic participants) and 28 controls (46% male; mean age 14.3 SD: 3.1 years) were enrolled. cSP duration was similar between groups ($F(2, 73)=0.55$, $p=0.582$). Log_{10} long interval intracortical inhibition (LICI) was reduced in symptomatic participants compared to healthy controls ($F(2, 59)=3.83$, $p=0.027$). Procedures were well tolerated with no serious adverse events.

Conclusions: TMS measures of cortical excitability are altered at one month in children with mTBI. Long interval cortical inhibition is decreased in children who remain symptomatic at one month post-injury.

Keywords: Transcranial magnetic stimulus, mild traumatic brain injury, pediatrics, cortical silent period, long interval intracortical inhibition

Introduction

Mild traumatic brain injury (mTBI) is a significant public health concern as it is both common, occurring in 350-799 per 100,000 per year[1–4], and 11-31% of children mTBIs have symptoms which last longer than 1 month: defined as post-concussion syndrome (PCS)[5,6]. PCS is a constellation of physical, emotional, and cognitive symptoms following mTBI[7] that significantly impacts the quality of life of the child and family[8]. The mechanisms underlying the pathophysiology of PCS are poorly understood[9–11], which significantly impedes the development of better diagnostic tools and treatments.

Traumatic brain injury results in dysregulated neurotransmitter release, altered receptor expression, and injury to interneurons and microcircuits, potentially leading to disruption in the functional balance between cortical excitation and inhibition. This is supported by both murine models of TBI[12,13], and adult human research[14–17]. Initially, TBI results in an uncontrolled glutamate release and a disruption of ionic balance across neuronal membranes, the extent of which is dependent on the severity of the injury[18,19]. Subsequent alterations in receptor expression occur, such as early changes in n-methyl-d-aspartate (NMDA) receptor subunit composition [20] and later shifts in γ-aminobutyric acid (GABA) subtype receptor subunits ratios[21,22].

Cortical excitation and inhibition can be interrogated *in vivo* in humans using transcranial magnetic stimulation (TMS)[23,24]. Using TMS methodologies, cortical inhibition has been found to be increased both acutely[25] and chronically in adult athletes recovering from mTBI

(e.g., increased cortical silent period (cSP)[26,27] and long interval intracortical inhibition (LICI)[27,28]). Whether such alterations in cortical inhibition occur in children, who have shorter cSP[29], different physiological responses to injury, and different recovery profiles[30,31], is unknown. Nor is it known how these physiological changes relate to clinical symptoms.

We explored cortical excitability following mTBI in children and its relationship with clinical symptoms to better understand mechanisms of symptom persistence and the variability in subject recovery. Specifically, we asked whether children with early versus late recovery differed in their neurophysiological parameters of cortical excitation and inhibition when compared to healthy controls of similar age and sex.

Methods

This prospective controlled cohort study was performed as part of PLAY GAME, a randomized controlled trial of melatonin for the treatment of PCS following childhood mTBI[32] (https://clinicaltrials.gov/ct2/show/NCT01874847). This study was approved by the University of Calgary Conjoint Health Research Ethics Board (REB13-0372).

Participants:

Children and adolescents (ages 8 to 18 years) presenting to the Alberta Children's Hospital with an mTBI were eligible. Mild TBI was defined as an impact to the head or body with a Glasgow Coma Score of 13-15 resulting in at least one of the following: an observed loss of

consciousness less than 30 minutes, or at least one acute symptom suggesting neurological dysfunction attributable to the injury (e.g., headache, confusion, vomiting, amnesia, balance problems)[8,33]. Concussion was considered part of the mTBI spectrum[34]. Exclusion criteria were: suspected child abuse; alcohol or drug use at the time of injury; inability to complete questionnaires; significant past medical or psychiatric history requiring medication; contraindications to TMS[35]; previous mTBI within 3 months or failure to recover from a previous mTBI; and/or use of neuroactive drugs. Untreated Attention Deficit Disorders (ADHD) or mild learning disorders were not excluded. Typically developing children (ages 8 to 18 years) were eligible if they satisfied exclusion criteria and had no history of TBI (healthy controls).

Children with mTBI were identified from a tertiary care pediatric Emergency Department (n=761) and eligible children with mTBI were contacted by telephone at 4 weeks post-injury (n=294). The recruitment process is shown in Figure 1. Parental consent and participant assent were obtained. The Post-Concussion Symptom Inventory (PCSI) was used to document symptoms. Participants who had clinically recovered were selected to be similar in age and sex to the symptomatic group. Controls were recruited from friends or siblings of the mTBI participants. Outcome was assessed at 4-6 weeks post-injury before enrolment into the treatment trial.

Figure 1: Participant recruitment flow

A flow chart of the recruitment of participants through each step in screening and final samples. Analysed participants are those whose thresholds permitted at least one TMS paradigm to be performed

Clinical Outcome measures

Post-concussion symptom inventory: This age-appropriate, standardized questionnaire provides ratings for 26 symptoms (Guttman scale: 0 to 6) and an overall rating of post-concussive symptoms[5,36]. It has 4 factor derived-domains: somatic, cognitive, affective, and sleep. Participants were asked to retrospectively report pre-injury symptoms at enrolment (baseline), and were considered *symptomatic* if they had an increase of two in two or more symptoms compared to baseline and a score greater than 0 to "Have you felt different from before your injury?" (score: 0 to 4) [5,32], or *recovered* if there was no increase in symptoms and a score of 0 to the "feeling different" question.

CNS Vital Signs: This is a computerized neuropsychological test battery with adequate test-retest reliability[37] and is a validated measure of cognitive skills in children with TBI[38]. The neurocognition index (NCI) is a summary score of the 5 domain scores: composite memory, psychomotor speed, reaction time, complex attention, and cognitive flexibility. All domain scores are normalized (mean: 100, SD: 15). The NCI was used to provide an overall estimate of cognitive function. As children may have an abnormal response to injury or illness, effort during cognitive testing was assessed using the test of memory malingering (TOMM)[39]. Children were excluded from regression analyses if they scored less than 45 on the test and re-test TOMM.

Transcranial magnetic stimulation protocol Participants and parents were first informed about TMS. Once comfortably seated, participants watched a movie of their choice during the TMS

session. Ag/AgCl EMG electrodes (Kendall; Chicopee, MA, USA, 1.5cm radius) were used to record surface EMG from first dorsal interosseous (FDI) muscles of both hands with a wrist ground band. EMG signals were amplified by 1000 and band-pass filtered from 20 to 2000 Hz and then digitized at a rate of 5000 Hz using CED 1401 hardware and Signal 6.0 software (Cambridge Electronic Design, Cambridge, UK). Using a Magstim BiStim 200 Transcranial Magnetic Stimulator (Magstim Company Limited, Carmarthenshire), stimuli were applied using an Alpha Branding Iron Range (70mm internal diameter) under image-guided neuronavigation (Brainsight2, Rogue Research Inc., Montreal) to define the FDI hotspot in the dominant motor cortex. The hotspot is the point where stimulation over the primary motor cortex produced the largest contralateral motor evoked potentials (MEPs). MEPs were recorded in Signal 4.0.6 (Cambridge Electronic Design Limited, Cambridge, England). Voluntary contraction was measured using an EMG oscilloscope (GwINSTEK GDS-1022, 25MHz, 250M Sa/s, Good Will Instrument Co, New Taipei City, Taiwan).

Single pulse paradigms

Rest motor threshold (RMT) was defined as the lowest stimulus intensity eliciting an MEP response of 50µV (the 50µV RMT) in 5 out of 10 consecutive trials. Suprathreshold test stimuli (TS) were defined by the 1000µV (1mV) motor threshold. Active motor threshold (AMT) was the lowest stimulus intensity eliciting 200µV during isometric FDI contraction at 20% maximum voluntary effort. Stimulus response curves (SRC) were generated using pseudorandomized stimulus intensities of 10% intervals between 100-150% of the 50µV RMT (rest) and AMT (active).

Cortical silent period (cSP) was the primary outcome based on previous adult mTBI studies[40]. Fifteen suprathreshold stimuli were applied (separated by 3s) to the dominant FDI hotspot during contralateral hand contraction at 20% of maximal effort[41]. The silent period was defined as the onset of disrupted EMG waveform after the MEP to the point where EMG activity exceeds 25% of the rectified pre-stimulus EMG. Ipsilateral silent period (iSP) was measured in the dominant FDI during 50% maximal contraction in the hand ipsilateral to stimulation (non-dominant hand).

Paired-pulse paradigms

Paired pulse TMS was used to evaluate cortical excitatory and inhibitory cortical circuitry. Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) stimulations were randomized. Here, a conditioning stimulus set to 90% of the 50μV RMT preceded a suprathreshold conditioning test stimulus of 120% of the 50μV RMT. The inter-stimulus interval was 2ms for SICI and 10ms for ICF. Ten conditioning-test stimuli pairs were applied for SICI and ICF and pseudorandomized with 10 unconditioned test stimuli. Long interval intracortical inhibition (LICI) was investigated with both the conditioning and test stimuli set to the 1000μV RMT, separated by 100ms. Ten conditioning-test stimuli pairs and 10 test stimuli alone were applied in pseudorandom order.

TMS Analysis

Data were processed using Matlab (MATLAB and Statistics Toolbox Release 2014b, The MathWorks, Inc., Natick, Massachusetts, United States) by an assessor blinded to group. The duration of cSP was defined as the period between the onset of the disrupted waveform after

the MEP and the point where the EMG activity returned to 25% of rectified background activity. iSP durations were defined as the onset of EMG disruption after the stimulation to point where the EMG activity returned to 25% of rectified background activity.

In the paired-pulse paradigms, peak-to-peak MEP amplitudes were calculated for each stimulus, then sorted into conditioned or unconditioned. The means of each state were calculated (unconditioned test stimulus amplitudes below 100μV and their corresponding conditioned states were removed, as they likely reflect issues with the neuronavigation goggles shifting). Paired pulse paradigms for each participant are expressed as a ratio of the mean conditioned response amplitude divided by their mean unconditioned response amplitude.

Safety and tolerability

At the end of each session, participants completed the pediatric TMS tolerability questionnaire, documenting and quantifying all potential adverse events (headache, nausea, dizziness, and neck pain) and ranking their TMS experience against 7 other common childhood experiences[42].

Statistical analyses

Analyses were performed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Graphs were created in Sigmaplot 13.0 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com). The sample size was estimated as 24 per group using the cSP data from Miller et al.[43]. Normality was tested using Shapiro-Wilks analyses. RMT at 50μV, AMT, rest SRC area under the curve, rest ICF ratio, LICI ratio were

transformed to a normal distribution using a $\log_{10}$ transformation. Group differences (CSP, iSP, SICI, ICF, and LICI) were assessed using analysis of variance (controls, recovered and symptomatic). Mixed models ANOVAs were used to compare between group differences for repeated measures paradigms and a Greenhouse-Geisser correction was applied where sphericity could not be assumed following Mauchly's test (MT, SRC, cSP). Tukey's post-hoc tests were used to correct for multiple comparisons between groups. Differences in group proportions were compared using chi squared tests. Exploratory analyses of the potential influence of gender, age, previous concussion, ADHD, PCSI score, and NCI on the outcome measures (cSP, SICI, LICI, iSP and ICF) were performed. Significant correlating factors ($p<0.1$) on univariate analysis were included in exploratory regression models to analyze the relationship between clinical symptoms (post-injury PCSI score, NCI), cSP and LICI, and mTBI whilst controlling for the potential effects of age, sex, ADHD[44] (including inattentive subtypes) and previous mTBI.

Results

Thirty-five symptomatic, 27 recovered, and 28 healthy control participants were enrolled. Groups were similar in age (overall mean age 14.16, SD 2.69 years), sex (42 males), handedness (77 right-handed), ADHD (n=3), and learning support requirements (n=5), see Table 1. A similar proportion of symptomatic and recovered participants had previous concussions, 22% ($\chi^2(4)=2.01$, $p=0.366$). Pre-injury PCS symptoms did not differ between groups ($H(2)=0.19$, $p = 0.909$). Injury characteristics are shown in Table 2 and were similar between groups. As

expected, the median post-injury PCSI score was higher in the symptomatic group: 35 (range: 6-122), compared to the recovered group: 3 (range: 0-26), $H(2)=4.81$, $p<0.001$.

Neurophysiology

TMS was well-tolerated with minimal adverse effects reported (see Table 3). Individual TMS paradigms were excluded if they could not be performed due to the participant's threshold. Thirteen participants had thresholds too high to complete rest SRCs, (3 control, 2 recovered, and 8 symptomatic). Test stimuli could not be evoked in one additional recovered participant. Two control, 1 recovered, and 2 symptomatic participants had thresholds too high to perform ICF and SICI.

Table 1: Pre-injury clinical and demographic details

Table 2: ***Injury characteristics and symptom scores in children with mTBI***

Table 3: Tolerability of TMS with subjective sensations

Table 4: Single pulse TMS paradigm data

The results of the single pulse paradigms are shown in Table 4, demonstrating that motor thresholds were similar between groups. Groups show no group X stimulation intensity interaction in rest ($F(4.52, 167.14)=1.09$, $p=0.368$)) or active SRCs ($F(4.48, 183.84)=1.36$,

p=0.244)), see Figure 2. Similarly, during active SRC, there was no group X stimulation interaction for cSP (F(4.53, 179.03)=0.58, p=0.702). cSP was dependent on the strength of the stimulation (F(2.27, 179.03)=419.58, p<0.01, see Figure 3) but did not differ between groups with increasing stimulus intensity (F(2, 79)=0.28, p=0.753). With the more commonly used practice or using 1000μV RMT, there also were no group differences (F(2, 73)=0.55, p=0.582). iSP was also similar between groups (F(2,70)=0.12, p=0.890) (Figure 4).

Figure 2: Rest and active stimulus response curves (SRCs) are shown for healthy controls, symptomatic and recovered groups.
(A) Line graph shows line graphs of resting stimulus response curve (SRC) amplitude for healthy controls, recovered, and symptomatic. (B) shows line graphs of the active SRC response amplitudes for the healthy controls, recovered, and symptomatic groups.

Figure 3: Cortical silent period paradigms.
(A) Boxplot of the cortical silent period (cSP) duration in milliseconds showed no differences between healthy controls and mTBI groups. (B) Line graph shows the mean and standard deviation of the $\log_{10}$ cSP with increased stimulation intensity during active stimulus response curve trials for healthy controls, recovered, and symptomatic groups with increasing stimulation intensity (no group X stimulus intensity interaction with healthy controls, F(4.53, 179.03)=0.58, p=0.702).
Boxplots show the group median as a black horizontal line inside the box. The top edge of the box is the third quartile, and the bottom of the box is the first quartile, with the group mean in the middle of the box. The box's whiskers denote the ends of the inner fence, or normal range of data. To calculate the inner fence, 1.5 times the interquartile range is subtracted or added to the first or third quartile, respectively. Outliers are shown as points.

Figure 4: Ipsilateral silent period
Boxplot of the ipsilateral silent period (iSP) were similar between healthy controls, recovered, and symptomatic groups, F(2,70)=0.12, p=0.890.

ICF (F(2, 56)=1.81, p=0.174) was similar between groups (Figure 5 and Table 5). SICI (Figure 6) was similar across groups (F(2, 56)=1.04, p=0.359). LICI differed between groups, see Figure 6 (F(2, 59)=3.83, p=0.027). Post-hoc analysis using Tukey's correction revealed that the symptomatic group demonstrated less $\log_{10}$ LICI effect compared to controls (p=0.027). Reverse

transformed LICI means for control, recovered and symptomatic were 0.31 (SD: 0.38), 0.44 (SD: 0.74), and 0.58 (SD:0.60), respectively.

Table 5: Paired pulse paradigms

Figure 5: Intracortical facilitation
Intracortical facilitation (ICF) ratio of conditioned stimulus amplitude over the test stimulus amplitude, separated by 10ms. Values above 1 (black line) are considered facilitation, while below 1 indicate inhibition. There were no group differences ($F(2, 56)=1.81$, $p=0.174$).

Figure 6: Inhibitory paired pulse paradigms
(A) Short interval intracortical inhibition (SICI): the ratio of conditioned stimulus to test stimulus at 2ms inter-stimulus intervals. Values above 1 (black line) are considered facilitation, while below 1 indicate inhibition. There are no differences between groups ($F(2, 56)=1.04$, $p=0.359$). (B) Log_{10} long interval intracortical inhibition (LICI): the log_{10} of the ratio of conditioned stimulus to test stimulus alone when inter-stimulus interval is set to 100ms. Values above 0 are considered facilitation, while below 0 indicate inhibition. There was a difference between groups in omnibus ANOVA tests ($F(2, 59)=3.83$, $p=0.027$), which post-hoc analyses revealed to be between healthy controls and PCS participants ($p=0.004$)

The influence of covariates

The correlation coefficient matrix is shown in Table 6. The presence of mTBI, ADHD, and LICI were included in a regression model to predict the PCSI score. The model was significant, predicting 26.2% (adjusted) of the variance ($F(3, 59)=8.34$, $p< 0.001$). The variables that significantly contributed to the model were ADHD (Beta=0.354, p=0.002), and mTBI (Beta=0.292, p=0.012). LICI was not predictive of symptoms (Beta=0.194, p=0.094). Factors influencing LICI were further explored in a regression model including TBI, gender, number of previous mTBIs, PCSI score, and the interaction effect between gender and PCSI score. The overall model was significant ($F(5, 61)=3.269$, $p<0.012$) and explained 16% of the variance.

When controlling for the significant interaction between gender and PCSI score (Beta = -.874, p = 0.041), LICI was predicted by gender (Beta 0.339, p=0.016) and PCSI score (Beta 1.071, p=0.012).

Table 6: Correlation matrix

Discussion

This is the first study to investigate cortical excitation-inhibition balance using TMS in children with different recovery patterns after an mTBI. We are also the first to demonstrate that TMS is well tolerated by children after an mTBI, and that any adverse events reported were mild to moderate and were not different between groups. This is similar to children with ADHD who also tolerate TMS [42], and who share a similar predisposition to injury as children with mTBI[45].

In our study, the motor thresholds and SRCs were similar between groups, which is consistent with the previous literature[27,46,47]. We also evaluated different measures of synaptic excitability, using silent periods and the MEPS of paired-pulse paradigms. ICF, a measure of net facilitation mediated via NMDA glutamate (excitatory) receptors, was similar between groups. SICI is a measure of net inhibition: the short-lasting inhibitory component of SICI is mediated by GABAa receptor activity. Contrary to our hypothesis of mTBI-induced increased local cortical inhibition underlying PCS, cSP durations (a GABAb receptor-mediated inhibition dependent effect) and SICI did not differ between control and mTBI groups regardless of recovery status. However LICI, which reflects long-lasting inhibition[23,24,48], was decreased in the

symptomatic mTBI group when compared to healthy controls, suggesting a decrease in $GABA_b$ receptor-mediated cortical inhibition.

Our findings of normal cSP duration and decreased LICI following mTBI is in contrast to results from the majority of adult studies, which have reported increased inhibition (cSP) after mTBI[25–27], although two small adult studies have also reported a normal cSP [16,46]. It is unlikely that our observations are due to differences in TMS protocols as we used previously described standard practices and methods[49]. And, the cSP durations in our control group were similar to reference data for children[29] and were correlated with other measures of cortical excitability (e.g., LICI and ICF).

Several factors can affect cortical excitability after TBI including age, time since the injury, severity of injury, ADHD, use of medications, and repeated mTBI[23,26,28,29,50–52]. A comparison between Miller et al.'s study and ours allows us to consider the effect of age and population on cortical excitability after mTBI[25]. Miller et al. found a prolonged cSP that was evident 72 hours after the mTBI that persisted at 2 months[25], whereas we found no difference in the cSP duration. Our cohort was very similar to Miller et al.'s cohort, including similar methods of eliciting cSP and a common analysis time point of 1 month post-injury[25]. Other than age (mean 14.1 vs. 20.8 years, respectively) and population (paediatric emergency department patients vs. adult concussion clinic, respectively), the cohorts were similar in sex (53% vs. 47%) and mechanism of injury (sport-related mTBI: 73% (11 of 15) participants in Miller's study compared to 60% in our study). Age and sex are significant predictors of

symptom persistence after a concussion, controlling for these factors between groups allowed us to examine the effect of mTBI and recovery on cortical excitability[53–56]. Notably, cSP duration was not correlated with age within our cohort perhaps because the age range (8 to 18 years) was not large enough to detect this. So, although children do have greater variability in their cSP durations [29] which could have decreased the power of our study [29,51], it is likely that age is a significant contributor to the differences in cSP duration observed between the two studies.

Chistayakov et al. found that injury severity may influence cortical excitability[17]. They report a cohort of adult participants who were admitted to hospital with mTBI. Similar to our study, participants with “minor head injury” (GCS 15, n=10) did not show an increase in cSP duration at two weeks post-injury whereas those participants with “mild head injury” (GCS 13-14, n=22) and moderate head injury (GCS 9-12, n=6) did show increased cSP durations[17]. Although this suggests that increases in cSP may be more likely in more severe injuries, it is also possible that this effect could be explained by the high proportion of diphenylhydantoin anticonvulsant medication use in the mild and moderate TBI groups (19 of 22 cases)[17,57]. A strength of our study was that we excluded any children treated with psychoactive or anticonvulsant medications.

Other studies that have found prolonged cSP focussed primarily on sports-related concussions, but do not define the severity of injury[16,25,27]. However, those studies show a strong effect of repeated concussions and sub-concussive events (events that resemble the mechanics of a

concussive event but do not result in symptoms) on cortical excitability. Tremblay et al.[26] and De Beaumont et al.[27] found increased cSP in adult Canadian athletes with multiple sport-related concussions examined more than 9 months post-injury. In our study, although the number of previous mTBIs was correlated with LICI on univariate analysis, it was not an independent predictor of LICI when TBI, PCSI score, and gender were taken into account. Increases in cortical inhibition in athletes with multiple concussive and sub-concussive events may take years to develop and reach detectable levels. Therefore, the effect of multiple concussions on cortical excitability in children over time is worthy of future study.

In contrast to our cSP results, there was evidence of decreased inhibition i.e. reduced LICI responses in children who remained symptomatic at one-month post injury, compared to healthy controls. LICI was modified by sex (more pronounced inhibition in females than males) and the severity of PCS symptoms. Although cSP and LICI are both considered to reflect GABAb receptor-mediated inhibition, LICI is thought to measure activity in different aspects of the inhibitory interneuronal circuit than cSP[58]. Previous reports of LICI alterations after TBI are varied, reporting a range of LICI responses between increased[26–28] and normal[16], to decreased[59]. For example, while Powers et al.[46] did not find differences between mTBI at one month post-injury and control subjects, most of the other TMS studies were performed at time points quite remote from the injury and in the setting of multiple mTBIs making it difficult to directly compare with our data. Therefore, although it is possible that inhibitory cortical interneuronal circuits may be preferentially affected in pediatric mTBI, our finding of decreased LICI and its relationship to PCS symptoms needs to be replicated in future studies.

We are the first to study iSP in mTBI. iSP is thought to be a measure of inhibition of the contralateral motor cortex via excitatory transcallosal pathways and is often prolonged in severe TBI[60]. These transcallosal tracts are of particular interest as they are susceptible to injury in TBI[61,62] and we have previously demonstrated altered interhemispheric connectivity in persistent post-concussion syndrome following mTBI in children[63]. The normal values of iSP after mTBI in our study suggests either no dysfunction in the transcallosal tracts or a compensated contralateral response. Future studies investigating iSP in the presence and absence of transcallosal injury could provide some insight about compensatory intracortical mechanisms following TBI.

The ICF paradigm is thought to reflect glutamatergic NMDA-mediated activity[23,64,65], which animal models have found to be dysregulated within hours of the injury, recovering by 24 hours[12]. In our study we found no differences in ICF between groups, which is in keeping with other studies of mild, moderate, and multiple TBIs[66,67]. These studies were performed longer after the injury than in our study, which may indicate that the normalization of NMDA receptor-mediated facilitation that is believed to underlie ICF[65] occurs by one month after injury in children.

Our study has several limitations. Firstly, only post-injury measures of cortical excitability were obtained. It is possible that cortical excitability may be different pre-injury in children at risk of mTBI, especially in females with higher pre-injury PCSI scores. Secondly, our study may be

underpowered to detect group differences given the increased variability of TMS parameters in children and given the smaller number of participants with LICI measurements. Thirdly, TMS is an indirect measure of cortical physiology. TMS paradigms were applied to a focal region of the cortex, which is used as a generalisation of the whole cortex. It is possible that cortical excitability varies in different regions of the brain especially after injury and that such generalization is incorrect. The sensitivity of TMS in mTBI could potentially be increased by correlating cortical excitability with the presence or absence of microstructural injury. Lastly, we did not exclude children with a history of attentional problems in order to increase the generalizability of our results to the group of children who sustain mTBI. However, this could also have increased the variability of our cortical excitability observations.

In summary, children are likely to differ from adults in their cortical excitation-inhibition balance following mTBI. Most TMS parameters of cortical excitation and inhibition are normal by one month post-mTBI. Long-lasting intracortical inhibition, however, is decreased in children who remain symptomatic which suggests a potential vulnerability of select inhibitory interneurons. Further research using sensitive TMS paradigms is required to validate these findings, and examine how cortical excitability changes over time and its relationship with cognitive and behavioural function.

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Figure1

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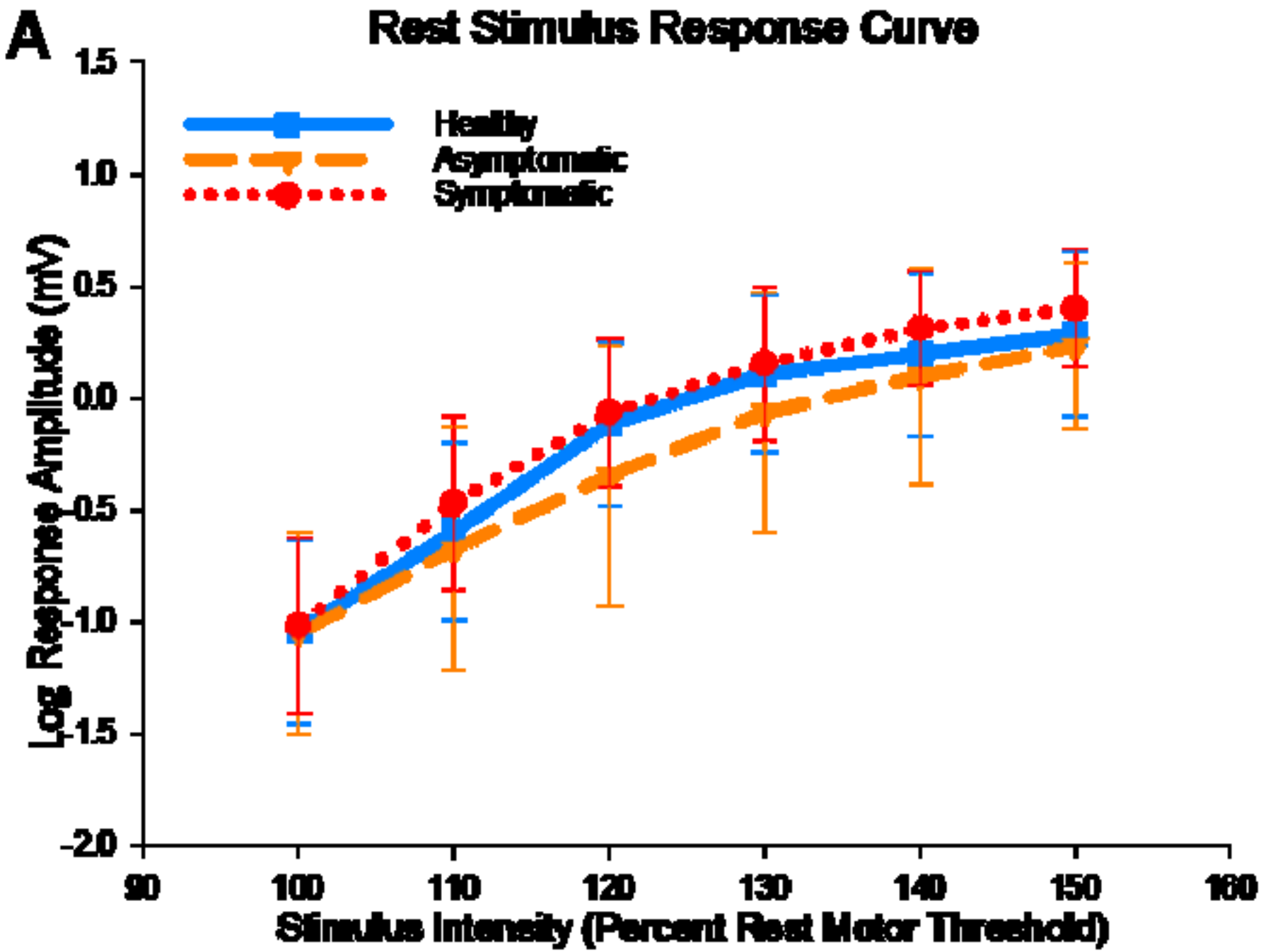

Figure2b

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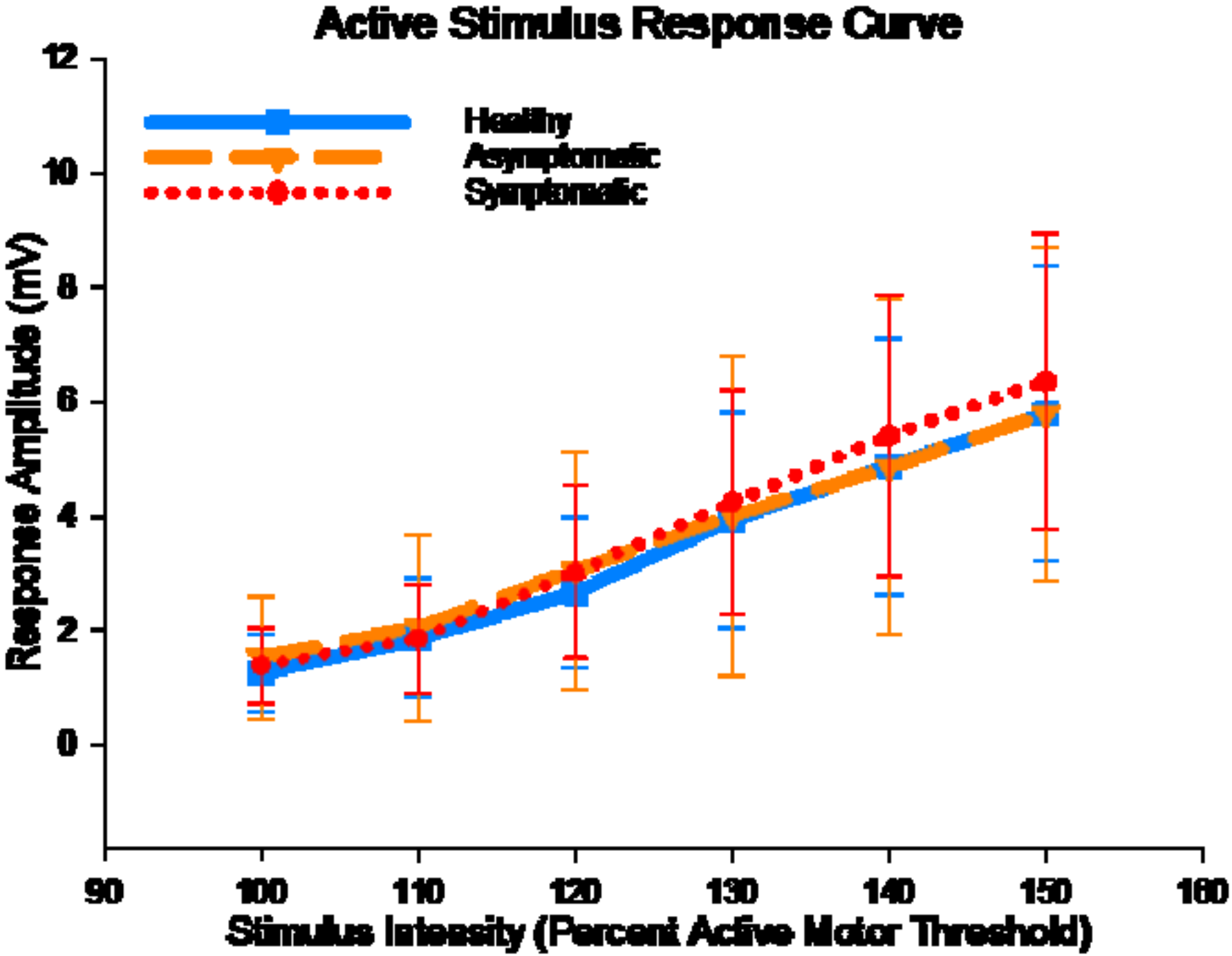

Figure3a

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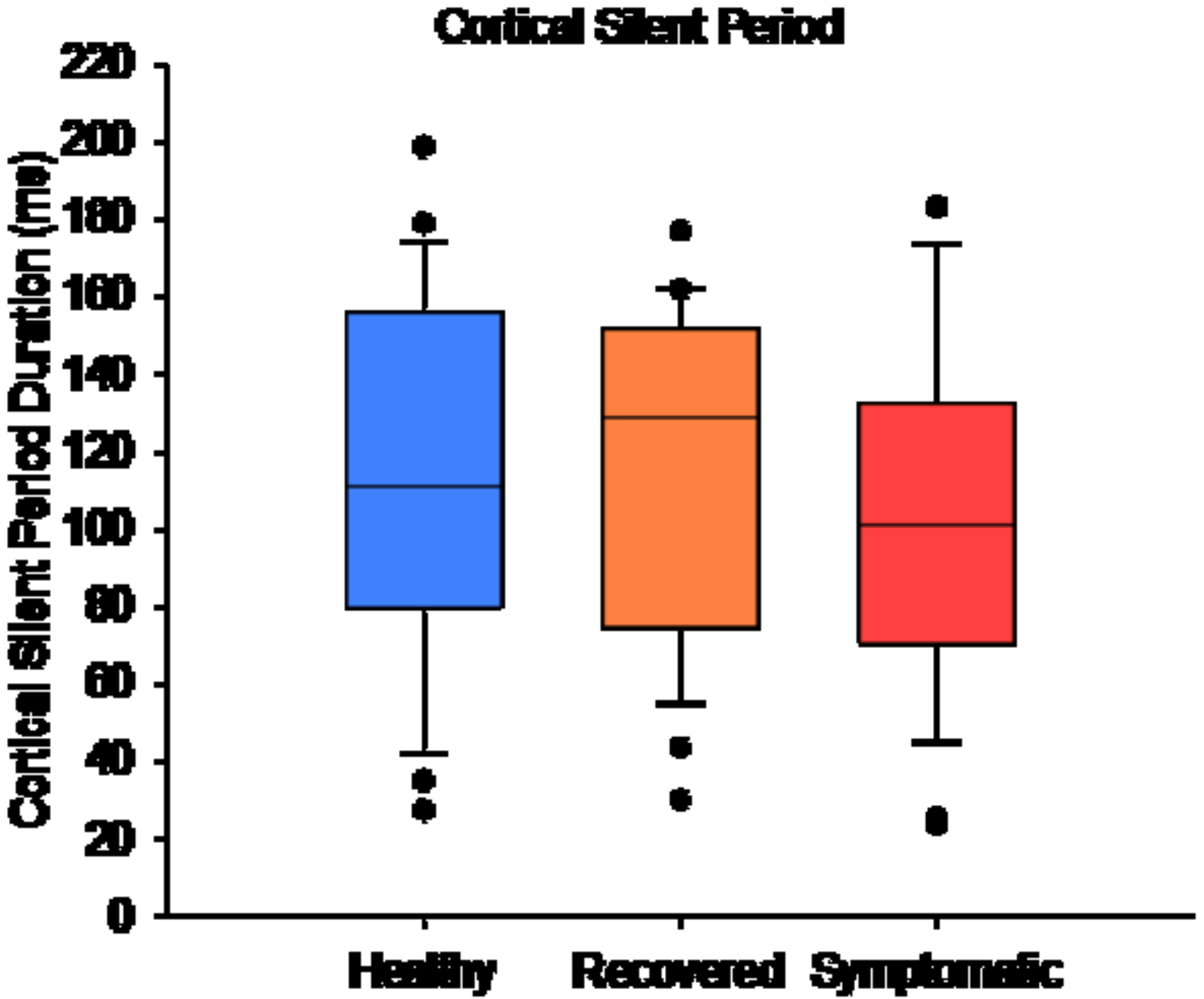

Figure3b

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Figure4

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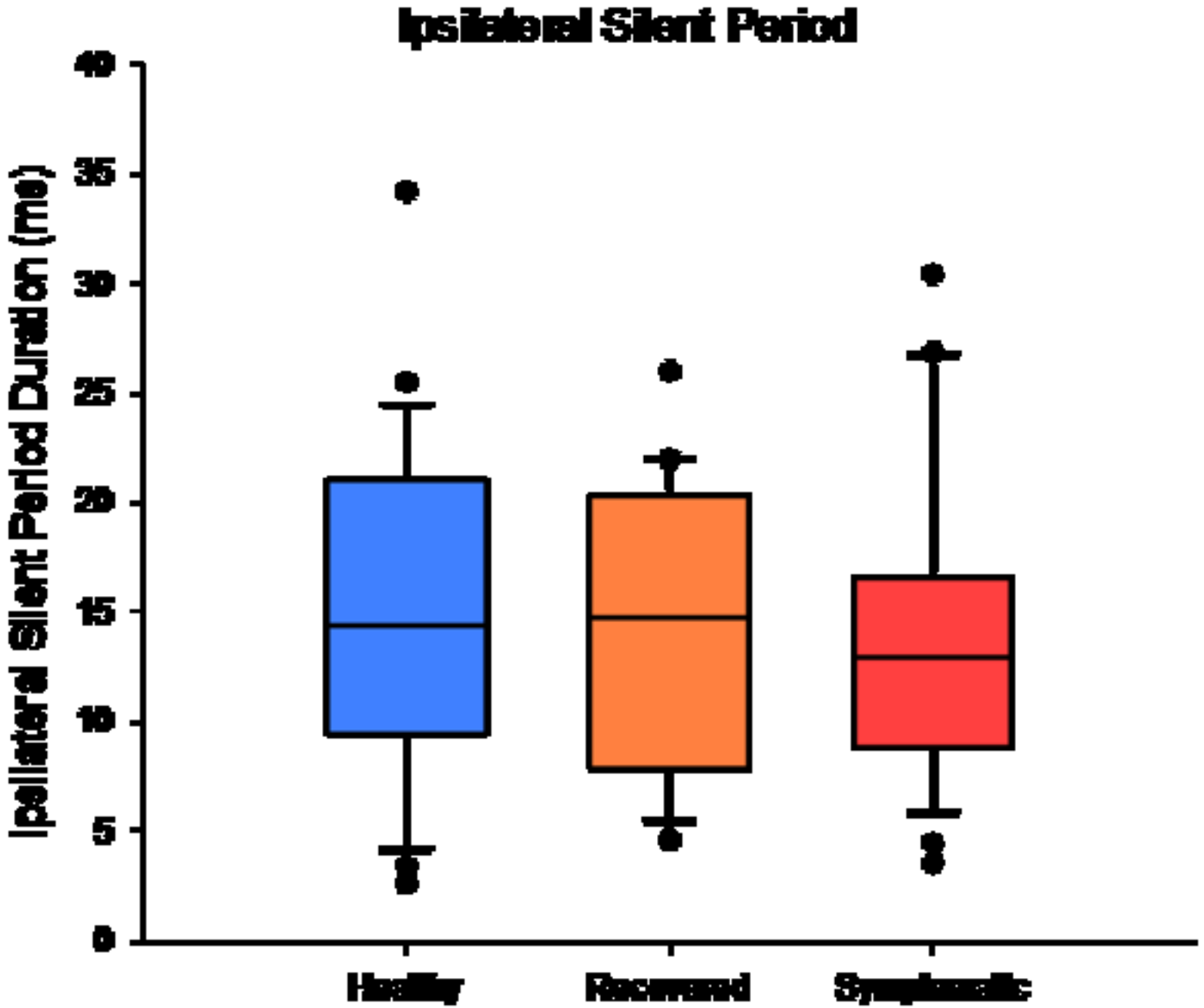

Figure5

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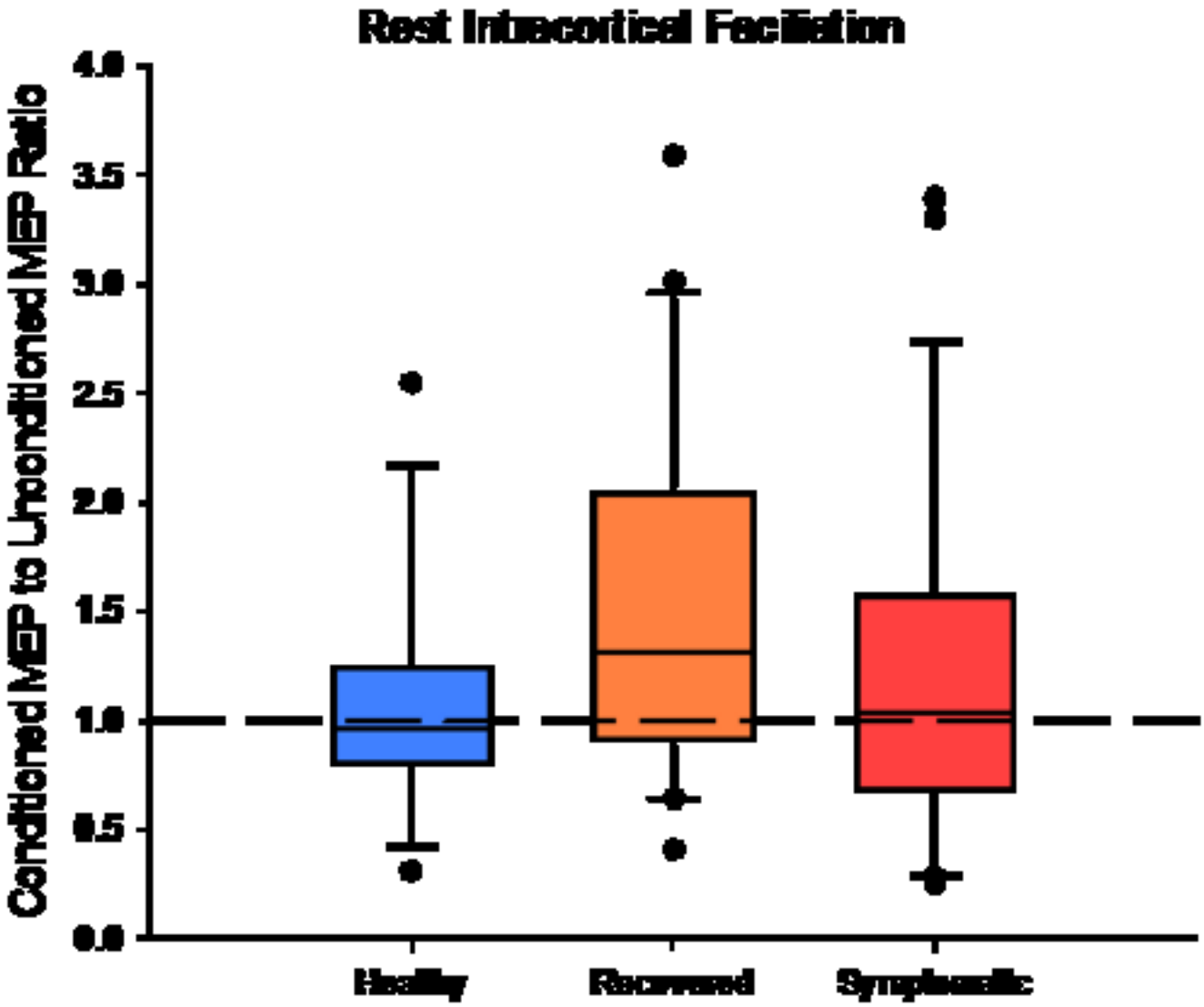

Figure6a

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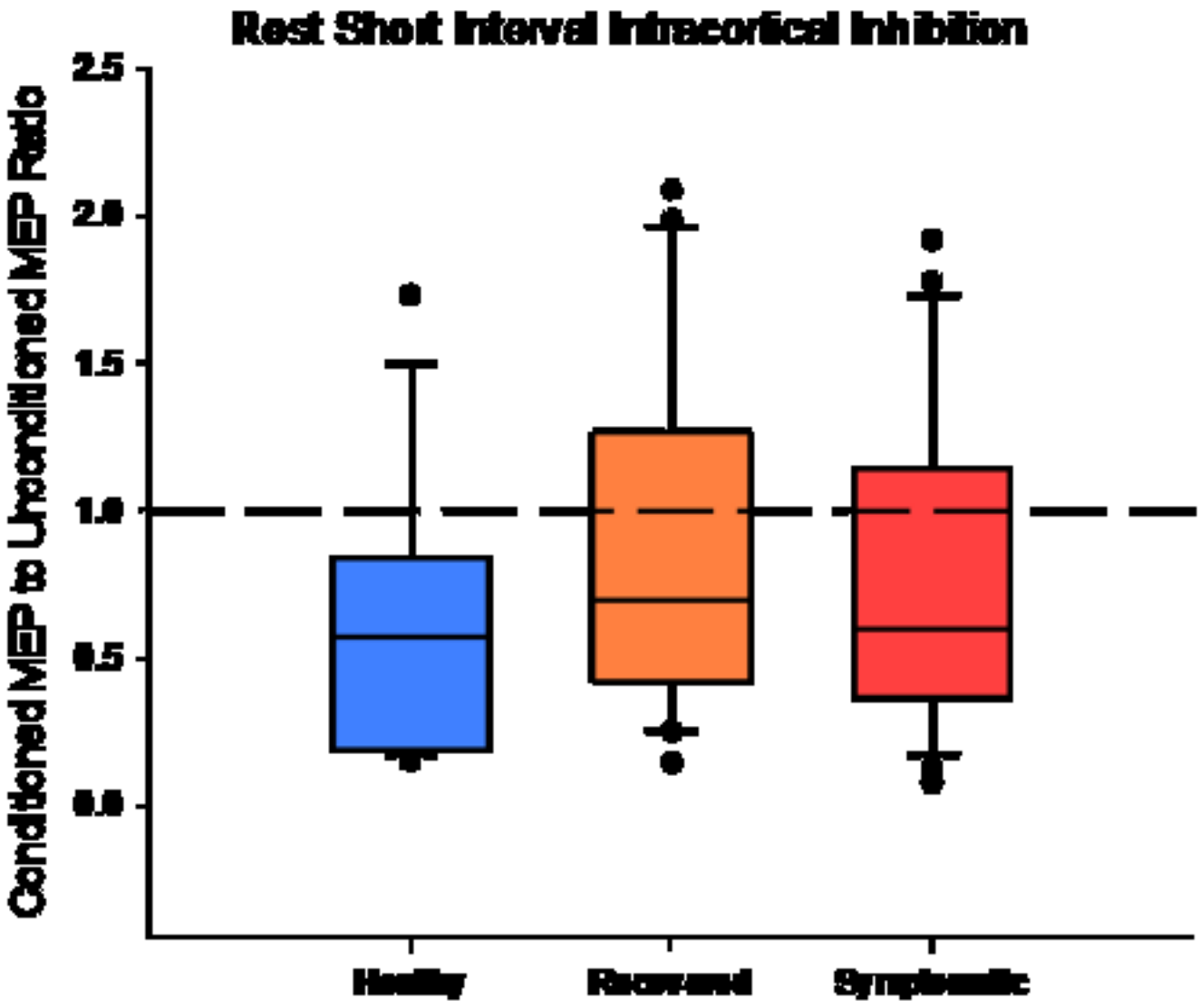

Figure6b

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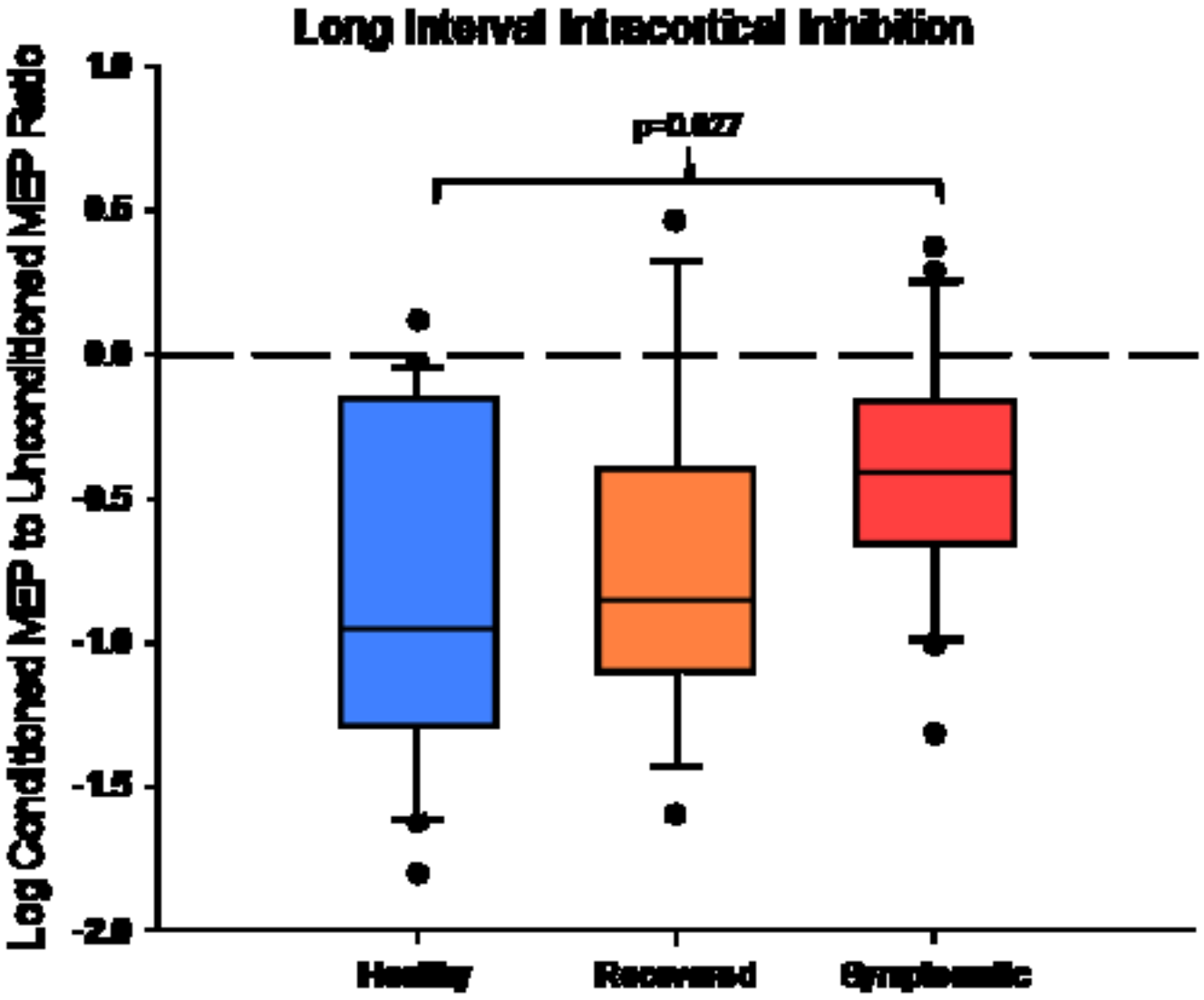

Table1

Table 1: Pre-injury clinical and demographic details

		Healthy (n=28)	**Recovered** (n=27)	**Symptomatic** (n=35)	χ^2	*p*
Mean age (SD)		14.31 (3.14)	14.13 (2.35)	14.06 (2.55)	-	-
Female		15	14	19	-	-
Left handed		3	3	7	1.43	0.489
ADHD		0	1	2	1.6	0.451
Learning Support		1	1	3	0.99	0.609
Previous Concussion	0	28	22	24	2.01	0.366
	1	-	2	7		
	≥2	-	3	4		
Median pre-injury PCSI (Range)		2 (0-29)	0 (0-9)	3 (0-46)	KW = 0.19	0.909

mTBI: mild traumatic brain injury; ADHD: Attention deficit disorder; PCSI: Post-concussion symptom inventory *Pre-injury PCSI scores were compared using Kruskal-Wallis test.

Table 2: ***Injury characteristics and symptom scores in children with mTBI***

	Recovered (n=27)	**Symptomatic** (n=35)	χ^2	***p***
Cause of Injury, n (%)				
•Sport-related	20 (74.0)	17 (45.9)		
•Fall	2 (7.4)	1 (2.9)		
•MVA	0 (0)	2 (5.8)		
•Other	1 (3.7)	2 (5.7)		
•Unknown	4 (14.8)	12 (34.3)		
Loss of consciousness, n (%)	3 (11.1)	3 (8.6)	1.91	0.385
Anterograde Amnesia, n (%)	6 (22.2)	4 (11.4)	3.54	0.171
Retrograde amnesia, n (%)	6 (22.2)	1 (2.9)	7.3	0.026
Time since injury, days (mean, SD)	39.89 (10.53)	39.56 (5.13)	0.16	0.873
Median post–injury PCSI (range)	3 (0-26)	35 (6-122)	U=4.81	<0.001*****

PCSI: Post-concussion symptom inventory; MVC: Motor vehicle collision;
mTBI: mild traumatic brain injury. Post–injury PCSI were compared using Mann-Whitney U

Table 3: Tolerability of TMS with subjective sensations

		Healthy	Recovered	Symptomatic	χ2	p
Headache	Mild	1	1	0	1.2	0.548
Neck Pain	Mild	0	2	3	4.31	0.365
	Moderate	0	0	1		
Tingling	Mild	1	1	6	5.6	0.061
Lightheaded/ Faint	Mild	0	0	1	1.74	0.42
Nausea	Mild	1	0	2	1.79	0.408
Median TMS Rating (range) (1 to 8)		5 (2-7)	4 (1-7)	4 (2-8)	16.05	0.311

Subjective symptom ratings and TMS ratings are compared using chi-squared tests

Table4

Table 4: Single pulse TMS paradigm data

	Controls		Recovered		Symptomatic			
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)		
Log_{10} RMT50uV	27	1.67 (0.11)	25	1.65 (0.10)	33	1.67 (0.12)	F(2, 82)=0.16	0.851
RMT1mV	25	59.64 (18.09)	23	58.35 (16.55)	27	52.78 (11.1)	F(2, 72)=1.46	0.239
Log_{10} AMT200uV	27	1.53 (0.11)	26	1.53 (0.12)	34	1.56 (0.13)	F(2, 84)=0.53	0.589
RSRC Curve	24	-	25	-	28	-	F(2, 74)=1.99	0.144
ASRC Curve		-	25	-	33	-	F(2, 82)=0.36	0.698
cSP	25	114.58 (46.15)	24	116.62 (41.12)	27	104.92 (41.94)	F(2,73)=0.55	0.582
Log cSP curve	27	-	25	-	33	-	F(2, 79)=0.28	0.753
iSP	24	15.03 (7.63)	24	14.69 (6.38)	25	14.07 (7.10)	F(2,70)=0.12	0.89

RMT: Rest motor threshold, AMT: Active motor threshold, RSRC: Rest stimulus response curve, ASRC: Active stimulus response curve, cSP: Cortical silent period, iSP: Ipsilateral silent period
Statistics shown are between groups analyses of variance.

Table5

Table 5: Paired pulse paradigms

	Healthy		Recovered		Symptomatic		Statistic	p
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)		
Log_{10} ICF	15	-0.03 (0.22)	20	0.12 (0.24)	24	-0.01 (0.30)	F(2, 56)=1.81	0.174
SICI	15	0.64 (0.47)	20	0.90 (0.60)	24	0.77 (0.54)	F(2, 56)=1.04	0.359
Log_{10} LICI	21	-0.83 (0.58)	20	-0.72 (0.54)	21	-0.40 (0.42)	F(2, 59)=3.83	*0.027

ICF: Intracortical inhibition, SICI: short interval intracortical inhibition, LICI: long interval intracortical inhibition

*Post-hoc analyses show symptomatic participants were significantly less inhibited than controls (p=0.027)

Table6

Table 6: Correlation matrix

		Group	Age	Gender	Number of previous mTBI	Attention Problems	Post PCSI	NCI	cSP	SICI	LICI	iSP1mV
Age	r	0.116	1									
	N	90	90									
Gender	r	-0.006	-0.058	1								
	N	91	90	91								
Number of previous mTBI	r	**0.235	0.026	-0.008	1							
	N	91	90	91	91							
Attention Problems	r	0.164	-0.015	-0.158	-0.059	1						
	N	91	90	91	91	91						
Post PCSI	r	**0.583	0.053	0.092	0.033	**0.311	1					
	N	91	90	91	91	91	91					
NCI	r	0.006	0.092	0.115	-0.039	**-0.212	-0.109	1				
	N	89	88	89	89	89	89	89				
cSP	r	-0.086	-0.048	**-0.324	-0.168	-0.178	-0.081	0.045	1			
	N	77	76	77	77	77	77	75	77			
SICI ratio	r	0.046	-0.153	0.105	0.03	-0.13	0.04	0.159	-0.114	1		
	N	60	59	60	60	60	60	58	59	60		
LICI ratio	r	0.3	0.118	*0.22	*0.212	0.116	**0.282	0.123	**-0.55	**0.382	1	
	N	63	62	63	63	63	63	61	63	52	63	
iSP1mV	r	-0.058	0.039	**-0.349	0.025	-0.083	-0.097	-0.043	**0.306	-0.07	-0.15	1
	N	74	73	74	74	74	74	72	74	57	61	74
RICF ratio	r	-0.023	0.041	-0.085	0.034	**-0.266	-0.144	0.083	*0.23	**0.359	0.032	-0.159
	N	60	59	60	60	60	60	58	59	60	52	57

* $p < 0.1$, ** $p < 0.05$

Abbreviations: r = Pearson's r; n = sample size; PCSI: Post concussion symptom inventory; NCI: Neurocognitive index; cSP: cortical silent period; SICI: Short interval intracortical inhibition; LICI: Long interval intracortical inhibition; iSP: ipsilateral silent period