Altered Bidirectional Plasticity and Reduced Implicit Motor Learning in Concussed Athletes

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Persistent motor/cognitive alterations and increased prevalence of Alzheimer’s disease are known consequences of recurrent sports concussions, the most prevalent cause of mild traumatic brain injury (TBI) among youth. Animal models of TBI demonstrated that impaired learning was related to persistent synaptic plasticity suppression in the form of long-term potentiation (LTP) and depression (LTD). In humans, single and repeated concussive injuries lead to lifelong and cumulative enhancements of gamma-aminobutyric acid (GABA)-mediated inhibition, which is known to suppress LTP/LTD plasticity. To test the hypothesis that increased GABAergic inhibition after repeated concussions suppresses LTP/LTD and contributes to learning impairments, we used a paired associative stimulation (PAS) protocol to induce LTP/LTD-like effects in primary motor cortex (M1) jointly with an implicit motor learning task (serial reaction time task, SRTT). Our results indicate that repeated concussions induced persistent elevations of GABAergic intracortical inhibition in M1, which was associated with suppressed PAS-induced LTP/LTD-like synaptic plasticity. This synaptic plasticity suppression was related to reduced implicit motor learning on the SRTT task relative to normal LTP/LTD-like synaptic plasticity in unconcussed teammates. These findings identify GABA neurotransmission alterations after repeated concussions and suggest that impaired learning after multiple concussions could at least partly be related to compromised GABA-dependent LTP/LTD synaptic plasticity.

Keywords: GABA system, neurophysiology, paired associative stimulation, sports concussion, synaptic plasticity, TMS

Introduction

Media exposure to career ending concussive injuries among high-profile professional athletes as well as recent associations made between a history of multiple concussions in former professional athletes and the development of both dementia pugilistica (Rabadi and Jordan 2001) and Alzheimer’s disease (Guskiewicz et al. 2005) significantly helped making this epidemic a major public health concern (Kelly 1999). Perhaps more alarming are recent estimates suggesting that approximately 5% of high school and collegiate football players sustain at least one concussion during the course of a single season (Guskiewicz et al. 2000), making sports injuries the most prevalent cause of mild traumatic brain injury (mTBI) among youth in the United States of America (Langlois et al. 2006). In light of the known association between mTBI and impaired learning (Roberts et al. 1990; Bigler 1996; Matser et al. 1998, 1999), a better understanding of the mechanism underlying lasting functional disturbances associated with sports concussions (Guskiewicz et al. 2005; De Beaumont et al. 2009) is imperative.

Paradoxically, neurocognitive evidence of pervasive learning/memory dysfunctions (Gaetz et al. 2000; Gaetz and Weinberg 2000; Bernstein 2002; Bleiberg et al. 2004; Gosselin et al. 2006; De Beaumont, Brisson, et al. 2007; De Beaumont et al. 2009) in concussed athletes has yet to find pathophysiological correlates that outlast short-lived neurometabolic disturbances typically resolving within 10 days (Giza and Hovda 2001). Animal studies conducted at the cellular level, however, suggest that impaired learning consecutive to the induction of single (Tang et al. 1997) and repeated (DeFord et al. 2002; Creeley et al. 2004) mTBI could be the result of persistent and cumulative suppression of hippocampal synaptic plasticity (Miyazaki et al. 1992; Reeves et al. 1995, D’Ambrosio et al. 1998; Sick et al. 1998; Albesni et al. 2000) in the form of long-term potentiation (LTP) and long-term depression (LTD).

Consistent with the central role of hippocampal LTP/LTD for the acquisition of new information and memory-related processes (Bliss and Collingridge 1993; Wolters et al. 2003), LTP/LTD in the primary motor cortex (M1) seems to play a pivotal role in motor skill learning (Pascual-Leone et al. 1994, 1995; Classen et al. 1998). In rat M1, motor training prevents subsequent LTP and enhances LTD (Rioult-Pedotti et al. 1998) without affecting synaptic modification range (Rioult-Pedotti et al. 2000). Based on the Bienenstock–Cooper–Munro (BCM) theory of bidirectional synaptic plasticity (Bienenstock et al. 1982), this is indicative of the involvement of LTP/LTD in motor skill learning. With the recent development of interventional paired associative stimulation (PAS) protocols that allow noninvasive investigation of LTP/LTD-like mechanisms in human M1 (Stefan et al. 2000; Ziemann et al. 2004), it has been suggested that human motor learning similarly occurs through LTP/LTD (Ziemann et al. 2004). This finding is in line with the notion that implicit learning of a motor sequence in a classic serial reaction time task (SRTT) (Willingham et al. 1989) is associated with the gradual expansion of M1 output maps (Pascual-Leone et al. 1995; Classen et al. 1998) via LTP/LTD-dependent synaptic strengthening of cortical horizontal connections (Sanes and Donoghue 2000).

Through its inhibitory effects on NMDA receptors, gamma-aminobutyric acid (GABA) modulates the expression of bidirectional plasticity as well as motor learning (Davies et al. 1991; Mott and Lewis 1991; Ziemann and Siebner 2008). In humans, the administration of GABA receptor agonist "Baclofen" suppresses PAS-induced LTP-like plasticity in M1 (McDonnell et al. 2007). This increase in GABA neurotransmission is thought to prevent LTP-dependent motor learning (McDonnell et al. 2007; Ziemann and Siebner 2008), a notion that finds support in animal studies (McNamara and Skelton 1996; Nakagawa and Takashima 1997). In addition to causing LTP...
suppression, the ingestion of Baclofen significantly increases M1 intracortical inhibition (McDonnell et al. 2006). Strikingly, recent transcranial magnetic stimulation (TMS) studies have shown that concussions in sports like football and ice hockey induce durable elevations in M1 intracortical inhibition that worsen with additional concussive injuries (De Beaumont, Lassonde, et al. 2007; De Beaumont et al. 2009). These findings suggest that excessive M1 intracortical inhibition could be the result of abnormally elevated GABA_A receptor-mediated inhibitory postsynaptic potentials (McDonnell et al. 2006). This prompted us to examine whether excessive GABA receptor activity found in concussed athletes could prevent bidirectional plasticity from occurring in M1.

Here, we tested whether sports concussions in university-level football players induce long-lasting impairments of LTP/LTD-like synaptic plasticity and whether abnormal GABA-mediated intracortical inhibition modulates M1 synaptic plasticity. Furthermore, in light of the link between M1 neuroplasticity and implicit motor learning, the effects of sports concussions were assessed in a classic SRTT procedure. Knowing that brain-derived neurotrophic factor (BDNF) polymorphisms can influence plasticity response and implicit motor learning (Kleim et al. 2006; Cheeran et al. 2008; Fritsch et al. 2010), genotype profiling was performed to control for BDNF polymorphism differences across groups. This study demonstrates that relative to unconcussed teammates, asymptomatic concussed university-level football players who returned to competition more than 9 months prior to testing presented suppressed LTP/LTD-like plasticity along with reductions in implicit motor learning. The extent of LTP/LTD suppression was found to be directly related to intracortical inhibitory dysfunction.

### Materials and Methods

#### Participants

All 32 participants were active players from Canadian university football teams aged between 19 and 27 years (mean age of 23.4 years; standard deviation [SD] 3.11). It was assumed that the level of physical activity was equivalent among participants since they were all teammates sharing highly similar training routines as well as practice/game schedules. Participants were included if they met all of the following criteria: no history of alcohol and/or substance abuse; no medical condition requiring daily medication; no previous history of psychiatric illness, learning disability, neurological history, or TBI unrelated to contact sports. None of them was, at the time of the study, regularly practicing any activity that involved sequential finger movements and all participants reported being right handed. The study was approved by the local ethics committee and all participants provided written informed consent prior to testing. Subjects received a financial compensation of $60 CDN for their participation.

The study included 2 groups. The first group consisted of 19 university-level football players who presented with no prior history of concussion. The second group included 13 university-level football players who reported having sustained at least 2 sports concussions (number of concussions ranged from 2 to 7; mean number of concussions was 2.87 ± 1.41) that took place more than 9 months prior to testing (mean time since last concussion: 13.74 ± 6.26 months). This interval since the last concussive event has previously revealed lingering concussion-related effects on TMS measures of corticospinal excitability in university football players equivalent to those who took part in the present study (De Beaumont, Lassonde, et al. 2007). Concussion history was based on medical records for accidents that occurred throughout the athletes' university years while concussions that occurred prior to university years were mostly self-reported. As part of a yearly medical checkup during the off-season as well as after sustaining a concussion, concussed athletes were subjected to a medical screening including cervical injuries that had to be negative prior to returning to competition. At the time of testing, concussed athletes were asymptomatic, reporting very few, if any, symptoms on the Postconcussion Symptoms Scale (mean 2.15; SD 2.08) (Maroon et al. 2000). The severity of concussions ranged from Grade 1 (concussion symptoms or mental status abnormalities on examination that lasted less than 15 min, no loss of consciousness [LOC]) to Grade 3 (LOC, either brief [seconds] or prolonged [minutes]) according to the American Academy of Neurology practice parameters (American Academy of Neurology Practice 1997); they all classified as mTBI on the Glasgow Coma Scale (scoring between 13 and 15). Experimental groups were equivalent according to age (F(1,31) = 0.395; P > 0.05; controls: 22.78 ± 1.88 years; concussed athletes: 23.41 ± 2.21 years) and level of education (F(1,31) = 0.712; P > 0.05; controls: 15.71 ± 1.47; concussed: 16.19 ± 1.83). A total of 2 concussed athletes were carriers of the val66met polymorphism of the BDNF gene associated with reduced synaptic plasticity and implicit motor learning while 4 controls carried this BDNF polymorphism. All 32 participants who joined this study completed sessions 1 and 2 while 3 of them (1 from the concussion group and 2 unconcussed athletes) withdrew from the experiment prior to session 3.

#### Timeline of Experiments

The experiment consisted of 3 testing sessions conducted at approximately the same time of day and that took place 1-6 months apart during football off-season. The first 90-min session included the administration of a concussion history questionnaire, a general health questionnaire, the Postconcussion Symptoms Scale (for more details, refer to this previously published paper, De Beaumont et al. 2009), and the PAS protocol for LTP generation. The second 45-min session performed more than 48 h after the first session consisted of a brief, semistructured assessment of new concussive injuries as well as potential changes in general health since the first session followed by the administration of the SRTT. The third 1-h session conducted more than a month after the SRTT session consisted of the administration of the PAS protocol designed to induce LTD-like effects.

#### Paired Associative Stimulation

Paired associative stimulis of 200 electric stimuli of the right median nerve at the wrist paired with single-pulse TMS of the hand area of the left motor cortex. The rate of paired stimulation was 0.25 Hz (total duration of the PAS protocol was approximately 13 min). Bipolar electrodes with cathode proximal were used to produce constant 1-ms current square pulses at an intensity of 300% perceptual threshold. TMS was delivered through an 8-cm figure-of-eight coil connected to a Mag Pro transcranial magnetic stimulator (Medtronic, Minneapolis, MN). The stimulating coil was placed flat on the skull with the handle pointing backwards and 45° away from the midline. A BrainSight frameless stereotaxic system (Rogue Research, Canada) was used to ensure stable coil positioning over the area that produced the largest motor evoked potentials (MEPs) in the right abductor pollicis brevis (APB) muscle. TMS intensity was adjusted to produce a MEP of approximately 1 mV peak-to-peak amplitude in the resting APB without median nerve stimulation. Surface electromyography was recorded from the APB of the right hand using a belly–tendon montage. The signal was amplified and filtered using a PowerLab 4/30 system (ADInstruments, Australia), with a 20 Hz–1000 Hz band-pass filter. Data were analyzed off-line on a G5 Macintosh computer using Scope 4 software (ADInstruments, Australia). PAS-induced LTD-like effects were obtained with TMS stimulation (central) following afferent electrical stimulation (peripheral) by 25 ms (PAS_{25ms}) while LTD-like effects were generated with peripheral stimulation delivered 10 ms prior to central stimulation (PAS_{10ms}). Participants were asked to count the number of median nerve stimulation pulses to keep their attention on the target hand to control for the known effects of attention on PAS-induced bidirectional plasticity (Stefan et al. 2004).
amplitude was expressed as a percentage of the unconditioned MEP collected prior to baseline CSP determination. The conditioned MEP interstimulus interval was 100 ms (McDonnell et al. 2006). Ten pairs be mediated by GABAB receptors. CSP duration was calculated at 2 the late phase, intracortical inhibitory mechanism that is thought to studied here, often lasting for a few hundred milliseconds, reflects (Ziemann et al. 1993). The extended duration of the silent period investigated resting MEP amplitude just before PAS as well as consecutive trials. The stimulus intensity that produced a MEP intensities (120%, 130% of rMT) were applied to the left M1. TMS intensities. Five single-pulse stimulations for each of 2 TMS described by Garvey et al. (2001). Baseline CSP was obtained prior to prestimulus time window recorded prior to each TMS stimulation. The duration of the CSP was calculated with the graphical method pre-TMS and baseline TMS measurements of intracortical inhibition, SRTT changes, baseline TMS measurements of intracortical inhibition, SRTT condition. CSP duration was calculated at 2 TMS intensities. Five single-pulse stimulations for each of 2 TMS intensities (120%, 130% of rMT) were applied to the left M1. Participants maintained a voluntary isometric contraction of the right APB at approximately 10% of their maximum strength. Continuous muscle contraction was assessed using the 100-ms prestimulus time window recorded prior to each TMS stimulation. The duration of the CSP was calculated with the graphical method described by Garvey et al. (2001). Baseline CSP was obtained prior to MEP(t1mv) determination, which was in turn performed just before PAS.

LTP-like Effects (Session 1) Measures of motor cortex excitability using TMS included resting motor threshold (rMT), MEP amplitude, and cortical silent period (CSP). MEP amplitude and CSP were performed before and after PAS(25ms) and the index of change was a ratio of motor cortex excitability modulation after PAS relative to Pre-PAS (baseline) data (ratio = (Post – Pre)/Pre) (Fig. 1).

rMT was determined as the minimum stimulus intensity required to produce a MEP in the relaxed APB of at least 50 μV in 5 of 10 consecutive trials. The stimulus intensity that produced a MEP amplitude of approximately 1 mV in resting APB was determined before PAS. Using this stimulator intensity, 15 trials were recorded to investigate resting MEP amplitude just before PAS as well as immediately after PAS. The mean amplitude was calculated from each trial at each time point (Pre vs. Post). The CSP comprises both spinal (early phase) and cortical (late phase) inhibitory mechanisms (Ziemann et al. 1993). The extended duration of the silent period studied here, often lasting for a few hundred milliseconds, reflects the late phase, intracortical inhibitory mechanism that is thought to be mediated by GABAB receptors. CSP duration was calculated at 2 TMS intensities. Five single-pulse stimulations for each of 2 TMS intensities (120%, 130% of rMT) were applied to the left M1. Participants maintained a voluntary isometric contraction of the right APB at approximately 10% of their maximum strength. Continuous muscle contraction was assessed using the 100-ms prestimulus time window recorded prior to each TMS stimulation. The duration of the CSP was calculated with the graphical method described by Garvey et al. (2001). Baseline CSP was obtained prior to MEP(t1mv) determination, which was in turn performed just before PAS.

LTP-like Effects (Session 3) Since the third LTD session was performed at least 1 month apart from the LTP session, baseline MEP and CSP measurement parameters adjustments were performed prior to PAS(10ms), protocol to control for between-day motor cortex excitability changes. In addition to MEP amplitude and CSP performed before and after PAS(10ms), long-interval intracortical inhibition (LICI) was introduced as an additional TMS measure of motor cortex excitability. To test LICI, TMS intensity of both conditioning (CS) and test (TS) stimuli was set at MEP(t1mv) and the interstimulus interval was 100 ms (McDonnell et al. 2006). Ten pairs of MEPs with a TS peak-to-peak amplitude of 0.4–1.5 mV were collected prior to baseline CSP determination. The conditioned MEP amplitude was expressed as a percentage of the unconditioned MEP amplitude to determine the inhibitory effect induced by the conditioning stimulus.

Serial Reaction Time Task Participants were seated on a straight back chair with elbows flexed at an angle of 90°. They performed a modified SRTT (Perez et al. 2007) running on SuperLab (version 4.0; Cedrus, San Pedro, CA). The GO signal was displayed on the computer screen and consisted of one asterisk and 3 dots evenly spaced on an invisible horizontal plane, all appearing simultaneously. The position of the asterisk varied across trials among the 4 possible locations and indicated the required key press (Perez et al. 2007). Participants were instructed to respond as fast and accurately as possible to the position of the asterisk by pressing the corresponding key with the predetermined finger (index finger for key 1, middle finger for key 2, ring finger for key 3, and little finger for key 4). A correct key press was required for the next trial to appear on the computer screen. Response time was defined as the time interval between stimulus presentation and the correct key press. Participants performed a total of 14 blocks separated by pauses and each block consisted of 10 presentations of the same 12-item sequence for a total of 120 key presses per block. They were instructed to perform the task only with their dominant hand and to keep your index finger on each predetermined key at all times. The 2 initial blocks consisted of stimuli presented in random order (random blocks) that differed from the predetermined repeating sequence. The first 2 random blocks (R1 and R2) were provided for participants to get familiar with the task. Blocks 3–7 and 9–13 corresponded to training blocks during which participants were presented with the following predetermined, repeating 12-item sequence (sequence A: 4-2-3-1-3-2-1-3-4-2-4). Learning blocks were named according to their respective order preceded by the letter "A." Sequence-specific learning was computed as the difference in median response time between the last sequence block (A10) and the last random block (R4) (Willingham et al. 2000). Total training-related learning was calculated as the median response time difference between the first sequence block (A1) and the last sequence block (A10).

BDNF rs6265 (val66met) Polymorphism Profiling DNA extraction from saliva samples was performed using Oragene OG-250s kits (DNA Genotek, Ottawa, Canada). Genotype profiling of BDNF rs6265 (val66met) polymorphism was performed with polymerase chain reaction (PCR) followed by pyrosequencing. Amplification was performed using a PCR approach, with the following primer pairs: forward biotine 5'-GGACCTGAGAGCGGTGAAT-3' and reverse 5'-CCGAACCTTCTGTTCTCCTAC-3'. Genomic DNA (250–500 ng) was amplified with 0.2 μM of each primer, 1 μM buffer (Qiagen kit), 0.4 mM dNTP, 1.0 mM MgCl₂, and 0.01 U of Qiagen Tag polymerase. Amplification was carried out on a Biometra TProfessional Basic thermocycler (Biometra, Göttingen, Germany) with the following conditions for 35 cycles: 30 s at 95 °C, 30 s at 61.2 °C, and 1 min at 72 °C. These 35 amplification cycles were preceded by a 3-min hot start at 95 °C and followed by a final 4-min extension to the last cycle at 72 °C. PCR products were visualized on a 1.2% agarose gel. The val66met polymorphism was subsequently determined via an established pyrosequencing protocol (Petersen et al. 2005) with oligo sequencing 5'-GGCTGACACTTCTGGAAGA-3'. The sequence to analyze was CA/GTGATAGAAG.

Statistical Analyses All values are expressed as means ± SDs. Demographic information and baseline intracortical inhibition measures were subjected to one-way between-group analyses of covariance (ANCOVAs) with age and level of education variables as covariates. Procedural learning ratios were subjected to between-group ANCOVAs with BDNF polymorphism, age, and level of education as covariates. LTP/LTD-like effects involving pre-PAS/post-PAS assessment of motor cortex excitability and intracortical inhibition were subjected to 2 (Groups) F0B4 2 (Pre-PAS/Post-PAS) mixed ANCOVAs with age, level of education, and BDNF polymorphism as covariates. Pearson correlations between measures of PAS-induced changes, baseline TMS measurements of intracortical inhibition, SRTT
learning, and concussion severity ratings were computed to draw potential associations between different measures used in this study. Partial eta squared were used to compute effect sizes.

Results

Baseline Motor Cortex Excitability
Baseline rMT and CSP duration obtained across testing sessions were highly similar in both groups (Table 1). There was no between-group difference on rMT ($F_{1,28} = 0.624; P = 0.792$; partial eta squared $\eta^2 = 0.003$). Increased GABAergic-mediated intracortical inhibition was found at baseline in athletes with a history of multiple concussions. Compared with their unconcussed counterparts, LICI was significantly enhanced (between-group ANCOVA: $F_{1,20} = 4.938; P = 0.037; \eta^2 = 0.183$; Fig. 2a) and CSP duration was significantly prolonged (main effect of Group: 120% of rMT: $F_{1,21} = 10.87; P = 0.002; \eta^2 = 0.269$; 130% of rMT: $F_{1,21} = 7.935; P = 0.009; \eta^2 = 0.213$; Fig. 2b). Increasing TS intensity significantly lengthened CSP duration in both groups (within-subject effect of Intensity) ($F_{4,84} = 60.696; P < 0.0001$) while the Intensity (120%, 130% rMT) $\times$ Group interaction was not significant ($F_{3,20} = 3.148; P = 0.091; \eta^2 = 0.092$). Adjusted MEP(1mV) size at baseline did not differ between groups ($F_{1,30} = 2.17; P = 0.205; \eta^2 = 0.044$).

PAS-Induced LTD-like Plasticity

Motor Cortex Excitability
A repeated measures ANCOVA with BDNF polymorphism as covariate revealed a highly significant PAS(25ms) $\times$ Group interaction ($F_{1,30} = 17.17; P = 0.002; \eta^2 = 0.364$) (Fig. 3a). Furthermore, between-group MEP size difference after PAS(25ms) was significant ($F_{1,30} = 4.97; P = 0.016; \eta^2 = 0.241$). Using paired-sample $t$-tests, our results indicated that PAS(25ms) induced a significant increase in MEP size (MEP pre-PAS vs. MEP post-PAS) in unconcussed athletes ($T_{18} = 2.38; P = 0.028$) after Tukey correction for multiple comparisons was applied. In sharp contrast, the effects of PAS(25ms) tended to inhibit motor cortex excitability in concussed athletes ($T_{12} = -2.31; P = 0.039$), but this failed to reach significance after Tukey correction for multiple comparisons.

Intracortical Inhibition
Previous studies have shown that PAS(25ms) significantly lengthens CSP duration in the general population (Stefan et al. 2000, 2004) of comparable age. This effect of PAS(25ms) on CSP duration across groups yielded a significant PAS(25ms) $\times$ Group interaction at both intensities (120% rMT: $F_{1,30} = 5.19; P = 0.028; \eta^2 = 0.147$; 130% rMT: $F_{1,30} = 4.04; P = 0.049; \eta^2 = 0.121$). Paired-sample $t$-tests revealed significant CSP prolongation after PAS(25ms) specific to unconcussed athletes (120% rMT: $T_{18} = -3.24; P = 0.004$; 130% rMT: $T_{18} = -2.23; P = 0.03$; Fig. 3b). CSP duration did not change among concussed athletes (120% rMT: $T_{12} = 0.44; P = 0.67$; 130% rMT: $T_{12} = 1.30; P = 0.22$; Fig. 3c).

PAS-Induced LTD-like Plasticity

Motor Cortex Excitability
A repeated measures ANCOVA PAS(10ms) $\times$ Group interaction with BDNF polymorphism as covariate revealed that the effects of PAS(10ms) were significantly different across groups ($F_{1,28} = 4.82; P = 0.037; \eta^2 = 0.189$; Fig. 3a). Moreover, between-group MEP size difference after PAS(10ms) was significant ($F_{1,28} = 4.88; P = 0.027; \eta^2 = 0.219$).

Using a paired-sample $t$-test, we demonstrated that PAS(10ms) induced significant LTD-like effects (MEP post-PAS vs. MEP pre-PAS) among unconcussed athletes ($T_{16} = -4.25; P = 0.0006$). In concussed athletes, however, PAS(10ms) did not modify motor cortex excitability ($T_{11} = 0.65; P = 0.53$).

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Note: CSP 120% refers to the duration of the CSP elicited by TMS pulses delivered at TMS intensity of 120% of rMT and CSP 130% refers to the duration of the CSP elicited by TMS pulses delivered at 130% of the rMT. Values are expressed as group means ± SD.

Figure 2. (a) LICI is expressed as the ratio CS $\rightarrow$ TS/T5. The intensity of both CS and TS was adjusted to induce a MEP of approximately 1 mV peak-to-peak amplitude (MEP(1mV)). Baseline LICI differed significantly between groups. (b) CSP duration (ms) when TMS of 2 different intensities (120% and 130% of the rMT) is applied to M1 while participants exert a voluntary isometric muscle contraction of the ARB muscle of the right hand at approximately 10% of maximum strength. Baseline CSP duration differed significantly between groups at both TMS intensities. Error bars represent standard error values and the asterisk (*) indicates that the effect was significant at $P < 0.05$. 

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Intracortical Inhibition
Pas(25ms) did not significantly modulate measures of intracortical inhibition. None of the PAS(25ms) × Group interactions reached significance (LIgi: F1,27 = 2.422; P = 0.14; ηp² = 0.101; CSP 120% rMT: F1,27 = 1.184; P = 0.270; ηp² = 0.043; CSP 130% rMT: F1,27 = 2.111; P = 0.186; ηp² = 0.078).

Implicit Motor Learning
The training block effect was significant when percent change in reaction times ((A1 - A10)/A1) for each participant was used to compute a between-group ANCOVA with age, level of education, and BDNF polymorphism as covariate (F1,51 = 6.381; P = 0.026; ηp² = 0.023; Fig. 3d).

The sequence-specific effect of learning was also significant when percent change in reaction times ((A10 - R4)/A10) for each participant was used to compute a between-group ANCOVA with BDNF polymorphism as covariate (F1,51 = 4.209; P = 0.046; ηp² = 0.074; Fig. 3d).

There was no group difference in mean response accuracy either in sequence blocks (concussion group: 90.7 ± 3.7; control athletes group: 92.3 ± 2.9; Fig. 3e).

Figure 3. (a) Effect of PAS (PAS(25ms) and PAS(10ms)) on the size of MEPs of the right APB muscle. The histogram illustrates the mean peak-to-peak amplitude (in mV) of MEPs recorded after both PAS(25ms) and PAS(10ms) relative to the constant, normalized baseline MEP amplitude set at 0. The intensity of the TS was adjusted prior to PAS to elicit a MEP of approximately 1 mV (MEP(1mV)) and was held constant after PAS to compute changes in motor cortex excitability. Note that PAS(25ms) induced significant MEP facilitation in controls while multiple concussion athletes show near significant MEP depression. In contrast, MEPT(1mV) induced significant MEP amplitude reduction only in the control athletes group. (b) Effect of PAS(10ms) on the duration of the CSP in control athletes. The histogram illustrates CSP duration prior to and after PAS(10ms). When the TS was set at 120% and 130% of the rMT. Note that CSP was significantly prolonged at both TS intensities. (c) Effect of PAS(25ms) on the duration of the CSP in multiple concussion athletes. The histogram illustrates CSP duration prior to and after PAS(25ms) when the TS was set at 120% and 130% of the rMT. Note that CSP duration was unchanged in the multiple concussion athletes group. (d) The histogram illustrates the significantly greater sequence-specific RT increase (in percent change) from the last training block (A10) to the immediately following random block (R4) in control athletes relative to multiple concussion athletes. This panel also depicts significantly greater RT reductions (in percent change) in control athletes relative to multiple concussion athletes after 10 training blocks (A1 vs. A10), (e) Response time (RT) in random and sequence blocks during the SRTT. The abscissa shows block type in temporal order, and the ordinate shows median RT. Note the significant, progressive RT shortening in sequence A (A1-A10 blocks) as well as the significant sequence-specific learning when controlling for task exposure (A10-R4) in both groups. (a-e) Error bars display standard error values. Asterisk (*) corresponds to P < 0.05.
controls: 91.6% \pm 3.7; F_{1,31} = 0.56; P = 0.337) or random blocks (concurrency group: 87.1% \pm 6.8; controls: 89.5% \pm 8.3; F_{1,31} = 0.94; P = 0.216).

**Correlational Analyses**

**Intracortical Inhibition and Bidirectional Plasticity**

In addition to correlating with one another, both CSP conditions were found to correlate with bidirectional associative plasticity in concussed athletes (CSP 120% rMT/LTP effects: $R = -0.487$; $P = 0.034$; CSP 130% rMT/LTP effects: $R = -0.658$; $P = 0.002$ [Fig. 4A]; CSP 120% rMT/LTD effects: $R = 0.649; P = 0.016$), such that those with longer baseline CSP duration displayed more suppressed LTP/LTD-like effects (MEP post-PAS - MEP pre-PAS). Finally, the degree of LTP and LTD suppression was highly correlated ($R = 0.824; P = 0.002$) among concussed athletes. Correlations between intracortical inhibition and bidirectional plasticity measures were also significant (CSP 120% rMT/LTP effects: $R = 0.480; P = 0.038$; CSP 130% rMT/LTP effects: $R = 0.464; P = 0.046$; CSP 120% rMT/LTD effects: $R = 0.572; P = 0.016$). LTP and LTD were also found to be significantly correlated in controls ($R = 0.592; P = 0.01$).

**Bidirectional Plasticity and Implicit Motor Learning**

In concussed athletes, LTP was strongly correlated with both training block effects (A10 vs. A1) ($R = 0.851; P = 0.0004$) and sequence-specific (A10 vs. A4) ($R = 0.771; P = 0.003$) implicit motor learning. For its part, LTD was correlated with sequence-specific learning ($R = 0.643; P = 0.033$). In contrast, correlations between bidirectional plasticity and implicit motor learning measures in unconcussed athletes only tended to be significant (training block effects and LTD (A10 vs. A1): $R = 0.411; P = 0.08$; sequence-specific learning and LTD (A10 vs. A4): $R = 0.351; P = 0.014$; training block effects and LTD (A10 vs. A1): $R = 0.458; P = 0.06$; sequence-specific learning and LTD (A10 vs. A4): $R = 0.473; P = 0.055$).

**Concussion Severity**

Concussion severity ratings of the most severe accident correlated significantly with baseline CSP duration at both TMS intensities (120% rMT: $R = 0.578; P = 0.021$; 130% rMT: $R = 0.493; P = 0.039$; baseline LICI: $R = 0.638; P = 0.026$; LTD effects: $R = 0.515; P = 0.029$; and LTD effects: $R = 0.726; P = 0.017$).

**Discussion**

Using PAS, this study shows that altered M1 intracortical inhibition in athletes with a history of multiple sports concussions is associated with compromised LTP/LTD-like synaptic plasticity. In addition, implicit motor learning involving the implicit acquisition of a repeated sequence was significantly reduced in concussed athletes and was found to correlate with synaptic plasticity alteration.

The major finding of the present study is the alteration of LTP/LTD-like plasticity in athletes with a prior history of multiple sports concussions. These data are in line with rat models of TBI where reductions of hippocampal LTP has been repeatedly found (Tang et al. 1997; DeFord et al. 2002; Creeley et al. 2004). Concussion-induced alterations of synaptic plasticity may be partly explained by increased GABAergic neurotransmission. GABA-mediated intracortical inhibition, measured with TMS-induced LICI and CSP, was significantly increased in concussed athletes and correlated with the degree to which PAS25 and PAS10 protocols induced LTP/LTD-like plasticity. These group differences on synaptic plasticity and implicit motor learning were found despite having controlled for the BDNF val66met variant, which is associated with clear reductions of electrophysiological markers of synaptic plasticity (Cheeran et al. 2008), reduced motor map reorganization (Kleim et al. 2006), and reduced motor skill acquisition (Fritsch et al. 2010).

Interestingly, Baclofen, a known GABA agonist, increases LICI (McDonnell et al. 2006) and suppresses LTP (McDonnell et al. 2007) in healthy humans. Consistent with the near significant reversal of the PAS25 effect found in concussed athletes, LTP-like plasticity switched to depression in 5 of 6 participants who had ingested Baclofen (McDonnell et al. 2007). Similarly, enhancing M1 intracortical inhibition with low-frequency (0.1 Hz) repetitive TMS (rTMS), which does not induce LTP/LTD itself, occludes subsequent PAS-induced LTP/LTD (Delvendahl et al. 2010). Taken together, these data suggest that persistent elevations in GABA-mediated intracortical inhibition contribute to the suppression of LTP/LTD-like plasticity presumably via blockade of NMDA receptors-mediated glutamatergic expression (Davies et al. 1991).
Although the underlying cause of increased GABAergic inhibition is unknown, the neurometabolic cascade of concussion, mainly characterized by glutamate excitotoxicity and ischemia-like reduction in cerebral blood flow (Giza and Hovda 2001), suggests a neuroprotective role against cell death for GABAergic neurotransmission (Perez-Pinzon 2007). Interestingly, animal studies have found endogenous elevations of GABAergic neurotransmission following the induction of a brief, sublethal ischemic episode (Dave et al. 2005; Kuramoto et al. 2007). This persistent upregulation of GABAergic receptor expression to reduce glutamate excitotoxicity induces a metabolic state of resistance against subsequent cerebral ischemia, which is referred to as ischemic preconditioning (IPC) (Barone et al. 1998). These findings suggest that similar excitotoxic/IPC can increase GABAergic receptor expression and may partly account for elevated levels of GABAergic inhibition following multiple concussions.

The close association between LTP/LTD-like effects and implicit motor learning found in concussed athletes suggests that altered GABA-mediated inhibition exerts its impact at least partly through its suppressing effects on bidirectional synaptic plasticity. This is reminiscent of both human studies and animal models of schizophrenia, where a similar reduction of LTP-like plasticity has been reported (Fatemi et al. 2000; Frantseva et al. 2008; Tang et al. 2009). In humans, MEP facilitation was absent following PAS(25ms) and performance on a rotary pursuit task (Nitsche et al. 2003; Antal et al. 2004), or PAS(25ms) and performance on a rotary pursuit task was reduced. Significantly, implicit motor learning and LTD-like plasticity were positively correlated, much like what was found here in concussed athletes. Interestingly, M1 intracortical inhibition is also altered in schizophrenia, although “decreased” inhibition appears to be the major net effect (Daskalakis et al. 2002; Fitzgerald et al. 2003). The relation between bidirectional plasticity and STTT learning was reduced in controls relative to that of concussed athletes. This suggests that abnormally elevated intracortical inhibition in concussed athletes exerts greater detrimental influence on implicit motor learning than slight variations occurring within a normal range of intracortical inhibition in a relatively small control sample.

As in synaptic plasticity, transient alterations of intracortical inhibition appear to modulate implicit motor learning (Nitsche et al. 2003; Antal et al. 2004; Kim et al. 2004). In healthy humans, increasing M1 excitability with high-frequency rTMS (Kim et al. 2004, 2006), intermittent theta burst stimulation (Huang et al. 2005), anodal transcranial direct current stimulation (Nitsche et al. 2003; Antal et al. 2004), or PAS(25ms) (Cirillo et al. 2009; Rajji et al. 2010) during or prior to motor learning is associated with significant task performance improvements. Furthermore, stroke patients make significant motor learning improvements with the application of transcranial brain stimulation techniques aiming either to reduce intracortical inhibition of the ipsilesional hemisphere (Hummel et al. 2005; Kim et al. 2006; Talelli et al. 2007) or to increase that of the unaffected hemisphere (Fregni et al. 2005; Fregni et al. 2006). Taken together, evidence for altered LTP/LTD plasticity and implicit motor learning deficits in concussed athletes contrast with their quickly resolving motor symptoms and typically quick return to competition (McCrorry et al. 2005). It is therefore plausible that adaptive compensatory mechanisms allow implicit motor learning in the presence of reduced LTP/LTD-like plasticity, and perhaps to a greater extent in highly trained, elite athletes whose performance levels are constantly relying on their capacity to adapt to rapidly changing sequences of action during play. In addition to changes in different muscle representations related to M1 synaptic plasticity in motor skill acquisition, the mere repetition of stereotyped movements was shown to activate plasticity in the spinal cord function. For instance, depression of H-reflexes in ballet dancers was shown to be closely associated with the acquisition of a motor task and not its performance (Nielsen et al. 1993). Moreover, the implication of other interacting brain plasticity mechanisms—synaptogenesis, growth of new synaptic connections and synapse remodeling, as well as neurogenesis—has recently been related to hippocampal acquisition of new information (Bruel-Jungerman et al. 2007). It therefore appears possible that adaptive changes at spinal levels together with other forms of brain plasticity mechanisms may help lessen the impact of altered bidirectional plasticity in concussed athletes.

Recent data have begun challenging the long-held belief that sports concussions are relatively benign events. For example, epidemiological studies have revealed that a history of 3 or more concussions is associated with a 5-fold increase in the prevalence of mild cognitive impairment, a condition that converts into dementia at an annual rate of 10–20% (Guskiewicz et al. 2005). Motor slowness was also recently found in former athletes who sustained their last concussion more than 3 decades earlier and who experienced quantifiable cognitive decline (De Beaumont et al. 2009). Interestingly, motor dysfunction in these individuals was associated with abnormally elevated levels of M1 intracortical inhibition, much like what is found in young concussed athletes. The presence of known dysfunctions that persist up to 30 years following the last concussive event opens up the possibility that early interventions may reduce the long-term effects of repeated concussions. Indeed, an important question that arises from the present data is whether increased GABAergic neurotransmission contributes to long-term postconcussion symptomatology. To this end, restoring GABAergic neurotransmission to preconcussion levels either locally with brain stimulation (Ziemann and Siebner 2008) or globally through pharmacology may provide significant insights into the pathophysiology of concussion and lead to intervention strategies. Another unresolved issue is whether LTP/LTD-related impairments in implicit motor learning are indicative of more generalized learning impairments that may exacerbate with aging. A better understanding of the sometimes subtle effects of sports concussions on brain function and their interaction with normal aging is needed considering their alarmingly high prevalence (Kelly 1999) and their potentially devastating effects on late-life cognitive/motor functions (Guskiewicz et al. 2005; De Beaumont et al. 2009).

In addition to increased GABAergic neurotransmission, reduced cortical excitability linked to alterations of M1 glutamatergic neurotransmission could conceivably contribute to aberrant synaptic plasticity found in concussed athletes. While these effects have yet to be documented beyond the acute postconcussion phase, M1 glutamate concentration was found to be reduced within 6 days postconcussion (Henry et al. 2010). However, considering that concussed athletes tested more than 9 months postinjury did not differ from unconcussed teammates on glutamate-mediated (Liepert et al. 1997; Ziemann et al. 1998) measures of intracortical facilitation (De Beaumont, Lassonde, et al. 2007), the long-term effects of alterations in M1 glutamatergic neurotransmission on synaptic plasticity would likely be limited. In parallel, one cannot
completely rule out alterations of the corticospinal tract in concussed athletes as a potential mediator of compromised M1 LTP/LTD induction. Indeed, while altered white matter integrity of the corticospinal tract has yet to be evidenced in concussed athletes specifically, decreased fractional anisotropy in the corticospinal tract was recently associated with mTBI (Kraus et al. 2007). mTBI white matter changes were reported to be primarily due to diffuse axonal damage as opposed to functionally disruptive myelin damage (Kraus et al. 2007). Assuming that corticospinal white matter integrity could have been altered after a concussion, this structural damage would, however, exert limited impact on measures of corticospinal tract excitability. Accordingly, recent evidence from our group suggests unaltered afferent and efferent conduction times, as well as normal somatosensory evoked potentials, in comparable asymptomatic, university-level football players (Tremblay et al. 2011). Preserved TMS measures of corticospinal tract excitability in concussed athletes despite potential white matter integrity losses mitigate its potential contribution to synaptic plasticity impairments reported herein. Moreover, there is a general consensus that overt cognitive functions alterations do not persist beyond the first week after the accident (Collins et al. 1999; McCrea et al. 2003; McCrory et al. 2009). Previous findings from our group showing comparable neuropsychological test performance between athletes who sustained their last concussion more than 9 months prior to testing and unconcussed teammates are consistent with this notion (De Beaumont, Brisson, et al. 2007). Similarly, athletes in this study had sustained their last concussion more than 9 months prior to testing and they were no longer experiencing cognitive difficulties, as systematically assessed with neuropsychological testing prior to obtaining medical clearance to return to play. It therefore appears improbable that cognitive function alterations either in episodic memory, attention, or executive functions could explain altered performance at the implicit motor learning task found in this study. PAS protocols conducted in human studies have looked at the duration of LTP/LTD-like plasticity effects and suggest that excitability alterations sometimes occur with a delay (Ziemann et al. 2004). One alternative explanation for aberrant bidirectional plasticity in concussed athletes could therefore be that LTP/LTD-like effects are delayed after PAS. Although not accounting for potential effects of time on PAS-induced plasticity (Ziemann et al. 2004), the present study suggests that at least for LTP/LTD-like effects recorded immediately after PAS, aberrant bidirectional plasticity was significantly linked to implicit motor learning impairment in concussed athletes. Nevertheless, further studies are necessary to address the extent to which cognitive factors and corticospinal tract integrity mediate the reported deficit in motor learning and associated reduction in bidirectional plasticity.

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**References**


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