ABSTRACT: We tested cortical motor evoked potentials (cMEPs) as a quantitative marker for in vivo monitoring of corticospinal tract damage in a murine multiple sclerosis model (experimental autoimmune encephalomyelitis, EAE). The cMEPs, previously standardized in naive C57BL/6 developing and adult mice, were studied longitudinally in adult EAE mice. Central conduction times (CCTs) increased significantly shortly before the earliest clinical signs developed (10 days postimmunization, dpi), with peak delay in acute EAE (20–40 dpi). In clinically stable disease (80 dpi), CCTs did not increase further, but cMEP amplitude declined progressively, with complete loss in >80% of mice at 120 dpi. Increase in CCT correlated with presence of inflammatory infiltrates and demyelination in acute EAE, whereas small or absent cMEPs were associated with continuing axonal damage in clinically-stabilized disease and beyond (>80 dpi). These results demonstrate that cMEPs are a useful method for monitoring corticospinal tract function in chronic-progressive EAE, and provide insight into the pathological substrate of the condition.

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MOTOR EVOKED POTENTIALS IN A MOUSE MODEL OF CHRONIC MULTIPLE SCLEROSIS

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Motor potentials (MEPs) evoked by transcranial stimulation are a noninvasive means for quantitative assessment of motor involvement in multiple sclerosis (MS).[13,15,19,25] They are useful for monitoring disease activity in patients with primary and secondary progressive MS[17] and may help predict the natural evolution of the disease.[9] However, little is known of the utility or otherwise of MEPs in experimental autoimmune encephalomyelitis (EAE), a preclinical model of MS with motor system involvement that can be induced in various strains of rodents. Depending on the immunization procedure, and type and amount of antigen administered, acute monophasic, relapsing-remitting, or chronic-progressive disease forms can be obtained.[12,22,28]

Because of the importance of EAE models for testing new MS therapies,[24] functional tests are required to monitor motor involvement, in the hope that they will prove more sensitive than clinical evaluation. In addition, studies providing neuropathological data to support neurophysiological findings in EAE are lacking.

After standardizing our transcranial electrical technique to obtain MEPs in developing and adult control mice, we performed longitudinal MEP monitoring in adult C57BL/6 EAE mice after immunization with myelin oligodendrocyte glycoprotein (MOG)-35–55. Our aim was to validate the use of longitudinal MEP monitoring as a quantitative surrogate for central nervous system (CNS) tissue loss, which occurs mainly by demyelination and axonal degeneration in this preclinical model of MS.

Abbreviations: CCT, central conduction time; CCT_{c-s}, CCT as difference between cMEP take-off and sMEP latency; CMAP, compound motor action potential; cMEP, cortical MEP; CNS, central nervous system; dpi, days postimmunization; EAE, experimental autoimmune encephalomyelitis; MEP, motor evoked potential; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PBS, phosphate-buffered saline; RMT, resting motor threshold; sMEP, spinal MEP; TES, transcranial electrical stimulation

Key words: axonal degeneration; demyelination; experimental autoimmune encephalomyelitis; motor evoked potentials; multiple sclerosis

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MATERIALS AND METHODS

Mice. Ten naive C57BL/6 newborn female mice were tested at 10, 20, 30, 45, 60, and 180 postnatal days to determine age-related MEP changes. Twenty-three naive C57BL/6 female mice aged 45–60 days

were employed as controls. Since MEP data at 60 days did not differ significantly between the two groups, the data were pooled to form a larger control group of 33 animals. We also tested 84 adult C57BL/6 mice with MOG35–55-induced EAE. These were sacrificed at 10 ($n = 19$), 20 ($n = 18$), 40 ($n = 17$), 80 ($n = 19$), and 120 ($n = 11$) days postimmunization (dpi). Five mice were randomly selected at each time for neuropathological investigations.

Induction of EAE. Chronic-progressive EAE was induced in the adult (42–56 days old) C57BL/6 female mice by subcutaneous challenge with 300 μl of 200 μg/ml of MOG35–55 (Multiple Peptide System, San Diego, California) in incomplete Freund's adjuvant containing 8 mg/ml Mycobacterium tuberculosis (strain H37Ra; Difco, Detroit, Michigan), as described elsewhere.[24] Pertussis toxin (Sigma, St. Louis, Missouri; 500 ng) was injected on the day of immunization and 2 days later.

Body weight and clinical scores (0, healthy; 1, limp tail; 2, ataxia with or without hindlimb paresis; 3, complete hindlimb paralysis with or without forelimb paresis; 4, tetraplegia; 5, moribund or dead) were recorded daily.

Neurophysiology. Mice were anesthetized with 0.02 ml/g body weight tribromoethanole (Avertine; Sigma) and placed under an infrared lamp to maintain body temperature above 34°C. To limit movement-related artifacts due to electrical stimulation-induced muscle twitch, the animals were restrained with tape in a rigid support.

Transcranial Electrical Stimulation (TES). Two monopolar 27G needle electrodes were used to produce bipolar stimulation. The cathode tip was inserted at the midline of the interaural line through the scalp to contact the periosteum of the bregmatic suture; the anode tip was inserted 3–4 mm lateral and anterior to the cathode, just anterior to the ear to contact the temporal bone. Thus, stimulation produced an electric field through the motor area.[8] Electrode position was adjusted if necessary to elicit cortical motor evoked potentials (cMEPs) with the lowest possible stimulus intensity.

The resting motor threshold (RMT) was defined as the lowest current intensity (mA) that allowed recording at least three responses of amplitude greater than 50 μV from six consecutive stimulation trials in the resting muscle. To stimulate all mice with equivalent current intensity, stimuli of 1.5 × resting motor threshold were always delivered.

MEP Recordings. Muscle responses from TES were recorded with a modified bipolar "belly–tendon" arrangement. The active needle electrode was inserted into muscle of the hindlimb footpad, and the reference electrode was inserted under the skin of the second digit. The distance between the two electrodes was usually less than 3–4 mm. The electromyographic signal was filtered through a 50-5 kHz bandpass filter and recorded with a Myohandy electromyograph (Micromed, Mogliano Veneto, Italy), at a sweep velocity of 20 ms and sensitivity of 500 μV. Because the response to TES was variable, at least 10 MEPs (Fig. 1A) were acquired and the average analyzed (Fig. 1B). The onset of the first, usually negative, deflection was taken as the cortical MEP (cMEP) latency. F1

Peripheral and Central Conduction Time Measurements. Spinal MEPs (sMEPs) were obtained by inserting a pair of stimulating needle electrodes into the lumbar spine, close to emergence of the sciatic nerve roots. The sMEP latency was always compared with the peripheral conduction time in each animal as calculated from the formula ($F\ wave_{lat} + CMAP_{lat} - 1)/2$,[27] where CMAP is the compound muscle action potential.

The difference between the cMEP latency (time for impulse propagation from motor cortex to hindlimb) and the sMEP latency (time for propagation from the motor root to hindlimb muscle) was defined as the central conduction time ($CCT_{c\text{-}s}$; Fig. 1B) and considered a measure of the propagation time between motor cortex and spinal cord.

The CMAP amplitude (Fig. 1B) obtained by stimulating the sciatic nerve at the ankle was also recorded and compared with the cMEP amplitude. To minimize the variation of muscle potentials inherent in the use to needle recordings in the serial cMEP evaluations, the amplitude of cortical responses was always expressed as a percentage of the CMAP (MEP/MAP ratio), and not as an absolute value.[30]

MEP Score. In addition to the standard MEP variables of threshold, latency, and amplitude, we also assessed corticospinal conduction abnormalities by assigning an MEP score in the range of 0–3, where 0 is normal, indicating that the CCT did not exceed the mean control CCT by more than 1 SD; 1 is a slightly prolonged CCT (between 1 and 2 SD of the control mean); 2 is a more prolonged increase in CCT (more than 2 SD above the control mean); and 3 indicates that cMEPs were not recorded and hence the CCT could not be determined. Thus, use of the MEP score meant mice with no cMEP (greatest functional impairment of corticospinal tract) were included in the evaluation.

Neuropathology. Animals were anesthetized and perfused transcardially with phosphate-buffered saline

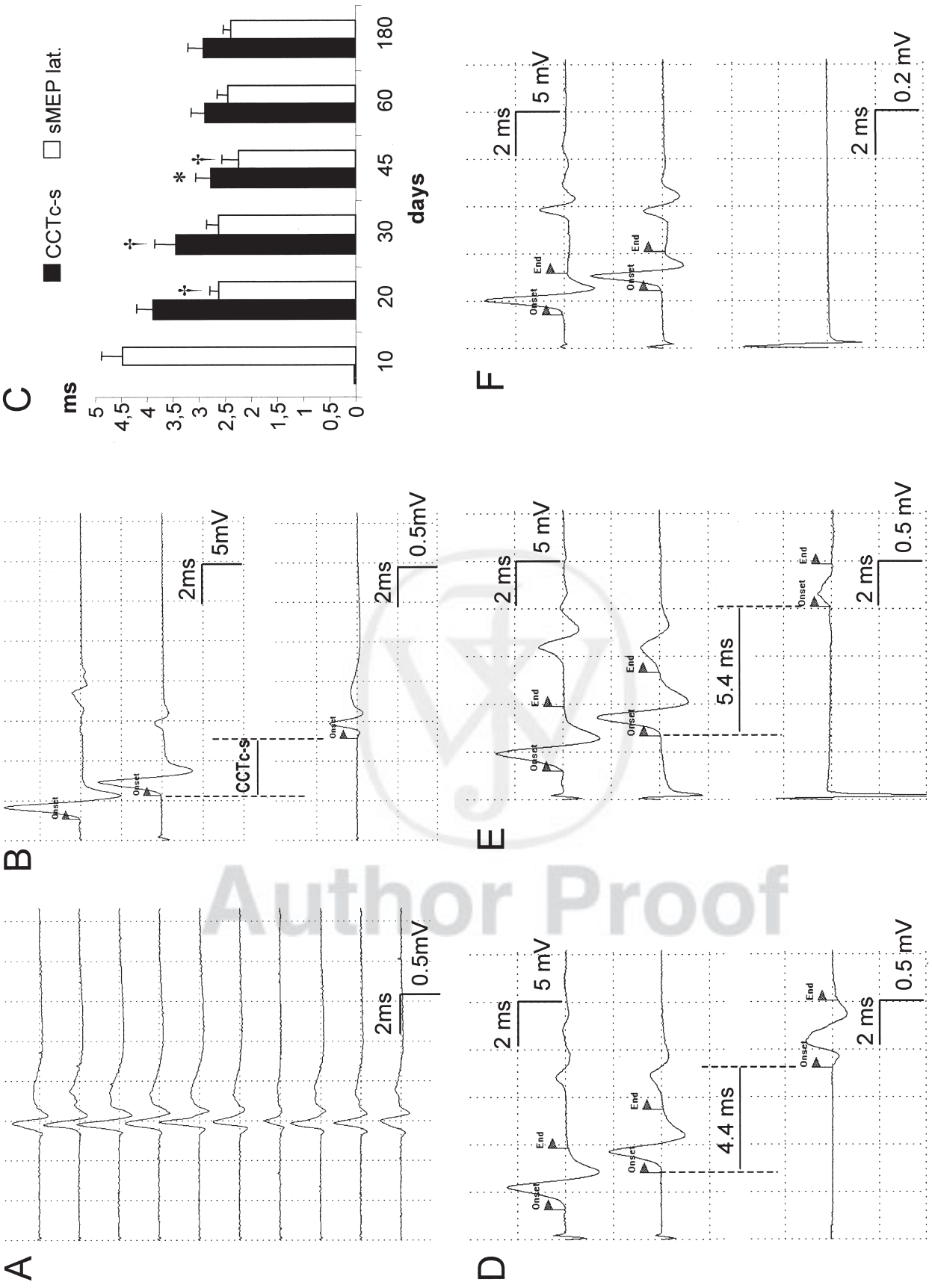

FIGURE 1.

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Table 1. Mean values for neurophysiological data over time in mice with EAE and in control mice.

Factors	Variables	Controls $n = 33$	10 dpi $n = 19$ (19)	20 dpi $n = 18$ (17)	40 dpi $n = 17$ (10)	80 dpi $n = 19$ (10)	120 dpi $n = 11$ (2)
CMAP	DML (ms)	1.12 ± 0.13	1.18 ± 0.13	1.14 ± 0.14	1.06 ± 0.15	1.08 ± 0.15	1.15 ± 0.15
	Amplitude (mV)	10.38 ± 3.36	10.85 ± 3.39	9.21 ± 2.34	11.72 ± 3.94	12.25 ± 4.21	13.70 ± 3.79
F wave	Latency (ms)	5.26 ± 0.36	5.34 ± 0.23	5.25 ± 0.32	5.09 ± 0.30	5.07 ± 0.43	5.09 ± 0.24
sMEP	Latency (ms)	2.41 ± 0.19	2.50 ± 0.16	2.51 ± 0.22	2.29 ± 0.20	2.31 ± 0.21	2.32 ± 0.14
	Amplitude (mV)	7.24 ± 2.84	7.24 ± 2.95	6.60 ± 2.08	7.28 ± 3.27	9.06 ± 3.40	9.08 ± 2.49
cMEP	RMT (mA)	7.85 ± 5.39	12.26 ± 14.15	46.06 ± 39.88*,§	35.20 ± 38.98*,§	27.00 ± 18.91*,§	NE
	Latency (ms)	5.25 ± 0.27	5.76 ± 0.42*	6.59 ± 1.03*,§	6.21 ± 0.69*,§	5.95 ± 0.23*	NE
	MEP/MAP	3.68 ± 2.44	4.00 ± 2.74	2.57 ± 2.18	1.82 ± 1.06†,§	2.00 ± 1.19†,§	NE
CCT	C−S (ms)	2.84 ± 0.27	3.26 ± 0.36*	4.08 ± 0.91*,‡	4.00 ± 0.67*,‡	3.73 ± 0.25*,‡	NE
	F wave (ms)	2.56 ± 0.26	2.99 ± 0.37*	3.90 ± 0.95*,‡	3.77 ± 0.64*,‡	3.44 ± 0.25*,‡	NE

CCT, central conduction time; CMAP, compound motor action potential; cMEP, cortical motor evoked potential; C−S, CCT measured by subtracting latency of spinal MEP from latency of cortical MEP; DML, distal motor latency; dpi, days postimmunization; F wave, CCT measured by means of F-wave latency; MEP/MAP, amplitude ratio of cortical (cMEP) to peripheral nerve response (CMAP) × 100; NE, not evaluated due to low number of mice; numbers in parentheses, mice with excitable cMEP and measurable CCT; RMT, resting motor threshold; sMEP, spinal motor evoked potential.
**$P < 0.001$ vs. controls; †$P < 0.05$ vs. controls; ‡$P < 0.001$ vs. 10 dpi; §$P < 0.05$ vs. 10 dpi.*

(PBS) followed by 4% paraformaldehyde. Spinal cords were removed and processed for pathological examination.[10] Paraffin-embedded 5-μm–thick transverse sections were cut and stained with hematoxylin and eosin, Luxol fast blue, or Bielschowsky to show inflammatory infiltrates, demyelination, and axonal damage, respectively. Damage was quantified using IM-50 Image Analyzer Software (Leica, Bannockburn, Illinois) on an average of seven spinal cord sections per animal and expressed as number of inflammatory foci per mm^2, percentage area of demyelination, or percentage area of axonal damage (per mm^2), respectively, in whole spinal cord sections, and in the ventral corticospinal tract only.

Statistical Analysis. Differences between means for parametric data were compared using Student's *t*-test. Nonparametric data were compared using either the Mann–Whitney or chi-squared test (Fisher's exact test). Associations between clinical score, MEP score, and CCT at each time-point were analyzed by Spearman's rank *(r)* correlation test.

RESULTS

We had no technical problems in obtaining stable MEP recordings from healthy mice. Transcranial stimulation depolarized the motor cortex at low current intensities (resting motor threshold 3–15 mA in most animals). The cMEPs, sMEPs, and CMAPs usually had a bi- or triphasic shape with clear take-off, allowing CCT and the MEP/MAP ratio to be calculated reliably (Fig. 1A, B).

Longitudinal MEPs in Control Mice. Unlike spinal stimulation, which provoked recordable sMEPs from mice at 10 days, transcranial stimulation did not evoke muscle responses in the youngest mice. High-threshold, long-latency cMEPs with a delayed CCT (3.88 ± 0.34 ms) first appeared at 20 days and reached adult values at 45 days (2.77 ± 0.29 ms, $P <$ 0.001 vs. 20-day-old animals). In spite of increasing body size, only small nonsignificant changes in CCT and sMEP latencies occurred at 60 and 180 days compared to 45 days (Fig. 1C).

FIGURE 1. Representative traces from individual animals. Traces illustrate MEPs in naive adult C57BL/6 mice (A, B) and in EAE mice over time **(D–F)**. The histogram **(C)** illustrates decreases in CCT and sMEP latencies as naive (control) mice mature. **(A)** cMEPs evoked with 10 consecutive transcranial stimuli at current intensity 6.0 mA (1.5 × resting motor threshold). **(B)** Top to bottom: CMAP evoked by stimulation of tibial nerve at ankle (F waves were found a few milliseconds after the CMAP); sMEP evoked by lumbar root stimulation; and average of the 10 cMEPs shown in panel **(A)**. The method of measuring $CCT_{c\text{-}s}$, as difference between cMEP take-off and sMEP latency, is also illustrated in panel **(B)**. **(C)** $CCT_{c\text{-}s}$ (black bars) and sMEP latencies (white bars) in control mice at 10 days (newborn), 20–30 days (developing), and 45, 60, and 180 days (adult). Means of 10 mice (± SD) in all cases. No cortical responses were evoked in newborn mice, even though peripheral responses were usually present, making it impossible to determine $CCT_{c\text{-}s}$. CCT and peripheral conduction did not change after 45 dpi. *$P < 0.001$ vs. 20 and 30 dpi; †$P < 0.05$ vs. previous time-point. Panels **(D–F)** show MEPs in EAE mice at 20, 80, and 120 dpi, respectively. Although CMAPs, F waves, and sMEPs were stable throughout the course of EAE, cortical responses changed markedly. Prolonged CCT with spared cMEP amplitude was the hallmark of acute EAE [20 dpi, **(D)**]; decreasing cMEP amplitude and severely prolonged CCT was typical at 80 dpi **(E)**, whereas complete cMEP disappearance was characteristic later [120 dpi, **(F)**].

Longitudinal MEPs in EAE. Neurophysiological findings in EAE mice are shown in Table 1. Representative traces from individual mice are shown in Figure 1D–F. Briefly, conduction variables obtained by stimulating the peripheral nerve (CMAP and F wave) and motor root (sMEP) in EAE mice tested throughout the course of the disease did not differ significantly from controls (Table 1). By contrast, cMEP variables changed in relation to the disease stage, as illustrated in Figure 2.

Figure 2A illustrates the monophasic natural history of MOG35–55-induced EAE. After a subclinical phase, the clinical score increased rapidly at 15–20 dpi, reached a peak (of 3) at 20–40 dpi, plateaued at slightly below peak (score 2.5) at about 40 dpi, and remained at the plateau to 120 dpi. By contrast, the CCT (Fig. 2B) increased early (evident at 10 dpi), before any clinically detectable motor impairment, followed by a peak 10 days later, at the time of maximum clinical deficit (20 dpi). Subsequently, CCTs substantially stabilized (40 dpi), then recovered somewhat (80 dpi). No evaluation of CCT mean value was possible at 120 dpi, because of the small number of mice (2 of 11) with cortical responses. The time trend of resting motor threshold paralleled that of CCT (Fig. 2C), with a peak at 20 dpi. The MEP/CMAP ratio changed later than CCT, and showed a significant decrease at 40 dpi (compared to 10 dpi and controls), which persisted at 80 dpi (Fig. 2D).

The proportion of EAE mice with the severest MEP score (level 3) increased progressively with time (Fig. 2E). Most animals (just under 80%) had an MEP score of 2 at 20 dpi, whereas over 80% had an MEP score of 3 (no cortical responses) at 120 dpi.

Clinical and Neurophysiological Correlations in EAE. Using Spearman's rank correlation coefficient, we found correlations between clinical score and CCT up to 40 dpi. The correlation was 0.57 ($P < 0.05$) at 20 dpi and stronger at 40 dpi ($r = 0.86$; $P < 0.01$). After the acute EAE stage (at 80 dpi), clinical score correlated with MEP score ($r = 0.74$; $P < 0.01$), but not with CCT. After 80 dpi, correlations between clinical and neurophysiological variables were no longer high or significant.

Neuropathological Features of EAE Mice. In the early subclinical phase (10 dpi), few infiltrating cells were found within the spinal cord of EAE mice. The number of infiltrating cells peaked at 20 dpi, and then declined progressively (Fig. 3A). Similarly, greatest areas of demyelinated axons in spinal cord were found at 20 dpi, the proportion of demyelinated fibers subsequently declining, although there was a small, nonsignificant increase at 80 dpi (Fig. 3B). Axonal damage was substantial at 20 dpi and significantly greater than at 10 dpi when it was undetectable (Fig. 3C), and persisted as an active process throughout the disease.

The corticospinal tract had higher proportions of demyelinating lesions and degenerating axons than sections of whole spinal cord (Fig. 3D, E). Major demyelination was observed at 20 dpi and 80 dpi in the corticospinal tract, in both cases followed by recovery (at 40 dpi and 120 dpi). By contrast, axonal damage was slowly progressive and continuous, being evident at 20 dpi and significantly greater at 120 dpi.

DISCUSSION

We were unable to elicit MEPs by transcranial stimulation in naive mice at 10 days of age, in agreement with studies on the growth of corticospinal axons in developing mice[11] and their rearrangement and plasticity after birth.[29] We did, however, detect sMEPs in newborn naive mice from 10 days of age, showing that the peripheral conduction system is already in place and suggesting that hypo/inexcitability of developing corticospinal axons was responsible for the early lack of response to cortical stimulation. At the earliest time that cMEPs could be elicited (20 days), CCTs were prolonged and gradually declined to adult values by age 45 days, when the developing corticospinal tract is thought to be fully myelinated.[1] No subsequent age- or weight-related changes in CCT were observed up to the latest observation time (day 180, Fig. 1C). Thus, the monitoring of TES-induced MEPs not only provides a window to corticospinal tract development in the naive mouse in vivo, but also shows that the CCT does not undergo significant changes in naive mice between 45 and 180 days of age, the age range of the EAE mice investigated in this study.

MEPs in a mouse model of CNS demyelination have been investigated previously,[16] but the method used differed substantially from our method in that potentials were evoked by stimulating the cervical spinal cord below the foramen magnum and did not distinguish between alterations in central and peripheral conduction. It is important to distinguish between central and peripheral conduction to localize the site of the functional abnormalities responsible for the weakness observed in EAE, particularly since central demyelination may coexist with spinal root demyelination in this animal model.[3] Indeed,

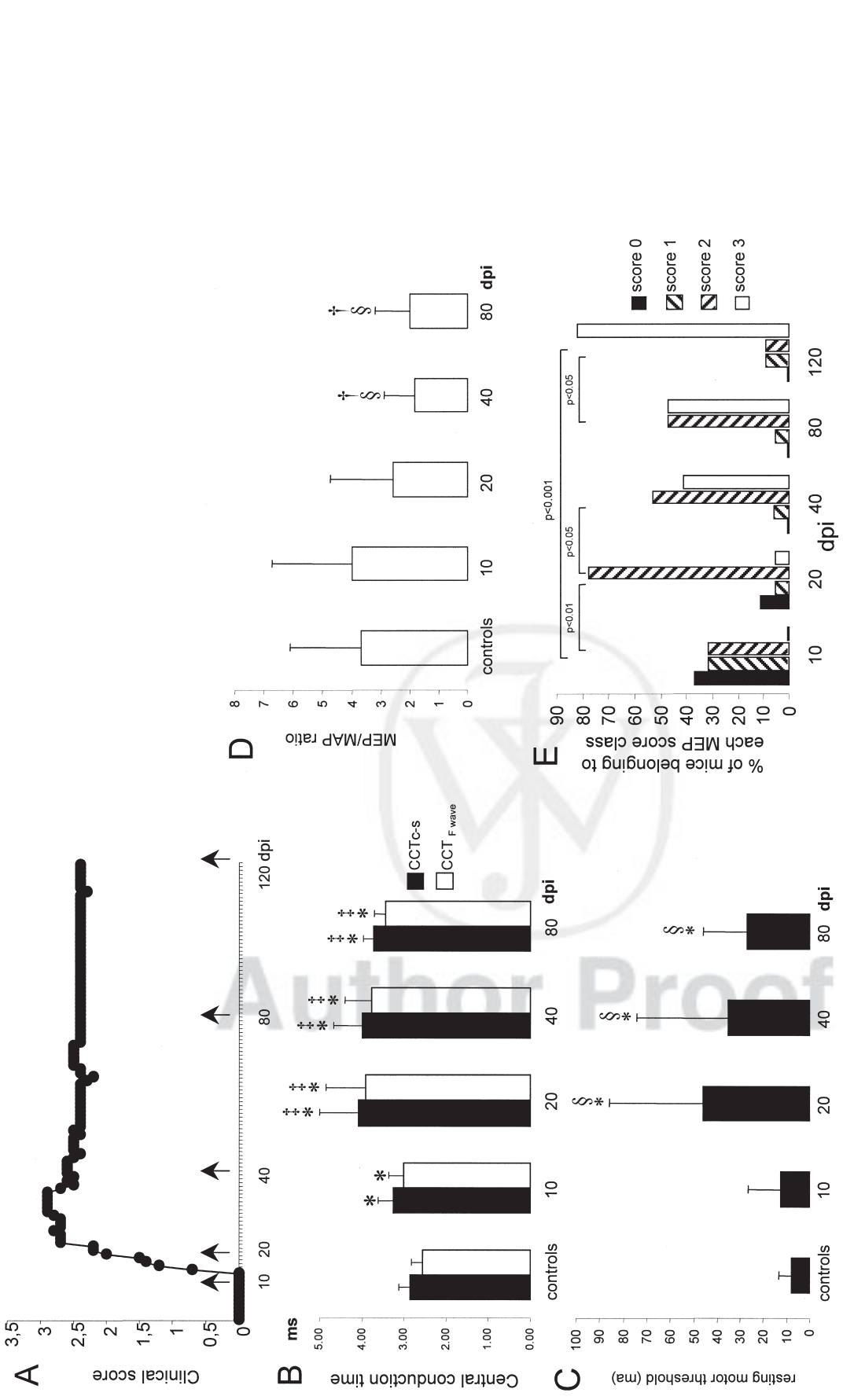

FIGURE 2. Changes of MEP variables, clinical score, and MEP score over time. **(A)** Natural history of MOG35–55-induced chronic-progressive EAE in adult C57BL/6 mice, from 0 to 120 dpi. **(B)** CCT changes. As expected, the two methods of measuring CCT (see text) were tightly coupled during disease course. Other cMEP variables showed different behavior: resting motor threshold peaked early [20 dpi, **(C)**], whereas MEP/MAP ratio did not significantly decrease before 40 dpi **(D)**. **(E)** Percentage distribution of EAE mice belonging to each class of MEP scores among mice at subsequent time-points of disease. The global percentage of mice with abnormal CCT (score 1–2) exceeded that of normal scoring mice since the subclinical EAE phase (10 dpi); score 2 represented the most frequent class of values either at 20 dpi or, at a lesser degree, at 40 dpi, and there were no mice scoring 0 since 40 dpi; these data demonstrated the notable sensitivity of CCT during the acute EAE phase. The increasing percentage of mice with score 3 (that is, absence of any cortical response) which exceeded 80% at the latest time-point (120 dpi) was the physiological hallmark of the chronic EAE stage. All values are means ± SD. $^*P < 0.001$ vs. controls; $\dagger P < 0.05$ vs. controls; $\ddagger P < 0.001$ vs. 10 dpi; $\S P < 0.001$ vs. 10 dpi.

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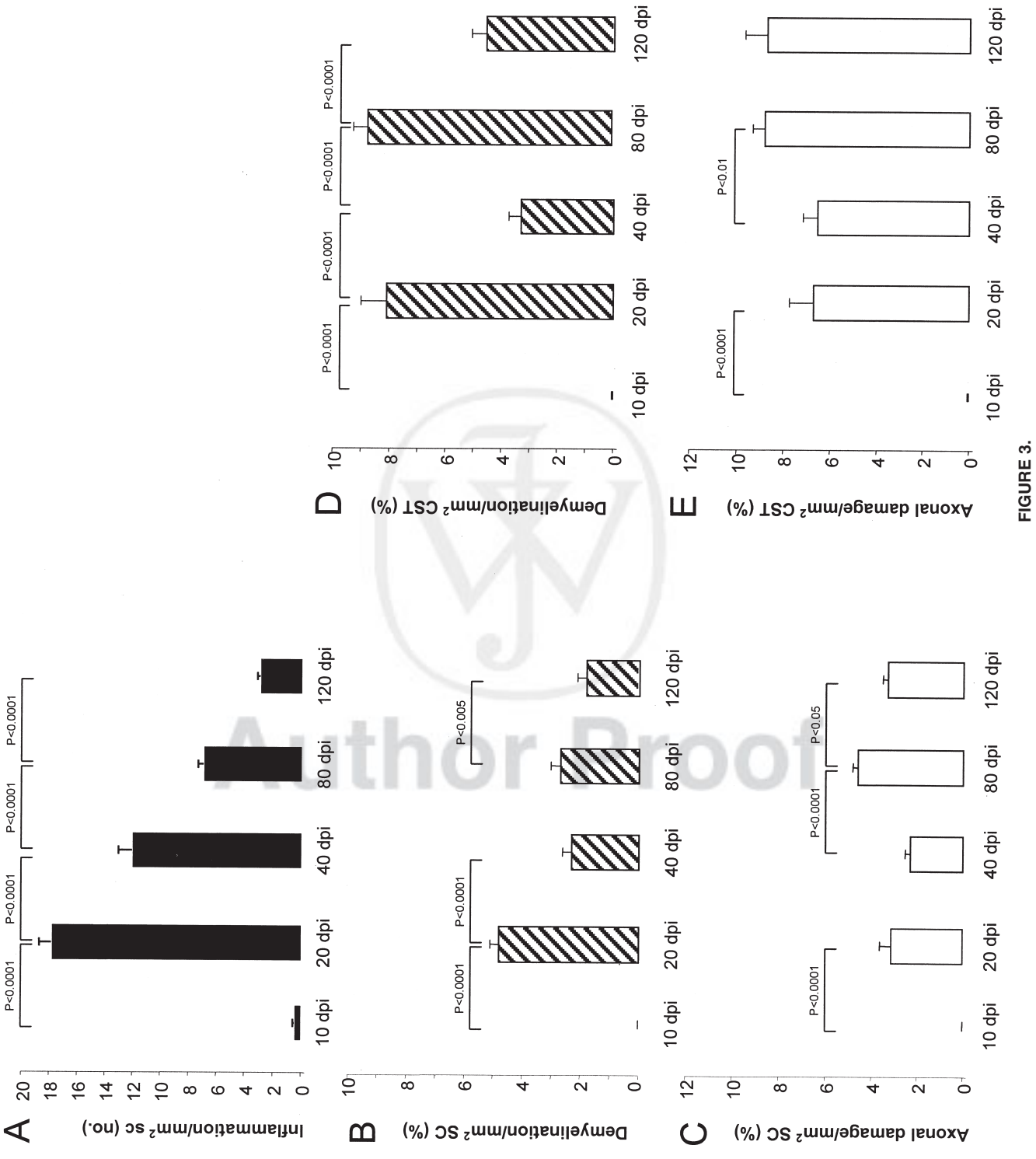

FIGURE 3.

the possibility that central and spinal root demyelination might coexist was the rationale for our investigation of peripheral conduction times in all the naive and EAE mice, both by direct stimulation of motor roots,[23] and also by assessment of F waves.[27] These investigations excluded the possibility that conduction blocks were present intrathecally (between the motoneuron cell body and the intervertebral foramen), slowing cortex–muscle conduction times without affecting spinal MEP latencies, and hence giving a false lengthening of CCT. In our EAE mice, peripheral conduction was never affected, and the MEP changes we observed reflected selective involvement of the corticospinal tract.

Longitudinal CCTs only partially correlated with clinical deficit. CCT and clinical score correlated well during the acute phase of EAE (20–40 dpi), but were independent of each other in the preclinical phase (10 dpi) (when CCTs were significantly lengthened, but weakness was not yet evident), and also during the transition from acute to chronic EAE (80 dpi), when clinical score recovered slightly to subsequently stabilize. At 80 dpi, MEPs in fact varied: in the mice in which cMEPs could still be elicited, there was a slight recovery of CCT; however, in a considerable proportion of animals, no cMEPs could be elicited (score 3 on the MEP scale) and, hence, CCT could not be calculated. As a consequence, the MEP score, but not CCT, correlated with clinical score at 80 dpi. After 80 dpi, correlations between clinical and neurophysiological variables could no longer be investigated because cMEPs could no longer be elicited in most animals. We therefore conclude that, as long as cMEPs are present, CCTs are the best disease descriptors, but that disease state is better described by MEP score during the intermediate EAE stage (80 dpi).

It is possible that the lack of correlation between MEPs and clinical scores in the earliest and final stages of EAE indicates that MEP reflect underlying pathological changes better than clinical score. Our neuropathological data on corticospinal descending pathways support this hypothesis. Thus, while demyelination fluctuated (Fig. 3D), as also reported in previous pathological studies,[18] damage to corticospinal tract axons was evident early (20 dpi) and progressed throughout the course of the disease. This progression of axonal damage parallels the progressive MEP/MAP ratio deterioration, and suggests how it might have occurred.

The corticospinal tract impairment evident in the subclinical (10 dpi) stage of EAE is plausibly related to the presence of inflammatory mediators, such as nitric oxide, which are able to produce conduction block even in normal axons.[26] Subsequently, demyelination and axonal damage are likely to have cooperated in promoting MEP alterations. Experimental studies have shown that focal demyelination of spinal cord may cause either a slowing of conduction or complete loss of transmission depending on the extent of the lesion.[20] Furthermore, multifocal demyelination and partial conduction blocks at different craniocaudal levels of the human corticospinal tract may prevent descending volleys from synchronizing or even from reaching the threshold for motoneuron discharge.[5] Thus, it would seem that inflammatory demyelination can explain not only the slowing of corticospinal tract conduction, but also the absence of cortical responses found in a proportion of the animals during the acute phase (up to 40 dpi).

Unfortunately, our in vivo model was unable to distinguish demyelination from axonal damage, as can be done in experimental models assessing conduction distal to the lesion; in such cases, conduction is preserved in demyelination and completely lost in axonal degeneration.[20] However, based on physiological data, we speculate that if early cMEP disappearance had been due to conduction block arising from demyelination (arrest of impulse progression in spared axons), significant CCT recovery would be expected once remyelination had taken place. However, no such recovery occurred, MEP/MAP ratios were decreasing, and the percentage of mice with absent cMEPs increased steadily over the EAE course. These longitudinal MEP abnormalities therefore provide physiological evidence that a subclinical degeneration of corticospinal tract axons, which is known to depress MEP amplitude in murine MS models,[16,21] was ongoing from the acute phase and continued through the clinically stable stages.

Both small amplitude and absent cMEPs could also be partly due to hypoexcitability of corticospinal pathways. Sequential resting motor threshold changes paralleled those of CCT (Fig. 2B, C), showing a sudden increase at EAE onset (20 dpi), at the

FIGURE 3. Neuropathological features of MOG35–55-induced EAE in C57BL/6 mice. Inflammatory burden **(A)**, demyelination **(B, D)** and axonal damage **(C, E)** were evaluated within either the whole spinal cord [SC, **(A–C)**] or the ventral corticospinal tract [CST, **(D, E)**] at cervical, mid-thoracic, and lumbar levels. Data are expressed as mean ± SEM of a minimum of five mice per group.

same time as the demyelination peak, followed by incomplete recovery. Increased resting motor thresholds to transcranial magnetic stimulation are a common finding in MS[4,30] particularly during clinical relapse,[2] and also in the terminal stages of amyotrophic lateral sclerosis,[7] suggesting that global corticospinal tract excitability, as indicated by increased motor threshold, can be decreased by both inflammatory demyelination and neuronal degeneration. These findings probably reflect a lack of resting motor threshold specificity, and do not allow us to distinguish impaired excitability of motor cortex neurons from conduction failure along corticospinal axons. Furthermore, magnetic stimulation stimulates in a different way than electrical stimulation[6,14] and may not be pertinent to the present experimental setting.

Thus, although individual MEP parameters are unable to identify the roles of demyelination and axonal damage, considering them together with the pathological alterations allows us to conclude that longitudinal changes in MEPs reflect a discernible pathological pattern. The peak of demyelination at 20 dpi coincided with high-threshold cMEPs, marked CCT slowing, but no decline in MEP/MAP (Fig. 1D). The progressive axonal damage attained its maximal expression at 120 dpi, as documented by the absence of cMEPs, the salient physiological feature in the late stage disease (Fig. 1F). At intermediate time-points (40–80 dpi, Fig. 1E) the picture was intermediate between the "demyelinating" and "axonal" patterns, being characterized by delayed CCT, severely reduced MEP/MAP ratio (beginning as early as 40 dpi), and absent cMEP in the most severe cases.

We conclude that MEP data are highly sensitive in detecting corticospinal tract impairment in both the acute and late stages of chronic progressive EAE and the technique is therefore likely to be extremely useful, particularly when used together with clinicopathological assessment, both for the validation of experimental therapies and for functional monitoring in animal models of CNS demyelination.

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AQ1: On Table 1, headings required for all columns; "Factors" and "Variables" inserted—please approve or supply replacement heads.