The Effect of Playing Career on Chronic Neurophysiologic Changes in Retired Male Football Players: An Exploratory Study Using Transcranial Magnetic Stimulation

Original Research

The effect of playing career on chronic neurophysiological changes in retired male football players. An exploratory study using transcranial magnetic stimulation.

Running title: Neurophysiology of neurotrauma exposure

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Abstract

Aim

Repetitive head impact exposure, from contact and collision sports, are increasingly being attributed to increased risk of neurodegenerative disease in aging athletes. This exploratory study investigated the association of playing career in retired professional contact sport athletes with cortical neurophysiology via transcranial magnetic stimulation (TMS).

Methods

This study used a cross-correlation design without a control group. Male athletes between the ages of 28-68 years (n=113; mean age [SD] 48.8 [9.7]) who had been retired from professional sport for a minimum of five years were recruited. Cortical excitability was measured using single pulse TMS for motor evoked potentials and paired pulse for short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI). Associations were assessed between TMS measures and concussion history, clinical symptom scores, total career length (including junior to complete retirement), and professional career length (elite competition only).

Results

Correlations showed significant associations between motor evoked potentials and clinical symptom reporting (*rho*: -0.21 – -0.38; *P*<0.01); and motor evoked potentials and short-interval intracortical inhibition with total career length (*rho*: 0.26 – -0.33; *P*<0.01). No significant correlations were observed between single and paired-pulse transcranial magnetic stimulation and professional career length (*rho*: 0.16 – -0.15), nor the number of concussions (*rho*: 0.17 – -0.17).

Conclusions

This exploratory study is the first to report pathophysiological outcomes in a cohort of retired professional athletes associated with total career exposure, rather than professional career exposure or concussion history. Without a control group comparison and cross-correlational design, these preliminary results should be viewed with caution, however, TMS assessment could be considered a viable biomarker in future studies of retired athletes classified with traumatic encephalopathy syndrome.

Keywords: Ageing athletes, professional football, neurotrauma, transcranial magnetic stimulation, pathophysiology, concussion, traumatic encephalopathy syndrome

Original Article

The effect of playing career on chronic neurophysiological changes in retired male professional football players. An exploratory study using transcranial magnetic stimulation.

Cumulative exposure to repetitive concussion and sub-concussion impacts are increasingly being attributed to abnormal ageing[1], neurological[2], and cognitive impairments[3], and increased risks of neurodegenerative disease[2, 4, 5]. Currently a definitive diagnosis of neurodegenerative disease, particularly chronic traumatic encephalopathy (CTE), can only be confirmed via neuropathology[6, 7]. However, in understanding the progression of CTE, descriptions of the clinical characteristics of CTE include reports of non-specific symptoms, cognitive, behavioural, mood and other psychiatric features, and motor impairments. These characteristics have contributed to the formation of a criteria for traumatic encephalopathy syndrome (TES)[6].

The TES clinical diagnostic criteria, which is currently in progress, acknowledges the need to include biomarkers[6]. While specific biomarkers have not yet been accepted, there is some promise from studies utilising transcranial magnetic stimulation (TMS) [8-10]. First reported in 1985[11, 12], TMS is a well-established technique that can non-invasively assess cortical physiology in health and disease[13]. In abnormal ageing and neurology, TMS has shown to have clinical diagnostic utility[14], including neurodegenerative diseases. Particularly in dementia, the loss of synaptic density and abnormalities in neurotransmission have been detected using TMS in Alzheimer's disease (AD) compared to healthy older adults[15]. Consequently, TMS has been touted as a potential prodromal biomarker for clinical diagnosis of AD or frontotemporal dementia[15-18], and may have potential as part of the overall criteria for TES.

In understanding long term concerns from repetitive neurotrauma, previous TMS studies in retired athletes have specifically focussed on concussion history, for example mean number of concussions, and the time since last reported concussion[8, 9, 19, 20]. These studies showed changes in cortical inhibition compared to age-matched controls but did not consider playing career length exposure. Similarly, a recent TMS study in contact sport athletes showed decreased cortical inhibition verses age-matched controls that worsened with ageing [10]. However, that study did not consider effects of playing career exposure (professional or total exposure) or concussion history.

Emerging evidence from different football codes have increased urgency to investigate the chronic neurophysiological effects of repetitive sub-concussive head impacts[21]. With evolving evidence that cumulative sub-concussive head impact exposure rather than concussion history may have a greater association on long term outcomes[4, 22]. The aim of this exploratory study was to investigate, in a cohort of retired professional contact sport athletes, if changes assessed with TMS were associated with playing history exposure. Using a single-group and cross-correlation design, our question was whether a career exposure of contact sport participation (*total* career length from junior to retirement versus *professional* career span only) was associated with neurophysiological changes as assessed by TMS. Secondary questions included if there were associations between TMS measures and participants' reporting of fatigue and related symptoms affecting daily activities[23, 24], and the number of concussions experienced over their career.

Materials & Methods

Retired male professional Australian football and rugby players (n=113; mean age [SD] 48.8 [9.6] years) were recruited for this study between 2018 to 2023 (see **table 1** for participant characteristics). Participants were excluded if they were retired less than five years or experienced any brain injury outside of contact sports participation (e.g., car or workplace accidents, fights or falls). Study participants reported no neurological, psychiatric, or neurodegenerative disease, sleep disorders (e.g., obstructive sleep apnoea), or current musculoskeletal injury, and provided written informed consent prior to data collection. Study protocols were approved by the University Human Research Ethics Committee (HEC18005).

Participants completed the *fatigue and related symptom scores* questionnaire [23, 24] to quantify ongoing clinical concerns with daily activities. Players responded to 15 questions using a scale from '0' (no concern) to '3' (greatest concern) regarding levels of fatigue and difficulties they may encounter that impact on daily activities. The total score from the questionnaire was used to correlate to TMS parameters.

Motor evoked potentials (MEPs) from single and paired pulse TMS where quantified following stimulation over the contralateral motor cortex[9, 10, 20, 25]. Surface electromyography (sEMG) recorded MEPs from 500 ms sweeps (100 ms pre-trigger, 400 ms post-trigger; PowerLab 4/35, ADInstruments, Australia) using two pre-gelled Ag/AgCl disposable surface electrodes (ADInstruments, Australia), placed over the first dorsal interosseous (FDI) muscle and a third ground electrode over the boney prominence of the fourth digit, of the participant's dominant hand adhering to the Non-Invasive Assessment of Muscles (SENIAM) guidelines for sEMG[26].

TMS was delivered using a R30 stimulator with a C-B 60 coil (MagVenture, Denmark). For identification of the 'optimal site' (defined as the site with the largest observed MEP) on the motor cortex projecting to the FDI muscle, participants wore a snugly fitted cap (EasyCap, Germany), positioned with reference to the nasion-inion and interaural lines. The cap was marked with sites at 1 cm spacing in a latitude-longitude matrix to ensure reliable coil position throughout the testing protocol[27].

Identification of active motor threshold (aMT) was determined, during a low-level isometric contraction of the first index finder (targeting the FDI muscle) against a force transducer (ADInstruments, Australia) at 10% of maximal voluntary contraction (MVC). MVC was completed following a warm-up of graded increases in muscular contractions of the FDI (three contractions at intensities of 20%, 50% and 75% of their perceived MVC). Following the warm-up, participants then conducted two MVC trials of 3 s duration, each separated by 60 s of rest. Continuous verbal encouragement to push against the force transducer as hard as possible was provided. The maximum transducer value was recorded as the MVC. A third trial was completed if the difference between the first two trials exceeded 5%[28].

The aMT was identified by delivering TMS stimuli (5% of stimulator output steps, and in 1% steps closer to threshold) at intensities from a level well below expected participant's threshold until an observable MEP of at 200 μV and associated cortical silent period (cSP) could be measured in at least five of ten stimuli[29].

Participants abducted and controlled a static contraction of the contralateral index finger to target the FDI muscle at 10% maximal voluntary contraction[9, 10, 20, 25, 27, 30] during data collection. Single pulse TMS was applied at 130%, 150% and 170% above aMT in order to

explore stimulus-dependent corticomotor connectivity. Paired-pulse short intracortical inhibition (SICI) at 3 ms interstimulus interval (ISI) was measured using a conditioning stimulus of 80% aMT and a test stimulus of 130% aMT, while long intracortical inhibition (LICI) was completed at 100 ms ISI using a suprathreshold conditioning and test stimulus at 130% aMT. Twenty single pulse stimuli (four sets of five pulses per set) were presented at random intervals, to avoid stimulus anticipation, four to eight seconds apart. For paired pulse (SICI and LICI), 20 stimuli were presented (four sets of five pulses per set) randomly six to 10 seconds apart. Between sets, a rest of 30 seconds was provided to reduce the possibility of muscular fatigue[30]. Examples of MEP waveforms are illustrated in **Figure 1**.

Single-pulse MEPs (**figure 1a**) were measured from the peak-to-peak amplitude of the waveform, and the cSP as the duration of 'silence' in the EMG signal following the MEP waveform onset to the return of uninterrupted EMG activity[31]. It is well accepted that inter-individual variability exists with MEP amplitudes and cSP duration[32]. As reasoned by Škarabot et al[33], the most influencing confounding factor on cSP duration is the intensity of TMS which influences the relationship the MEP has on cSP duration. Consequently, in order to analyse the balance between excitatory and inhibitory mechanisms with TMS and to reduce interparticipant variability, we utilised a ratio measure of calculating the cSP duration/MEP amplitude size (**figure 1a**), first reported by Orth and Rothwell in 2004[32]. While this ratio is useful for intervention studies where post-intervention changes in the MEP or cSP would indicate excitability or inhibitory changes [33], we have previously used this method in cross-sectional studies comparing retired contact-sport athletes, with and without ongoing symptoms, to age-matched controls[9]. Further, to mitigate any influence of motor threshold differences on changes in cSP, we constructed stimulus-response curves at TMS intensities of

130%, 150%, and 170% above aMT [33, 34]. Area under the recruitment curves (AURC, **figure 1b**) calculated MEP:cSP ratios at all stimulus intensities, reflecting the input-output properties of the corticomotor pathway. The AURC was calculated via the method of trapezoidal integration method described by Carson et al[35]. This method involved plotting the MEP amplitude against the stimulus intensity and calculating the area under the resulting curve using the trapezoidal rule. Carson et al[35] have argued the AURC is an extremely reliable measure of the state of corticospinal projections to hand and forearm muscles. In studies these authors reported that the AURC method has face and concurrent validity and demonstrates high reproducibility with intra-class correlation for FDI muscle of 0.96. We have used this method in previous TMS studies of retired athlete cohorts[9, 10].

SICI (**figure 1c**) was calculated as a ratio of the paired-pulse MEP at 3ms to the single-pulse MEP at 130% aMT. LICI (**figure 1d**) was calculated as a ratio of the suprathreshold test stimulus to the conditioning stimulus[36]. As data was found to be non-normally distributed, relationships between variables were explored using Spearman's *rho*. Data is presented as means (±SD) and alpha was set at 0.05, apart from cSP:MEP ratios where alpha was corrected as 0.017 due to comparisons at 130%, 150% and 170% aMT.

<Figure 1 here>

Results

All participants recruited completed TMS testing with no adverse effects. **Tables 1** and **2** show descriptive data and correlations respectively. All participants had reported starting to play their sport at 9.9 ± 2.4 years of age and retiring from their sport at 30.9 ± 4.5 years. All had reported at least one concussion in their career. For self-report fatigue and related symptoms,

72% of retired athletes scores were above the clinical cut-off as suggested by Johansson et al[23, 24].

Significant, but small to moderate, correlations were found between total career length and cSP:MEP ratios, AURC, and SICI (*rho* -0.29 – -0.34, 0.25 respectively; *P*<0.05; **table 2** and **figures 2 and 3**). Similarly, small to moderate significant correlations were observed between symptom scores and cSP:MEP ratios and AURC (*rho* -0.21 – -0.38, *P*<0.05; **table 2**). No significant correlations were reported between the number of concussions recorded, or the length of the individual's professional career on cSP:MEP ratios, SICI or LICI (**table 2** and **figures 2 and 3**). Further no significant correlations were seen between symptoms scores with number of concussions, or career length (total or professional).

<Tables 1 and 2 here>

<Figures 2 and 3 here>.

Discussion

The aim of this exploratory study was to investigate, using a cross-correlational design, in a cohort of ageing retired professional contact sport athletes, the association of TMS neurophysiological outcomes with career exposure. While previous studies have reported changes associated with concussion history in retired contact sport athletes,[8-10] we found differences in intracortical inhibition was significantly correlated with *total* career length and symptom reporting, but not with *professional* career length, or the number of recorded concussions. This is the first study showing findings associated with career experience, supporting recent studies also reporting associations of exposure and risk of CTE[4, 22].

Previous TMS studies have reported alterations in cortical inhibition associated with healthy ageing[10, 37-40] suggesting age-related changes in ɣ-aminobutyric acid (GABA) mediated cortical activity. This study, conversely, showed decreased cortical inhibition in a cohort of contact sport athletes, supporting previous concussion studies[9, 10, 20, 25]. Of note, however, is that TMS data correlated to exposure rather than concussion history. While the correlations are small to moderate, our results from this exploratory study provide further evidence of altered physiology in GABAergic receptor activity in cohorts exposed to repetitive neurotrauma. However, as TMS is an indirect measure, the mechanisms of repetitive sub-concussive trauma affecting GABAergic activity are postulated. In acute trauma studies, it has been shown that physical trauma alters depolarizing actions of GABA contributing to maladaptive signal transmission[41-44]. It has been recently stated that repetitive sub-concussive head impact research using TMS is needed [21]. The data in our TMS study suggests that repetitive and regular sub-concussive impacts experienced by athletes in contact sports over multiple seasons are cumulative, and consequently contribute to maladaptive long-term potentiation (LTP) changes altering pre/post synaptic processing[10, 45]. While we appreciate that the majority of LTP work stems from experimental stimulation studies[45], LTP has also been shown not only in neurological and psychiatric conditions but also with interventional methods such as exercise[46]. It is plausible that our findings reflect, in this cohort, the experiences of individuals who have experienced tens of thousands of sub-concussive impacts[4] over their playing lifetime. Further research extending on this work, using spatio-temporal techniques such as magnetoencephalography to illustrate multi-region functional LTP connectivity, could confirm these initial findings.

Conversely, the finding of no correlations between the number of concussions and TMS data was surprising. Previous studies[8, 9, 19, 20, 25] investigated cortical physiology changes in light of concussion history only. While a majority of these studies found differences between athletes and age-matched controls[8, 9, 20, 25], what the data from our study suggests is that previous findings could have been ascribed to career exposure rather than concussion history. Future studies investigating long term outcomes in these cohorts should record career history that includes when participant's started playing contact sports, if they played at professional or semi-professional levels and had access to full-time medical support, and when they completely retired from their sport further to their concussion history.

A secondary finding was the significant correlation of higher symptom scores with decreased cortical inhibition. Although self-reported in nature , high reliability of the scale (Chronbach's alpha of 0.94[23]) gives us confidence that participants did not exaggerate their responses, as we would not have seen a significant correlation of symptom scores to TMS data. Moreover, our data concurs with previous studies showing differences in TMS excitability and inhibition between groups (described as 'symptomatic' and 'asymptomatic')[9] separated by the cut-off score as recommended by Johansson and colleagues[23, 24].

TMS neurophysiological measures are currently being actively studied as clinical biomarkers for a variety of neurologic and psychiatric conditions. Cortical excitability and inhibition from TMS has been suggested as an objective biomarker and clinical neurophysiological signature to support clinical diagnoses[47]. While exact mechanisms are needed to be elucidated, changes in excitability or inhibition suggest alterations in cholinergic activity affecting cognition and/or motor outputs. Being an exploratory study, we did not take cognitive or motor data. However previous TMS studies in similar cohorts (retired contact sport athletes) have shown slowing in

response times in cognitive[48], and bradykinesia in motor tasks[8] with TMS showing alterations in cortical inhibition. Single- and paired-pulse TMS protocols have recently been able to show differences between AD and frontotemporal dementia (FTD) illustrating the clinical utility of TMS as a non-invasive biomarker in cognitive neurology[14, 18, 47]. However, further study is required utilizing TMS in those with suspected CTE, using the current TES research criteria[6], to discriminate from other neurodegenerative diseases.

Several limitations of this exploratory study include the cross-correlational and non-control design. Given emerging evidence of exposure risk our aim was to undertake a preliminary investigation if there were any associations with single- and paired-pulse TMS on concussion and career exposure history. While we found associations in exposure history, we caution against specific clinical translation of our data due to moderate correlations presented and encourage further studies to investigate career exposure on TMS neurophysiological measures.

A further limitation is acknowledging the limitations of self-report of concussion history, but aimed to only record concussions where the player recalled being assessed by the club doctor and missed the following week of competition[25]. This may have contributed to our findings of no correlations between concussion history and TMS data insofar that it was not possible to determine the quality and quantity of concussion associated problems, such as the number and severity of symptoms. Similarly, in terms of little data for this cohort on recovery times was not available. Indeed, it was not possible to account for medical care and/or athlete behaviour (on- or off-field with recovery and regeneration) differences in players during professional vs. non-professional periods of their career. Similarly, we were not able to account for post-career lifestyle risk factors and current physical activity patterns that may

contribute to our findings presented here. Using a cross-correlation study design, we recognize the difficulty in controlling for extraneous factors given the relationship of post-career lifestyle, frequently seen in retired players of contact or collision sport, that may impact on long-term pathophysiology[49]. Future studies aim to incorporate clinical assessments to account for potential risk factors that may confound TMS data.

A final limitation of this study is that TMS with sEMG is a measure of corticomotor physiology. Although cSP, SICI, and LICI evoked potentials represents intracortical networks, we caution on generalizing these findings to non-motor regions. Further, our SICI and LICI protocols were limited to only 3 ms and 100 ms ISIs respectively. Future studies should aim to include paired pulse TMS with electroencephalography, previously reported in those with persistent post-concussion symptoms[37], and a panel of ISIs for SICI and LICI, as well as utilizing intracortical facilitation, which has been undertaken in recent dementia studies[16, 18].

In conclusion our findings, demonstrating differences in cortical physiology associated with total career exposure and symptoms scales, compared to professional career exposure or the number of concussions, suggest that TMS evoked potentials would be a suitable prodromal biomarker to complement clinical assessment and neuropsychological components in developing diagnostic criteria for TES. We call to progress this research further, so that objective measures can assist diagnostics for those with a history of neurotrauma suspected of CTE.

Data availability

The data generated during and/or analysed during the current study are not publicly available due to potential ability to identify professional athletes based on exposure data. However, these data are available from the corresponding author upon reasonable request and with relevant University Human Research Ethics approval.

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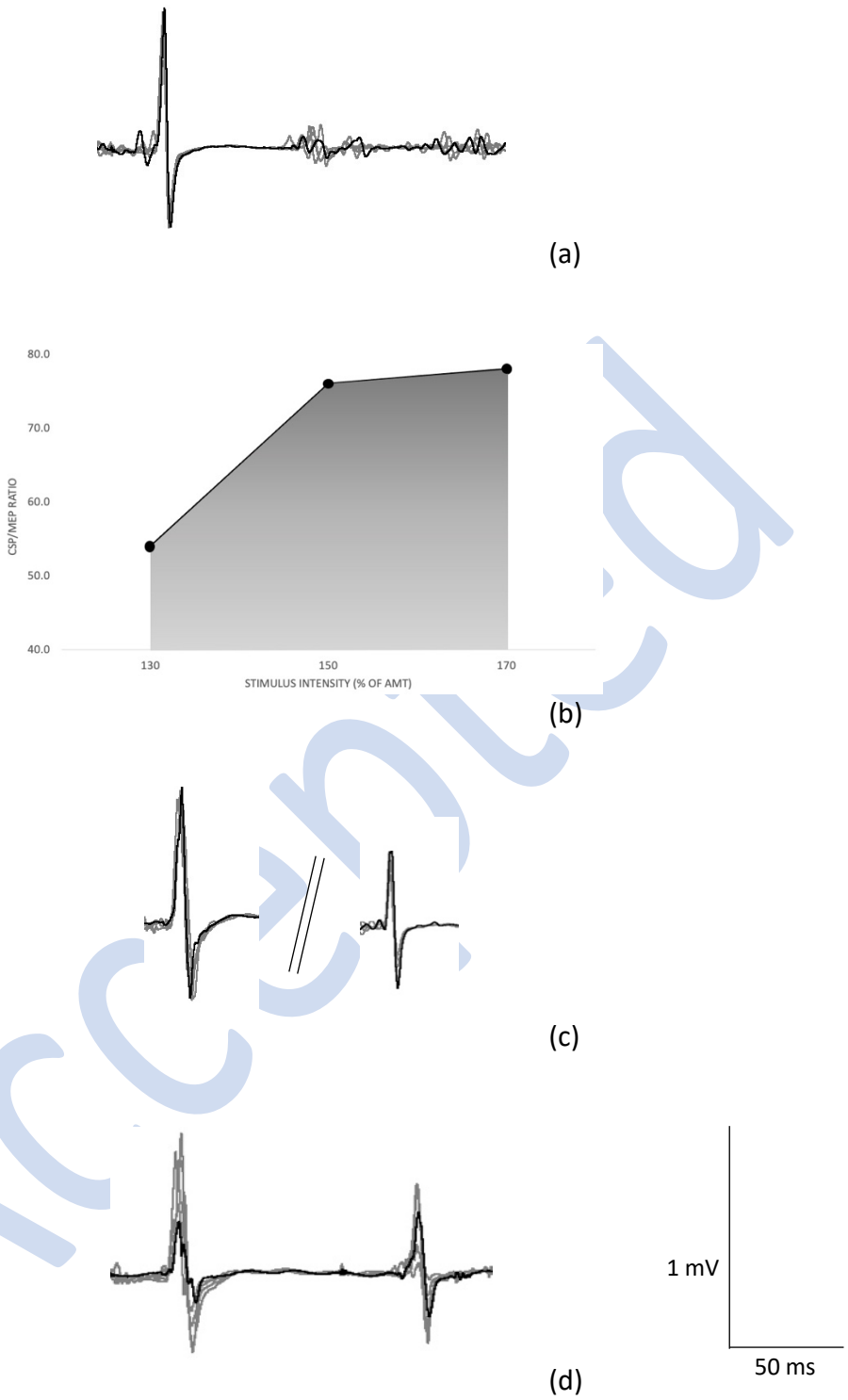

Figure 1. Representative data for dependent variables. **(a)** single pulse motor evoked potential (MEP) waveform and subsequent cortical silent period (cSP), expressed as a ratio of cSP duration value/MEP amplitude value; **(b)** Area Under the Recruitment Curve (AURC) calculated using the method described by Carson et al (35), and expressed as arbitrary units (AUs); **(c)** short interval intracortical inhibition (SICI) where the inhibited paired-pulse MEP (3ms interval) waveform (right) is calculated as a ratio of the single pulse MEP waveform (left); and **(d)** long-interval intracortical inhibition (LICI) where the two pulses, conditioning (left waveform) and test stimulus (right waveform) MEP are delivered (100 ms interval) and calculated as a ratio of the test/conditioning amplitudes.

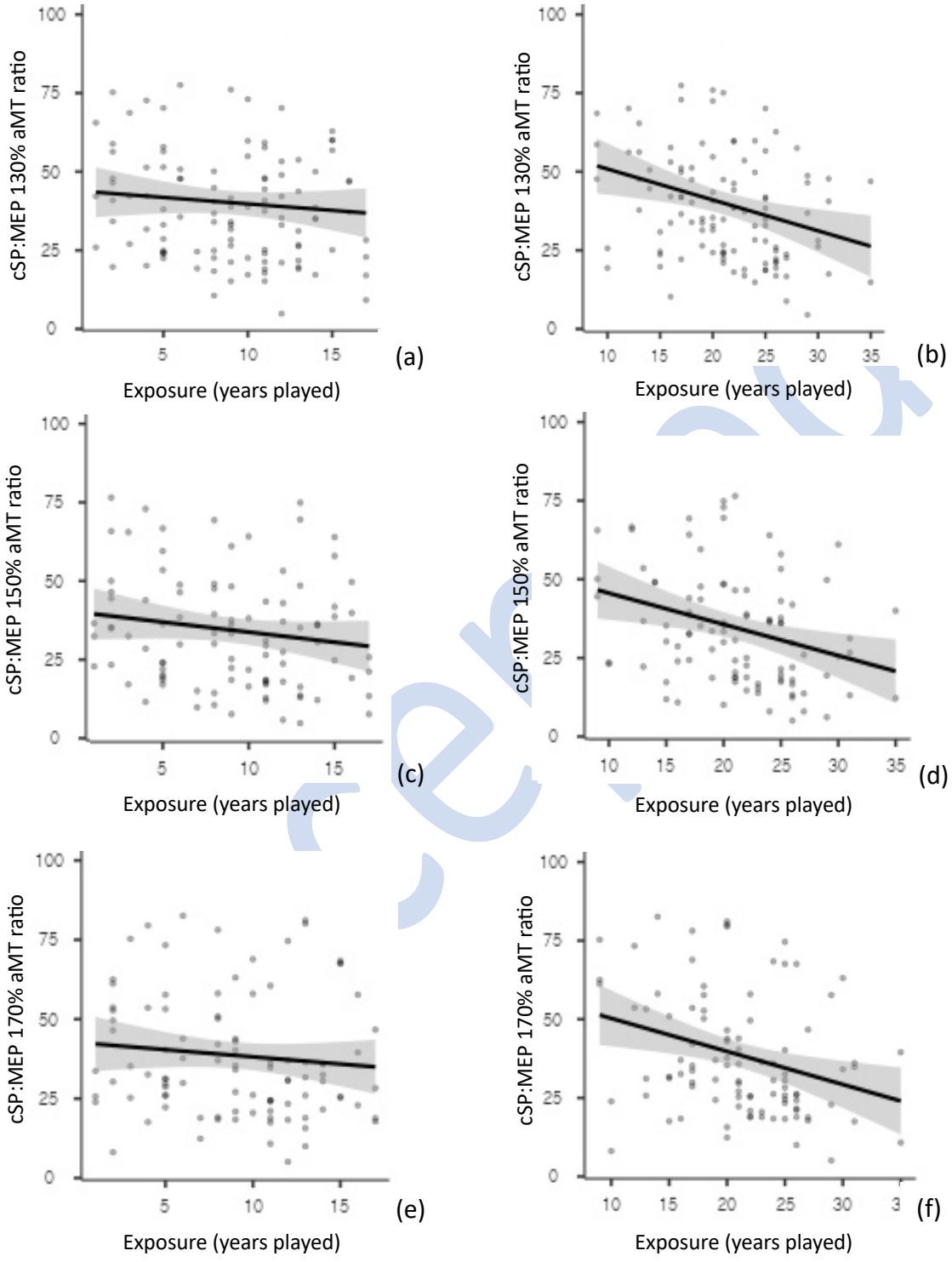

Figure 2. Scatterplots for single-pulse cSP:MEP ratios at 130%, 150%, and 170% above active motor threshold (aMT) for professional career time (a, c, e) and total career time (b, d, f).

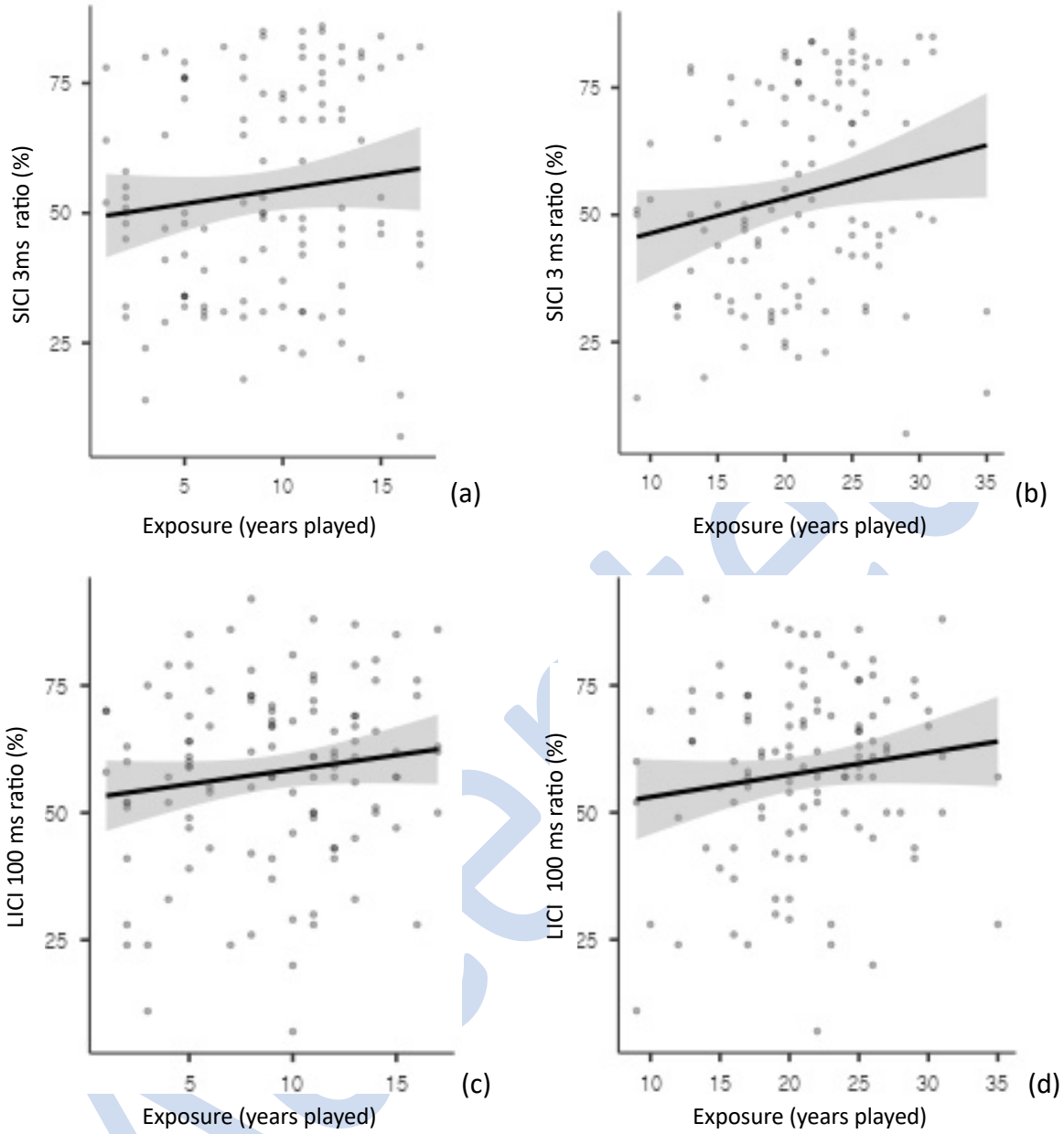

Figure 3. Scatterplots for paired-pulse SICI and LICI ratios for professional career time (a, c) and total career time (b, d).

Table 1. Participant characteristics (n=113, all male)

Characteristic	Mean [SD]	Range [min-max]	Median [IQR]
Age (Years)	48.8 [9.7]	28 – 68	50.0 [42 – 55]
Total career length (Years)	21.0 [5.7]	9 – 35	21.0 [17 – 25]
Professional career length (Years)	9.0 [4.3]	5 – 17	9.0 [5 – 12]
Number of concussions diagnosed	7.5 [5.5]	1 – 30	7.0 [3 – 10]
Last concussion recorded (years)	19.3 [10.0]	5 – 35	19.0 [11 – 25]
Fatigue and related symptom score (max score 44)	18.2 [7.0]	2 – 37	18.5 [14.5 – 24]
aMT (% stimulator output)	35.7 [7.5]	23 – 65	35.0 [30 – 40]
cSP:MEP ratio 130%aMT	0.44 [0.21]	0.07 – 1.19	0.41 [0.27 – 0.55]
cSP:MEP ratio 150%aMT	0.41 [0.20]	0.10 – 1.32	0.39 [0.24 – 0.51]
cSP:MEP ratio 170%aMT	0.39 [0.21]	0.51 – 1.20	0.33 [0.24 – 0.51]
cSP:MEP AURC (AU)	2043 [1138]	429.00 – 5559.00	1812 [1183 – 2637]
SICI ratio	0.55 [0.20]	0.14 – 0.86	0.51 [0.37 – 0.75]
LICI ratio	0.58 [0.18]	0.07 – 0.92	0.61 [0.49 – 0.70]

(IQR: interquartile range; MEP: motor evoked potential; cSP: cortical silent period; aMT: active motor threshold; AURC: area under the recruitment curve; AU: arbitrary units; SICI: short intracortical interval; LICI: long intracortical interval).

Table 2. Correlations (rho) between TMS variables and concussions recorded, fatigue and related symptom scores, and career length (total and professional).

	Concussions recorded	FRSS	Total career length (years)	Professional career length (years)
aMT (% stimulator output)	-0.071	-0.048	0.104	-0.033
	P=0.453	P=0.614	P=0.271	P=0.731
MEP:cSP ratio 130%aMT	-0.174	-0.319	-0.321	-0.106
	P=0.066	P<0.001	P<0.001	P=0.265
MEP:cSP ratio 150%aMT	-0.166	-0.377	-0.331	-0.152
	P=0.100	P<0.001	P<0.001	P=0.134
MEP:cSP ratio 170%aMT	-0.157	-0.274	-0.331	-0.148
	P=0.118	P=0.006	P<0.001	P=0.142
MEP:cSP AURC (AU)	-0.012	-0.213	-0.290	-0.138
	P=0.898	P=0.024	P=0.002	P=0.145
SICI ratio	0.158	0.144	0.263	0.164
	P=0.095	P=0.129	P=0.005	P=0.076
LICI ratio	0.176	0.168	0.112	0.096
	P=0.062	P=0.075	P=0.238	P=0.314

(FRSS: fatigue and related symptom score; MEP: motor evoked potential; cSP: cortical silent period; aMT: active motor threshold; AURC: area under the recruitment curve; SICI: short intracortical interval; LICI: long intracortical interval).