Abnormal Corticospinal Excitability in Traumatic Diffuse Axonal Brain Injury

Montse Bernabeu,^{1*} Asli Demirtas-Tatlidede,^{2*} Eloy Opisso,¹ Raquel Lopez,¹
Jose M^a Tormos,¹ and Alvaro Pascual-Leone²

Abstract

This study aimed to investigate the cortical motor excitability characteristics in diffuse axonal injury (DAI) due to severe traumatic brain injury (TBI). A variety of excitatory and inhibitory transcranial magnetic stimulation (TMS) paradigms were applied to primary motor cortices of 17 patients and 11 healthy controls. The parameters of testing included resting motor threshold (MT), motor evoked potential (MEP) area under the curve, input-output curves, MEP variability, and silent period (SP) duration. The patient group overall revealed a higher MT, smaller MEP areas, and narrower recruitment curves compared to normal controls ($p < 0.05$). The alterations in excitability were more pronounced with an increase in DAI severity ($p < 0.005$) and the presence of motor impairment ($p < 0.05$), while co-existence of focal lesions did not affect the degree of MEP changes. MEP variability was significantly lower in the group with motor impairment only ($p < 0.05$). The intracortical inhibition, as revealed by SP duration, did not exhibit any significant differences in any of the patient groups. In conclusion, our findings expand the concept that impairment of the excitatory and inhibitory phenomena in the motor cortex does not proceed in parallel and demonstrate distinct patterns of aberrations in TBI. Furthermore, these data suggest that alterations in the corticospinal excitatory mechanisms are determined predominantly by the severity of DAI, and show a significant relationship with clinical motor dysfunction following severe trauma diffusely affecting the motor cortical connections. In severe TBI, motor and functional recovery might be linked to restitution of normal corticospinal mechanisms, indexed by normalization of the cortical excitability parameters.

Key words: corticospinal excitability; diffuse axonal injury; transcranial magnetic stimulation; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a common cause of acquired neurological insult secondary to physical trauma to the brain, most frequently due to traffic accidents, falls, violence, and sports injuries (Maas et al., 2008; Butcher et al., 2007). It primarily affects the young population (Sorenson and Kraus, 1991), and has enormous personal and social consequences (Mills et al., 1992). Disabilities resulting from TBI correlate with the severity of injury, and loss of motor function is one of the most devastating of a number of serious cognitive, behavioral, and sensorimotor impairments (Willemse-van Son et al., 2007). The number of victims of TBI continues to increase each year, and it has been predicted that TBI will become the third leading cause of death and disability in the world by the year 2020 (Murray and Lopez, 1997). Therefore

further research addressing the underlying pathophysiological mechanisms is imperative to guide development of better rehabilitation strategies for TBI.

Diffuse axonal injury (DAI) occurs due to abrupt angular acceleration or deceleration motions of the head, which frequently leads to stretching and widespread disruption of axonal fibers and tissue-tear hemorrhages, and results in a biochemical cascade of toxic substances (Gennarelli et al., 1998). This form of injury is generally present in severe TBI, and causes generalized degeneration of the white matter, including major intra-hemispheric and commissural white matter tracts (Adams et al., 1982). The severity of the damage from DAI is critical for determining the degree of motor functional impairment, and is the main component affecting the level of the decline in motor functional outcomes seen after severe TBI (Katz et al., 2004).

¹Guttmann University Institute for Neurorehabilitation-UAB, Badalona, Spain, and ²Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center, Boston, Massachusetts.

*These authors contributed equally to this work.

In the last two decades, transcranial magnetic stimulation (TMS) has been widely used for noninvasive electrophysiological evaluation of the human brain, and provides insights into the excitability and the functional integrity of the corticospinal system in a number of neurological disorders and brain pathologies (Kobayashi and Pascual-Leone, 2003). Excitatory and inhibitory phenomena, as revealed by the motor threshold (MT) measurements, motor evoked potential (MEP) characteristics, and cortical silent period (SP) durations have been frequently used to explore changes in motor cortical excitability in several conditions, including mild to moderate TBI (Chistyakov et al., 1998, 1999, 2001; De Beaumont et al., 2007). Prior studies focusing on minor to moderately injured TBI patients (Chistyakov et al., 1998, 1999, 2001) have mostly detected changes in motor cortical excitability during the second week post-trauma, with a trend to return to normal levels after 3 months of follow up. The normalization of changes in MT and MEP parameters was significantly related to the clinical recovery (Chistyakov et al., 1998). With regard to cortical inhibition, de Beaumont and colleagues have recently reported that repeated mild TBIs permanently alter intracortical inhibitory mechanisms as assessed by TMS-induced SP. Of interest, the abnormalities were positively correlated with the severity of concussions (De Beaumont et al., 2007).

Current data identifying changes in the cortical excitability due to DAI, on the other hand, are sparse, and the extent of motor cortical reorganization present after severe TBI remains largely unknown. Two studies in which evaluations were performed on chronic DAI patients with clinically normal motor function did not find significant differences in MT (Fujiki et al., 2006) or MEP amplitude (Jang et al., 2005). To date, the only study that assessed corticospinal function in severe TBI included post-comatose patients with brain injury due to anoxia or trauma, and reported significant differences in MT levels in the group persistently unresponsive to simple verbal commands and multimodality sensory stimulation (Moosavi et al., 1999).

The understanding of functionally relevant adaptive changes and aberrant neurophysiological mechanisms following cerebral injury constitutes a key step toward improving outcome prediction and promoting optimal motor functional recovery in individuals with TBI. In this study, we used various single-pulse TMS measures in an attempt to evaluate the integrity and excitability of the excitatory and inhibitory cortical motor phenomena in DAI due to severe TBI. We predicted that motor cortical reorganization might reveal changes as a function of motor impairment and the severity of axonal injury following severe TBI. In order to test the excitation in the motor cortex, we studied (1) MT, as a measure of membrane excitability and anatomical features related to corticospinal tract function (Reid et al., 2002); (2) MEP area under the curve, which offers information regarding the excitability of the motor cortex, conduction abnormalities along the corticospinal pathway, and the consistency of conduction velocities of the involved axonal fibers (Kiers et al., 1995; Weber, 1997); (3) MEP variability, reflecting intrinsic oscillations and fluctuations in the excitability of the motor cortex and the role of mid-threshold neurons (Steriade et al., 1990; Kiers et al., 1993); (4) input-output curves, indicating the strength and integrity of the corticospinal pathways and MEP area as a function of stimulus intensity (Abbruzzese

and Trompetto, 2002); and (5) SP was studied for assessment of motor cortical inhibition. The silent period is defined as the interruption in the background EMG activity during a voluntary contraction in response to a single-pulse TMS, and its later cortical portion is considered to depend on the long-lasting intracortical inhibitory mechanisms of the motor cortex (Roick et al., 1993).

Methods

Subjects

Seventeen patients (17 males; mean age (SD): 25.8 years (5.37), range: 20–41 years) with severe TBI were included in this study conducted at the Guttman University Institute for Neurorehabilitation between 2002 and 2006. Severe TBI was defined in accordance with a common classification system and included a Glasgow Coma Scale score (GCS) of ≤ 8 on admission, loss of consciousness for >24 h, and post-traumatic amnesia of >1 day (Rao and Lyketsos, 2000). Patients with severe TBI meeting the following criteria were enrolled: (1) age between 18 and 50 years, (2) ability to understand commands, (3) a minimum of 6 months post-TBI, (4) having completed the post-traumatic amnesia (PTA) phase, and (5) the presence of DAI on neuroimaging. Patients with contraindications for MRI or TMS (Wassermann, 1998), including previous history of other head trauma, diagnosis of post-traumatic epilepsy, peripheral nerve injury, unstable medical condition (prior to or following TBI), and a history of alcohol or drug abuse in the prior 3 years were excluded.

Patients were classified separately according to neuro-radiological and clinical findings. All patients underwent complete neurological examinations and their Medical Research Council (MRC) scores were recorded. According to the loss of motor function in the corresponding upper extremity, each hemisphere was evaluated individually and two subgroups were formed: (1) paretic ($n=20$ hemispheres) and (2) non-paretic ($n=14$ hemispheres). On the basis of radiological findings on CT scan or MRI, initially four subgroups were differentiated: (1) DAI only ($n=20$ hemispheres), (2) combined (DAI + focal lesions) ($n=14$ hemispheres), (3) severe DAI (sDAI) ($n=22$ hemispheres), and (4) mild and moderate DAI (mDAI) ($n=12$ hemispheres). Comparisons between the combined and DAI-only groups did not reach significance for any of the selected parameters ($p > 0.5$); consequently these two groups were merged and the patients were analyzed according to the degree of DAI severity. DAI classification was made according to widely accepted criteria (Adams et al., 1989), and included three stages: involvement of subcortical white matter from the parasagittal regions of the frontal lobes, the periventricular temporal lobes, and less likely the parietal and occipital lobes and the internal and external capsules (stage I: mild); involvement of the corpus callosum in addition to the white-matter areas of stage I (stage II: moderate); and rostral brainstem involvement in addition to the areas associated with stage II (stage III: severe). The mean (SD) DAI severity in our population was 2.41 (0.87) according to this classification.

Patient characteristics are summarized in Table 1. Motor vehicle accidents (car or motorcycle) were the cause of the injury in all cases. For the overall group, mean GCS at admission was 4.8, and the mean duration after TBI was 19.7 months. Only four patients had a PTA period under 12 weeks

TABLE 1. PATIENT DEMOGRAPHIC DATA

Patient	Duration after TBI (months)	PTA period (days)	GCS	DRS	MRC (R/L)	Focal lesion location	DAI type	M
1	12	149	4	3	5/4	N	III	V
2	42	102	8	4	5/5	B (frontobasal)	I	N
3	14	165	6	8	4/3	B (frontotemporal)	III	O
4	23	215	4	4	2/5	N	III	V, F
5	6	120	6	4	3/4	N	III	V, F
6	17	197	5	9	4/3	N	III	N
7	7	94	3	2	4/5	U (temporal)	III	T
8	6	28	6	1	5/5	U (frontobasal)	I	N
9	7	10	7	4	5/4	U (temporal)	I	N
10	16	175	6	7	3/4	U (frontal)	II	N
11	22	183	4	5	4/5	U (thalamic)	II	N
12	10	62	4	1	5/5	U (frontal)	I	P
13	11	70	3	7	3+/3	U (frontoparietal)	III	O
14	18	122	6	1	5/4	B (frontobasal)	III	O
15	74	135	3	4	5/1	U (parietal)	III	N
16	32	123	3	11	4/3–	N	III	V, F
17	39	174	4	9	1/5	N	III	VN

TBI, traumatic brain injury; PTA, post-traumatic amnesia; GCS, Glasgow Coma Scale score; DRS, disability rating scale; N, none; B, bilateral; U, unilateral; DAI, diffuse axonal injury; MRC, Medical Research Council score; R, right; L, left; M, medications; V, valproate; O, Other; F, fluoxetine; T, trazodone; P, phenytoin; VN, venlafaxine.

(mean PTA period: 124.3 days), which indicates very severe cognitive and behavioral impairments. In order to provide a thorough characterization of the population, a neuropsychological evaluation particularly emphasizing the most commonly affected domains in TBI was performed and included the following: (1) immediate attention and verbal working memory (Digits-Forward and Digits-Backward), (2) verbal memory (Test Barcelona), and (3) attention and executive function domains (Trail Making Tests A and B, Verbal Fluency, Sustained Attention, Stroop, and Wisconsin Card Sorting Tests) (Lezak, 1995; Peña-Casanova et al., 1997). Consistent with their injuries, 72% displayed attentional problems, 88% had prominent encoding and retrieval memory impairment, and 90% showed dysexecutive syndrome characterized by impairments in planning, organizing, abstract reasoning, and problem solving.

Medications taken at the time of testing are specified in Table 1. Importantly, four patients with severe DAI were under valproate treatment during the time of testing. To clarify possible drug-induced effects on cortical excitability, valproate ($n=8$ hemispheres) and no-valproate groups ($n=15$ hemispheres) with severe DAI were compared and no significant differences favoring the effect of valproate were found in any of the measures ($p>0.3$).

The control group consisted of 11 male subjects (mean age (SD): 37.9 years (11.1); range: 23–58 years) with normal neurological examinations and no history of neurological disease or trauma to the head. The study was approved by the local institutional review board. Written informed consent was obtained from all participants before being enrolled in the study.

EMG recordings and transcranial magnetic stimulation

Electromyography (EMG) was recorded from the first dorsal interosseus (FDI) muscle using pairs of standard Ag/AgCl electrodes. The patients were instructed to keep their hands relaxed, and the EMG activity in the target muscle

was monitored to confirm complete muscle relaxation. EMG recording continued for 500 msec following each TMS stimulus. EMG signals were amplified using a conventional electromyography machine (Dantec Neuromatic 2000; Dantec, Skovlund, Denmark) with a band pass of >2 Hz and <10 kHz. The signals were digitized using a CED 1401 plus interface (Cambridge Electronic Design Ltd., Cambridge, England) and stored on a PC using Spike2 software for offline analysis.

TMS was administered via a commercially available figure-of-8 coil using a Magstim Super Rapid Stimulator with a maximum stimulator output of 2 Tesla (Magstim Company, Dyfed, UK). MEPs could not be evoked in single hemispheres of two patients with paresis, even at the maximum output intensity of the machine; therefore the overall number of hemispheres tested was 32. In another patient SP measurement could not be tested due to the patient's inability to maintain constant contraction.

Resting motor threshold (RMT). Resting motor threshold (RMT) was determined for the FDI muscle and was defined as the minimum TMS intensity (expressed as a percentage of maximum stimulator output) capable of eliciting five MEPs of at least $50 \mu V$ amplitude in 10 consecutive trials (Rossini et al., 1999). The coil was held 45° tangential to the scalp with the handle pointing back. The center of the coil was moved until the position that produced the largest MEP response on FDI was located, and this position was used throughout the experiment. Stimulation was performed at rest at all times.

MEP parameters. In this study, evaluation of MEP parameters comprised (1) MEP area under the curve, (2) MEP variability, and (3) input-output curves. Mean MEP area under the curve was estimated for each hemisphere at 120% RMT stimulation intensity. The results of five consecutive single stimuli delivered at 10-sec interstimulus intervals (ISIs) were averaged. MEP variability was estimated using coefficient of variation ($CV = SD/\text{mean}$) calculations. For this evaluation, a

total of 60 pulses (ISI 2 sec) were delivered consecutively at 110% RMT. Ultimately, individual input-output curves were assessed for each hemisphere. Single TMS pulses were applied at 80%, 100%, 120%, and 140% of RMT, and five responses were recorded for all stimulus intensities. MEP areas under the curve were measured and averaged to characterize the value for each stimulus intensity.

Cortical silent period (SP). The silent period was defined as the pause in the EMG until the recommencement of baseline activity. Ten consecutive single stimuli (ISI 10 sec) were applied to the contralateral motor cortex during steady isometric contraction of the FDI at approximately 10% of maximum muscle strength. Stimulations were performed at 110% of the RMT intensity.

Data analysis

EMG recordings were measured and analyzed off-line by two blinded investigators with a PC using Spike 2 software. Statistical analysis was carried out by a staff statistician using SPSS v. 15.0 (SPSS Inc., Chicago, IL). Due to the small sample size, nonparametric tests were employed for statistical inference in order to be more conservative and decrease outlier confounds. The groups were initially compared using nonparametric multiple comparison tests (Kruskal-Wallis), and the p -value was adjusted using the Bonferroni method. The Mann-Whitney U test was then performed for significant comparisons. Statistical significance was set at $p < 0.05$.

Results

Overall, TMS was tolerated well with only minor side effects, including a mild transient headache in one patient and

neck pain in another. The degree of DAI severity was significantly related to the clinical motor outcome as indexed by the MRC scores ($p < 0.05$).

Resting motor thresholds

MTs were significantly higher in the patient group ($p < 0.01$) than in the healthy controls, and showed more pronounced changes in the paretic group ($p < 0.001$) and the sDAI group ($p < 0.0001$). When compared according to the severity of DAI, the mean MT for the sDAI group was significantly higher than that of the mDAI group ($p < 0.01$). Other between-group comparisons for MT did not reach statistical significance ($p > 0.05$) (Fig. 1 and Table 2).

MEP area

Mean MEP area under the curve was significantly less in the patient group compared to controls ($p < 0.05$). When analyzed in subgroups, the sDAI ($p < 0.005$) and paretic ($p < 0.05$) groups showed significant differences compared with controls. The between-group comparisons were significant between the sDAI and mDAI groups ($p < 0.05$), while the other groups did not exhibit significant differences ($p > 0.05$).

MEP variability

MEP variability was not significantly different in the overall patient group compared with controls ($p > 0.05$) (Fig. 2). Among the subgroups, only the paretic group showed less variability ($p < 0.05$), and the variability was significantly different than that of the non-paretic group ($p < 0.05$). Other group comparisons did not reveal significant differences ($p > 0.05$).

FIG. 1. Mean (A) resting motor threshold values presented in percentages of the maximum stimulator output. (B) MEP area under the curve. (C) MEP variability expressed as coefficient of variation. (D) Cortical silent period duration for all groups (control group, patient group, paretic group, non-paretic group, severe DAI group, and mild and moderate DAI group) (* $p < 0.05$).

TABLE 2. MEAN (SE) CORTICAL EXCITABILITY PARAMETERS GROUPED ACCORDING TO THE CLINICAL OUTCOMES AND DEGREE OF DAI SEVERITY

	Control	Patient	Paretic	Non-paretic	sDAI	mDAI
Motor threshold (%)	57.95 (1.46)	66.91 (2.2)*	69.95 (3)*	62.79 (3)	72.29 (2.7)*	57.50 (2.1)
MEP area (mV.ms)	15.86 (2.4)	10.41 (1.7)*	9.20 (2.3)*	11.79 (2.9)	7.95 (2)*	14.11 (3.1)
MEP variability	0.55 (0.05)	0.46 (0.04)	0.38 (0.04)*	0.56 (0.07)	0.4 (0.05)	0.57 (0.07)
Input-output curves (mV.msec)						
80%	5.42 (0.98)	3.92 (0.5)	3.38 (0.5)	4.57 (1)	2.65 (0.3)*	5.94 (1.0)
100%	8.53 (1.2)	6.39 (1.1)	6.19 (1.7)	6.65 (1.05)	4.72 (1)*	9.18 (2.2)
120%	15.86 (2.4)	10.41 (1.7)*	9.20 (2.3)*	11.79 (2.9)	7.95 (2)*	14.11 (3.1)
140%	36.7 (3.5)	27.54 (4.9)	24.55 (6.7)	30.83 (7.3)	23.83 (8.1)	30.33 (6.1)
Silent period (s)	0.0923 (0.0079)	0.1049 (0.0087)	0.1037 (0.0085)	0.1063 (0.0162)	0.1022 (0.008)	0.110 (0.018)

sDAI, severe DAI, mDAI, mild and moderate DAI.

* $p < 0.05$.

Input-output curves

Recruitment curves followed the expected pattern in all groups, exhibiting a gradual enlargement in MEP areas with increasing stimulation intensity. The curves were clearly broader in controls compared to the patient group (Fig. 3). Comparisons showed significant differences between sDAI patients and controls ($p < 0.005$), and sDAI and mDAI patients ($p < 0.005$) for 80%, 100%, and 120% of RMT intensities. For the overall patient and paretic groups the dif-

ferences were only significant for 120% RMT intensity ($p < 0.05$), while other comparisons failed to reach significance. At 140% RMT intensity comparisons were not significant for any of the groups. However, it is important to note that the number of stimulated hemispheres was much lower for the patients than the controls for 140% RMT ($N_{\text{controls}} = 38$, $N_{\text{patients}} = 23$). This was due to the higher RMT of the patients, which often prevented reaching the required stimulation intensity before reaching the maximum output of the machine.

FIG. 2. Superimposed MEPs demonstrate distinct patterns of variability in motor responses: (A) control, (B) mild DAI without paresis, (C) severe DAI without paresis, and (D) severe DAI with paresis.

FIG. 3. Input-output curves for patient subgroups according to the degree of paresis (above), and according to the severity of DAI (below), in comparison with the control group. The mean MEP areas under the curve for increasing TMS intensities are expressed as a percentage of the RMT (* $p < 0.05$).

Silent period

SP durations were slightly longer in patients compared to healthy controls; the between-group comparisons, however, were not significant ($p > 0.7$).

Discussion

In the present study, various measures of corticospinal excitability showed significant differences between patients with severe TBI and controls, while the SP duration, thought to primarily reflect intracortical inhibition, was not significantly altered. These results suggest that DAI following severe TBI differentially affects inhibitory and excitatory mechanisms in the motor cortex. In addition, our findings provide evidence that alterations in corticospinal excitability reveal a significant relationship with clinically demonstrable motor impairment in chronic DAI.

Results from this work also suggest that corticospinal output is primarily affected by the severity of DAI, with no

apparent relation to focal lesions. Indeed, in contrast to the typical scenario seen in stroke, injury to sensorimotor pathways is generally deemed to result from bilateral widespread foci of axonal injury in DAI (Katz et al., 2004). Subsequent to injury, lost synaptic spaces responsible for diffuse injury to the motor pathways are reoccupied by collateral sprouting of the intact adjacent axons, enabling proper synaptic reorganization and usually good recovery (Povlishock and Katz, 2005; Steward, 1989). However, following severe damage, lesions tend to be more condensed and deep, affecting not only the motor fibers, but also related neural networks (Blumbergs et al., 1989). In such instances the injury is more severe and the clinical outcome is naturally worse; this study also demonstrated a significant relationship between severe DAI and motor dysfunction. Here, in line with our assumptions, neurophysiology demonstrated no significant cortical excitability changes in patients with mild to moderate DAI, and provided further evidence confirming good recovery. On the contrary, MEP parameters revealed significant abnor-

malities in the group with severe DAI. These findings suggest that the severity of DAI plays a key role in the aberrations of cortical excitability, in addition to the clinical motor outcome.

This study represents the first detailed evaluation of several excitatory and inhibitory phenomena in a group of patients, all of whom had DAI due to severe TBI. The detected neurophysiological changes in the MEP parameters most likely resulted from desynchronized repetitive firing of multiple descending volleys, or less effective temporal summation of excitatory post-synaptic potentials, as these components seem particularly sensitive to cortical or axonal injuries (Chistyakov et al., 2001). Moreover, the integrity of corticospinal fibers is clearly a major determinant of the characteristics of TMS-induced MEPs. MEP measurements in patients with recovered motor function showed non-significant alterations compared to controls. This presumably reveals that the integrity of corticospinal fibers is preserved in patients with no lasting motor deficits, and that the heterogeneity is attributable to the recovered axons (Jang et al., 2005). Given these findings, one might speculate that acute TBI leads to disruption of cortical excitability mechanisms, while recovery of motor deficits is associated with their normalization, leading to restoration of motor cortical excitability and corticospinal efferents. It is thus possible that evaluation with TMS early after TBI might allow one to predict which patients will and which will not recover motor function.

While it has been proposed that the SP reflects an interruption of the cortical drive by activation of descending inhibitory volleys or GABAergic and dopaminergic cortical inhibitory mechanisms (Hallett, 1995), its exact physiology remains to be elucidated. Tiagabine, a cellular GABA reuptake inhibitor that activates both GABA_A and GABA_B receptors, increases the SP duration (Werhahn et al., 1999), while the effects of the relatively selective GABA_B-agonist drug baclofen have led to contradictory results (Siebner et al., 1998; McDonnell et al., 2006). In any case, basic neurophysiology studies indicate that GABA receptors mutually influence each other, and GABA_B-inhibitory post-synaptic potentials are compromised by the concomitant activation of GABA_A receptors (Lopantsev and Schwartzkroin, 1999). A preferential vulnerability of the GABAergic receptor systems after trauma has been discussed by de Beaumont and associates (2007), who recently reported significantly increased SP durations following repeated concussions. In a study by Chistyakov and colleagues (2001), acute mild-to-moderate injury TBI patients showed prolonged SP durations when measured at 130% RMT, but not at lower intensities, in contrast with their MEP findings. The authors concluded that the mechanisms affecting the excitatory and inhibitory components likely involved dissociated impairments, and suggested a more severe brain injury might be required for significant changes in SP. In the present study, we largely confirm and expand on the findings by Chistyakov and colleagues (2001), suggesting that excitatory and inhibitory processes in the motor cortex may be affected differently by severe chronic TBI.

There are several limitations to this study that warrant consideration. Several of our patients were on CNS drugs, which might contribute to changes in cortical excitability. However, cortical excitability measures of these patients did not reveal significant differences compared with those who

did not use such medications. Therefore, although this study was not designed to search for drug-induced changes, we believe that the reported findings on cortical motor excitability are unlikely to represent drug-related effects in our patient population. In a few studies, contraction force has been reported to affect SP duration (Catano et al., 1997), hence the use of a digital force gauge for continued monitoring would have been ideal for optimization of this factor. A very recent study suggests that SP durations evoked by an intensity of $\geq 130\%$ RMT are more reliable (Damron et al., 2008), and Chistyakov and colleagues (2001) reported prolonged SP durations in mild-to-moderate TBI patients only with a TMS intensity of 130% RMT. Therefore, additional stimulation intensities for our SP determinations might have been desirable. Further assessment of the anatomy of the white matter tracts using diffusion tensor imaging could also provide additional valuable information in estimating the real extent of DAI (Xu et al., 2007; Sugiyama et al., 2007; Yasokawa et al., 2007). Correlating such measures with our neurophysiological determinations would surely be most informative.

In conclusion, we have demonstrated that mechanisms related to the excitatory and inhibitory components of motor output appear to be affected independently after severe TBI. While no changes were detected in SP duration, neurophysiological alterations in the MEP parameters were shown to have a significant relationship with the severity of DAI and clinical motor findings. From a clinical standpoint, this study supports that neurophysiological assessment may provide valuable diagnostic information that is complementary to the clinical examination in patients with severe TBI. We suggest that in severe TBI, motor and functional recovery might be linked to restoration of normal corticospinal and intracortical mechanisms, as indicated by normalization of the cortical excitability parameters. Longitudinal studies in patients with TBI will be valuable to assess this hypothesis further, which if confirmed might offer prognostic surrogate markers and suggest novel therapeutic strategies.

Acknowledgments

Supported in part by a BBVA Translational Research Chair in Biomedicine, and a mentoring grant from the National Institutes of Health (K 24 RR018875), and the Instituto de Salud Carlos III Research Grant, Non-Invasive Brain Stimulation and Robot-Assisted Rehabilitation to Improve TBI recovery (PI082004). Special thanks go to Dr. Felip Orient, who assisted in collecting part of the data. We would also like to take this opportunity to thank the anonymous peer reviewers, whose reviews greatly helped to improve the manuscript.

Author Disclosure Statement

No competing financial interests exist.

References

- Abbruzzese, G., and Trompetto, C. (2002). Clinical and research methods for evaluating cortical excitability. *J. Clin. Neurophysiol.* 19, 307–321.
- Adams, J.H., Doyle, D., Ford, I., Gennarelli, T.A., Graham, D.I., and McLellan, D.R. (1989). Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* 15, 49–59.

- Adams, J.H., Graham, D.I., Murray, L.S., and Scott, G. (1982). Diffuse axonal injury due to nonmissile head injury in humans: an analysis of 45 cases. *Ann. Neurol.* 12, 557–563.
- Blumbergs, P.C., Jones, N.R., and North, J.B. (1989). Diffuse axonal injury in head trauma. *J. Neurol. Neurosurg. Psychiatry* 52, 838–841.
- Butcher, L., McHugh, G.S., Lu, J., Steyerberg, E.W., Hernández, A.V., Mushkudiani, N., Maas, A.I., Marmarou, A., and Murray, G.D. (2007). Prognostic value of cause of injury in traumatic brain injury: results from the IMPACT study. *J. Neurotrauma* 24, 281–286.
- Catano, A., Houa, M., and Noël, P. (1997). Magnetic transcranial stimulation: clinical interest of the silent period in acute and chronic stages of stroke. *Electroencephalogr. Clin. Neurophysiol.* 105, 290–296.
- Chistyakov, A.V., Hafner, H., Soustiel, J.F., Trubnik, M., Levy, G., and Feinsod, M. (1999). Dissociation of somatosensory and motor evoked potentials in non-comatose patients after head injury. *Clin. Neurophysiol.* 110, 1080–1089.
- Chistyakov, A.V., Soustiel, J.F., Hafner, H., Elron, M., and Feinsod, M. (1998). Altered excitability of the motor cortex after minor head injury revealed by transcranial magnetic stimulation. *Acta Neurochir.* 140, 467–472.
- Chistyakov, A.V., Soustiel, J.F., Hafner, H., Trubnik, M., Levy, G., and Feinsod, M. (2001). Excitatory and inhibitory corticospinal responses to transcranial magnetic stimulation in patients with minor to moderate head injury. *J. Neurol. Neurosurg. Psychiatry* 70, 580–587.
- Damron, L.A., Dearth, D.J., Hoffman, R.L., and Clark, B.C. (2008). Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *J. Neurosci. Methods* 173, 121–128.
- De Beaumont, L., Lassonde, M., Leclerc, S., and Théoret, H. (2007). Long-term and cumulative effects of sports concussion on motor cortex inhibition. *Neurosurgery* 61, 329–336.
- Fujiki, M., Hikawa, T., Abe, T., Ishii, K., and Kobayashi, H. (2006). Reduced short latency afferent inhibition in diffuse axonal injury patients with memory impairment. *Neurosci. Lett.* 405, 226–230.
- Gennarelli, T.A., Thibault, L.E., and Graham, D.I. (1998). Diffuse axonal injury: An important form of traumatic brain damage. *Neuroscientist* 4, 202–215.
- Hallett, M. (1995). Transcranial magnetic stimulation. Negative effects. *Adv. Neurol.* 67, 107–113.
- Jang, S.H., Cho, S.H., Kim, Y.H., You, S.H., Kim, S.H., Kim, O., and Yang, D.S. (2005). Motor recovery mechanism of diffuse axonal injury: a combined study of transcranial magnetic stimulation and functional MRI. *Restor. Neurol. Neurosci.* 23, 51–56.
- Katz, D.I., White, D.K., Alexander, M.P., and Klein, R.B. (2004). Recovery of ambulation after traumatic brain injury. *Arch. Phys. Med. Rehabil.* 85, 865–869.
- Kiers, L., Clouston, P., Chiappa, K.H., and Cros, D. (1995). Assessment of cortical motor output: compound muscle action potential versus twitch force recording. *Electroencephalogr. Clin. Neurophysiol.* 97, 131–139.
- Kiers, L., Cros, D., Chiappa, K.H., and Fang, J. (1993). Variability of motor potentials evoked by transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol.* 89, 415–423.
- Kobayashi, M., and Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol.* 2, 145–156.
- Lezak, M.D. (1995). *Neuropsychological Assessment*, 3rd ed. Oxford University Press: New York.
- Lopantsev, V., and Schwartzkroin, P.A. (1999). GABA_A-dependent chloride influx modulates GABA_B-mediated IPSPs in hippocampal pyramidal cells. *J. Neurophysiol.* 82, 1218–1223.
- Maas, A.I., Stocchetti, N., and Bullock, R. (2008). Moderate and severe traumatic brain injury in adults. *Lancet Neurol.* 7, 728–741.
- McDonnell, M.N., Orekhov, Y., and Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp. Brain Res.* 173, 86–93.
- Mills, V.M., Nesbada, T., Katz, D.I., and Alexander, M.P. (1992). Outcomes for traumatically brain-injured patients following post-acute rehabilitation programmes. *Brain Inj.* 6, 219–228.
- Moosavi, S.H., Ellaway, P.H., Catley, M., Stokes, M.J., and Haque, N. (1999). Corticospinal function in severe brain injury assessed using magnetic stimulation of the motor cortex in man. *J. Neurol. Sci.* 164, 179–186.
- Murray, C.J.L., and Lopez, A.D. (1997). Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *Lancet* 349, 1498–1504.
- Peña-Casanova, J., Guardia, J., Bertran-Serra, I., Manero, R.M., and Jarne, A. (1997). Shortened version of the Barcelona test (I): subtests and normal profiles. *Neurologia* 12, 99–111.
- Povlishock, J.T., and Katz, D.I. (2005). Update of neuropathology and neurological recovery after traumatic brain injury. *J. Head Trauma Rehabil.* 20, 76–94.
- Rao, V., and Lyketsos, C. (2000). Neuropsychiatric sequelae of traumatic brain injury. *Psychosomatics* 41, 95–103.
- Reid, A.E., Chiappa, K.H., and Cros, D. (2002). Motor threshold, facilitation and the silent period in cortical magnetic stimulation, in: *Handbook of Transcranial Magnetic Stimulation*. A. Pascual-Leone, N.J. Davey, J. Rothwell, E.M. Wasserman, and B.K. Puri (eds), Oxford University Press Inc: New York, pps. 97–111.
- Roick, H., Giesen, H.J. and von Benecke, R. (1993). On the origin of the postexcitatory inhibition seen after transcranial motor cortex stimulation in awake human subjects. *Exp. Brain Res.* 94, 489–498.
- Rossini, P.M., Berardelli, A., Deuschl, G., Hallett, M., Maertens de Noordhout, A.M., Paulus, W., and Pauri, F. (1999). Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology. *Electroencephalogr. Clin. Neurophysiol. Suppl.* 52, 171–185.
- Siebnér, H.R., Dressnandt, J., Auer, C., and Conrad, B. (1998). Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle Nerve* 21, 1209–1212.
- Steriade, M., Gloor, P., Llinás, R.R., Lopes de Silva, F.H., and Mesulam, M.M. (1990). Report of IFCN Committee on Basic Mechanisms. Basic mechanisms of cerebral rhythmic activities. *Electroencephalogr. Clin. Neurophysiol.* 76, 481–508.
- Steward, O. (1989). Reorganization of neural connections following CNS trauma. Principles and experimental paradigms. *J. Neurotrauma* 6, 99–152.
- Sorenson, S., and Kraus, J. (1991). Occurrence, severity, and outcomes of brain injury. *J. Head Trauma Rehabil.* 6, 1–10.
- Sugiyama, K., Kondo, T., Higano, S., Endo, M., Watanabe, H., Shindo, K., and Izumi, S. (2007). Diffusion tensor imaging fiber tractography for evaluating diffuse axonal injury. *Brain Inj.* 21, 413–419.
- Wassermann, E.M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive

- Transcranial Magnetic Stimulation, June 1996. *Electroenceph. Clin. Neurophysiol.* 108, 1–16.
- Weber, R.J. (1997). Nerve conduction studies, in: *Practical Electromyography*, 3rd ed. E.W. Johnson, and W.S. Pease (eds), Williams & Wilkins: Baltimore, pps. 131–195.
- Werhahn, K.J., Kunesch, E., Noachtar, S., Benecke, R., and Classen, J. (1999). Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J. Physiol.* 517, 591–597.
- Willemse-van Son, A.H., Ribbers, G.M., Verhagen, A.P., and Stam, H.J. (2007). Prognostic factors of long-term functioning and productivity after traumatic brain injury: a systematic review of prospective cohort studies. *Clin. Rehabil.* 21, 1024–1037.
- Xu, J., Rasmussen, I.A., Lagopoulos, J., and Häberg, A. (2007). Diffuse axonal injury in severe traumatic brain injury visualized using high-resolution diffusion tensor imaging. *J. Neurotrauma* 24, 753–765.
- Yasokawa, Y.T., Shinoda, J., Okumura, A., Nakayama, N., Miwa, K., and Iwama, T. (2007). Correlation between diffusion-tensor magnetic resonance imaging and motor-evoked potential in chronic severe diffuse axonal injury. *J. Neurotrauma* 24, 163–173.

Address correspondence to:

Alvaro Pascual-Leone, M.D., Ph.D.

Berenson-Allen Center for Noninvasive Brain Stimulation

Beth Israel Deaconess Medical Center

330 Brookline Avenue KS-158

Boston, MA 02215

E-mail: apleone@bidmc.harvard.edu