Improving Motor Evoked Potential as preclinical marker of cortico-spinal conduction

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Introduction

Motor Evoked Potential (MEP) is the electrical response recorded from muscle, spinal cord or peripheral nerves after magnetic or electrical stimulation of the motor cortex. MEP can be used to check neurological disease progression that involves the cortico-spinal tract, such as demyelinating neuropathies and multiple sclerosis (MS). Pathological alterations in motor conduction can be detected by MEP recording, resulting in delayed and/or decreased responses. Experimental Autoimmune Encephalomyelitis (EAE) is the most common animal model of MS, characterised by MEP delayed latency and/or decreased amplitude, usually associated with demyelination and axonal loss. Among MEP outcomes, resting motor threshold (RMT), onset latency and peak-to-peak amplitude are taken into consideration to evaluate the neurophysiology of the cortico-motor pathway. RMT is the lowest current intensity able to generate MEP responses in 50% of trials and reflects the excitability of cortico-spinal connections. MEP latency denotes the time taken by descending impulses to reach the recorded muscle, assessing conduction velocity along the motor pathway. MEP peak-to-peak amplitude provides an indirect quantitative measure of the number of axons conducting impulses from the motor cortex to the muscle. Therefore, a satisfying repeatability of MEP measurements becomes crucial to highlight cortico-motor conduction changes during disease course and treatment. In this work, we analysed RMT, latency and amplitude of MEP elicited in C57BL/6 mice by subcutaneous needle, epicranial screw or surface cup electrodes at five consecutive timepoints. Repeatability indices were calculated for all MEP parameters to assess the stability and reliability of the techniques over time. Finally, we compared intramuscular needle with surface electrode recordings of MEP obtained by surface stimulation, to observe if more stable and reproducible electrophysiological outcomes could be obtained, further ameliorating the repeatability over time.

Methods

Animals

Fifty C57BL/6 mice (25 males and 25 females), 8 weeks aged, were included in these experiments.

Experimental protocol

In the first experiment, animals were divided in three groups of 10 mice each (5 males and 5 females), depending on the type of stimulating electrode used to obtain MEPs. In particular, MEPs were elicited through TES delivered by either subcutaneous needles (nMEP), epicranial screw (sMEP) or surface cup (cMEP) electrodes. For the second experiment, cMEPs were recorded in two groups of 10 animals each (5 males and 5 females), depending on the type of MEP recording electrode (intramuscular needle or surface plate). In both experiments, five MEP recording sessions (one every 72 hours) were conducted in the different groups to assess RMT, latency and amplitude. Finally, repeatability indices were calculated to declare which method was the most reliable.

Epicranial screw electrode implantation

Three days before the first MEP recording session, sMEP mice were anaesthetized with an intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. The scalp was removed and a stainless-steel screw (0.9 mm diameter) was implanted with glass ionomer dental cement over the primary motor cortex (M1), 2 mm right to the bregma.

MEP recording

Mice were anaesthetised with 90 mg/kg ketamine *plus* 10 mg/kg xylazine and body temperature was maintained at 37 °C using a homeothermic heating pad. For sMEP, the epicranial screw implanted over the skull was used as cathode, while the anode consisted of a sponge (5.2 cm^2) soaked with conductive gel placed under the mouse head. For nMEP, two monopolar needle electrodes (stainless steel, 27 gauge, 12 mm length) were used to induce bipolar stimulation. The cathode was inserted through the scalp at the midline of the interaural line; the anode was placed 3-4 mm lateral and anterior to the cathode to produce an electric field through the motor area. For cMEP, a removable cup electrode (Ag/AgCl, 6 mm Ø) was the cathode applied on the shaved scalp over the motor cortex (3-4 mm anterior to the interaural line and 1.5-2 mm right to the midline), while the anode was the soaked sponge described previously. For intramuscular MEP recording, an active needle was inserted into the centre of the left hind limb footpad; for surface MEP recording, the active electrode was a customised 1 mm Ø tin plate applied on the left hind limb footpad. For both intramuscular and surface MEP recordings, the reference electrode was a needle inserted under the skin of the second digit. In order to elicit MEPs, square wave current pulses of 50 µs width were delivered at a frequency of 0.3 Hz. Firstly, RMT was determined as the lowest current intensity (mA) that allowed the recording of at least three responses out of six consecutive stimulation trials with amplitude > 50 µV in the resting muscle. To stimulate all mice with comparable current intensity, stimuli of 1.5 × RMT were delivered. Three averages of ten MEPs were analysed in terms of RMT (mA), latency (ms) and peak-to-peak amplitude (µV).

Statistical analysis

RMT, latency and amplitude values were examined using two-way ANOVA for repeated measures entering "stimulating electrode" as the "between subjects" main factor (3 levels: sMEP, nMEP and cMEP) and "time" as the "within subjects" main factor (5 levels: t1-t5). Subsequently, to compare two different MEP recording electrodes, RMT, latency and amplitude values were examined using two-way ANOVA for repeated measures entering "recording electrode" as the "between subjects" main factor (2 levels: intramuscular needle and surface plate) and "time" as the "within subjects" main factor (5 levels: t1-t5), followed by post-hoc protected t-tests. The inter-session Bland-Altman coefficient of repeatability (CR), inter-session standard error of the mean (SEMw), inter-session coefficient of variation (CoVw) and inter-session relative standard error (RSEw) were used to measure repeatability of both stimulating and recording methods. Data were considered significant at $p < 0.05$

Results

Technical feasibility of nMEP, sMEP and cMEP

nMEP and cMEP recording sessions were completed from t1 to t5 without missing data. Conversely, three mice from sMEP group were excluded due to early detachment of epicranial screw electrodes. Implants of the remaining seven mice detached around t5, therefore MEP analysis over time was performed from t1 to t4. nMEP, sMEP and cMEP waveforms were similar in terms of morphology, with onset and peaks clearly distinguishable over the background noise (**Fig 1**).

RMT, latency and amplitude with different stimulating electrodes

At two-way ANOVA for repeated measures, no significant effect of "time", "stimulating electrode" and "time*stimulating electrode" interaction were found for MEP RMT, latency and amplitude ($p > 0.05$; **Fig 2**).

Repeatability of nMEP, sMEP and cMEP

Regarding MEP RMT and latency, one-way ANOVA did not detect significant differences between nMEP, sMEP and cMEP in terms of CR, SEMw, CoVw and RSEw ($p > 0.05$; **Fig 3A-B**). Concerning MEP amplitude (**Fig 3C**), one-way ANOVA did not notice significant differences between nMEP, sMEP and cMEP in respect of CR, SEMw and RSEw ($p > 0.05$), but significant differences emerged for CoVw ($p = 0.046$). Post-hoc analysis revealed that, compared to nMEP, CoVw of cMEP was significantly decreased ($p = 0.016$).

Technical feasibility of cMEP recorded with intramuscular needle or surface plate electrodes

cMEP acquisitions using intramuscular or surface recording electrodes were completed from t1 to t5 without missing data. cMEP waveforms obtained with both types of recording electrodes were similar in terms of morphology, with onset and peaks clearly distinguishable over the background noise (**Fig 4**). At visual inspection, cMEP amplitude from surface electrode was lower than that obtained with intramuscular needle.

RMT, latency and amplitude of cMEP acquired with different recording electrodes

At two-way ANOVA for repeated measures, no significant effect of "time", "recording electrode" and "time*recording electrode" interaction were detected for cMEP RMT and latency ($p > 0.05$; **Fig 5A-B**). Concerning cMEP amplitude, no significant effects of "time" and "time*recording electrode" interaction were reported. On the contrary, a significant effect of "recording electrode" ($p < 0.0001$) was noticed. Post-hoc analysis revealed a significantly lower cMEP amplitude for surface compared with intramuscular recording at all timepoints (t1: $p = 0.013$; t2: $p = 0.003$; t3: $p = 0.042$; t4: $p = 0.0001$; t5: $p = 0.002$; **Fig 5C**).

Repeatability of intramuscular and surface cMEP recording

Concerning cMEP RMT and latency, no significant differences emerged between intramuscular and surface recording in respect of CR, SEMw, CoVw and RSEw ($p > 0.05$; **Fig 6A-B**). For cMEP amplitude (**Fig 6C**), significant decreases were found in surface compared to intramuscular recording in terms of CR ($p = 0.0001$), SEMw ($p = 0.0001$), CoVw ($p = 0.011$) and RSEw ($p = 0.011$).

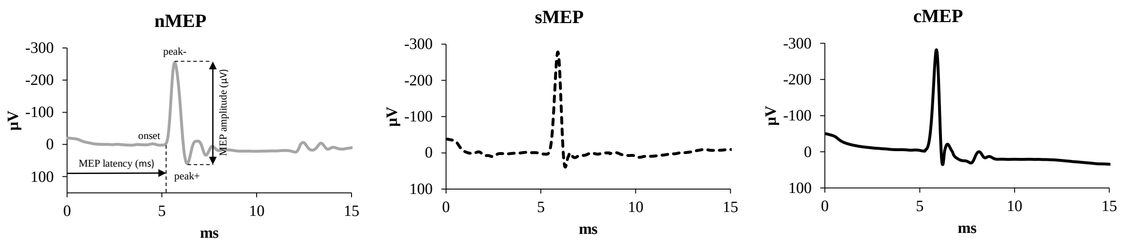

Figure 1. *Examples of MEP waveforms elicited by needle (nMEP, in which onset, peak- and peak+ are highlighted), screw (sMEP) and cup (cMEP) electrodes..*

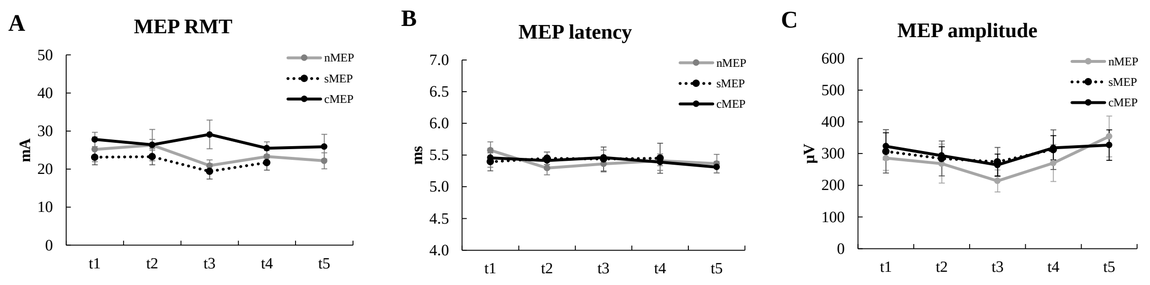

Figure 2. *MEP RMT (A), latency (B) and amplitude (C) measured in mice stimulated with needle (nMEP: n = 10), screw (sMEP: n = 7) or cup electrodes (cMEP: n = 10) at different timepoints. Data are expressed as mean ± SEM.*

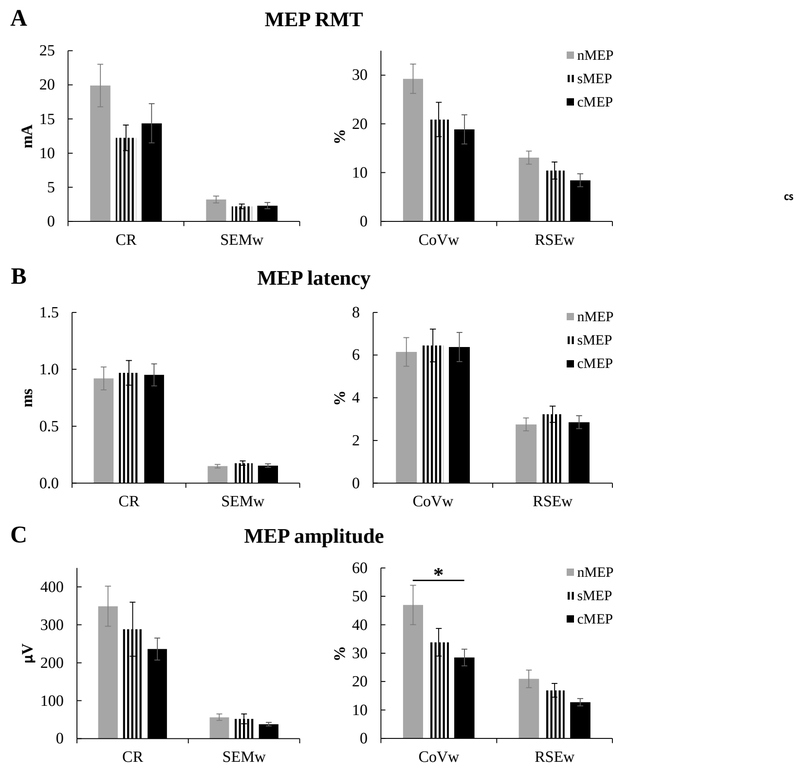

Figure 3. *Repeatability indices of MEP RMT (A), latency (B) and amplitude (C) obtained from mice stimulated with needle (nMEP: n = 10), screw (sMEP: n = 7) or cup electrodes (cMEP: n = 10). Data are expressed as mean ± SEM (*: $p < 0.05$).*

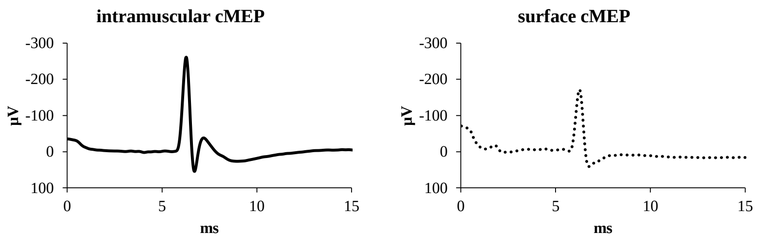

Figure 4. *Examples of MEP waveforms elicited by cup electrode and recorded with intramuscular needle (intramuscular cMEP) or surface plate (surface cMEP).*

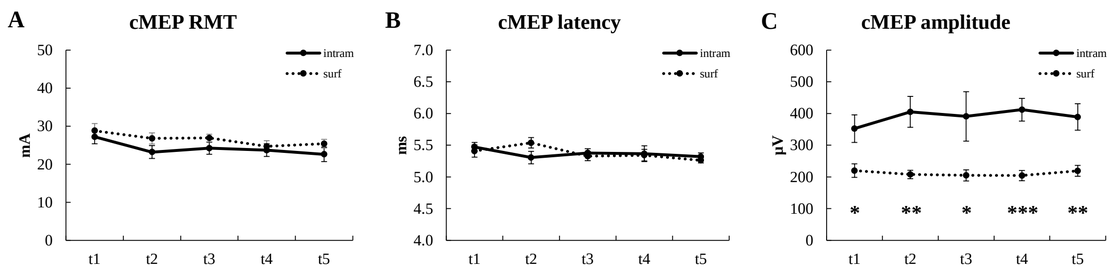

Figure 5. *cMEP RMT (A), latency (B) and amplitude (C) measured in mice recorded with intramuscular needle (intram: n = 10) or surface plate (surf: n = 10) at different timepoints. Data are expressed as mean ± SEM. Asterisks indicate significant differences between the two groups at each timepoint (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).*

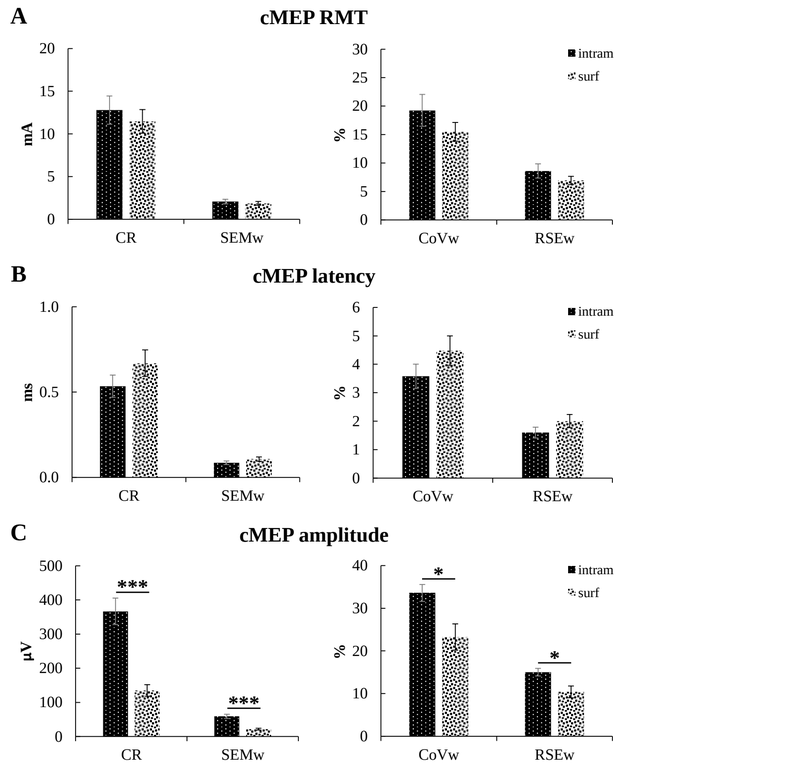

Figure 6. *Repeatability indices of cMEP RMT (A), latency (B) and amplitude (C) obtained from mice recorded with intramuscular needle (intram: n = 10) or surface plate (surf: n = 10). Data are expressed as mean ± SEM (*: $p < 0.05$; ***: $p < 0.001$).*

Conclusions

The first experiment demonstrated the interchangeability of the three different types of stimulating electrodes, since no significant differences in terms of MEP waveform, RMT, latency and amplitude were noticed over time. However, epicranial screws tended to detach from the skull, forcing to exclude three out of ten animals from the study, with the remaining seven mice that did not complete all the five recording sessions. In addition, invasive surgery could induce excessive suffering and stress, which are not indicated in studies involving animal models of autoimmune disorders, such as EAE. MEP amplitude presented a significantly lower CoVw in mice stimulated with surface cup electrode compared with subcutaneous needles. The intra- and inter-session variability of needle positioning under the scalp may reduce MEP amplitude repeatability, together with the relative small stimulated area of the motor cortex that could increase the variability of the motor tract that is activated. The electrical stimulation of a quite extended brain area that included the motor cortex, together with a wide and deep electrical field generated by the combination of surface cup and the relatively large sponge could have facilitated the activation of the whole motor cortex, without excessive between- and within-subjects fluctuations. In the second experiment, MEP amplitude obtained with surface electrode was significantly decreased with respect to intramuscular needle recording, probably because of both the presence of cutaneous tissue that could attenuate the electrical signal, and the summation of different responses deriving from several muscle fibers that contribute to the averaged evoked potential. Regarding the repeatability indices calculated from intramuscular or surface recording, it is noteworthy that for MEP amplitude, there was a significant improvement of all the repeatability indices calculated from surface with respect to intramuscular recording, confirming the hypothesis that the contribution of several muscle fibers recorded together with minimal variations in surface electrode positioning could substantially decrease MEP amplitude variability. In conclusion, after exploring different stimulation and recording protocols, this study showed that the combination of TES through surface cup electrode and surface MEP recording significantly improved MEP repeatability, assuring a valid protocol to explore the cortico-motor function in mice with important implications in clinical electrophysiology when testing murine models of neurological diseases.

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