Supplemental Material

Serum S100B measurement

Serum was obtained by centrifuging the blood samples for 10 min at 4000 rpm, and was kept frozen at -80°C until studied. Serum samples were analyzed for human S100B using commercial enzyme-linked immunosorbent assays kits (EMD Millipore, Billerica, MA, USA). The assays were performed in duplicate following the manufacturer's instructions. 50 μL of diluted standard, quality controls, and serum samples were incubated onto an antibody-coated microplate and incubated for 2 h on an orbital microplate shaker at 500 rpm. The wells were then washed five times with 300 μL of wash buffer. Then, 100 μL of antibody solution was added to each well and incubated for 90 min, with shaking at 500 rpm. After five washes, 100 μL of conjugate was added and the plate was incubated for 30 min, with shaking at 500 rpm. After five washes, 100 μL of substrate solution was coated and the plate incubated for a further 15 min in the dark. Then, 100 μL of stop solution was added and the absorbance was measured at 450 nm on a spectrophotometer. The S100B levels were determined using a standard curve of S100B. Mean coefficient variation was 3.4 ± 2.6% between duplicates. To minimize assay variation, the same analyst analyzed all serum samples on the same day in the same laboratory batch. S100B concentrations are expressed in pg/mL and the limit of detection was 2.74 pg/mL.

Neuromuscular excitability and activation

Peripheral nerve stimulation was used to measure peripheral voluntary activation (peripheral VA), muscle contractility (peak twitch), muscle excitability (M-wave), and spinal excitability (H-wave). The femoral nerve of the dominant leg was stimulated with a constant-current, high-voltage stimulator (DS7AH, Digitimer, Hertforshire, UK). The anode, a self-adhesive electrode (10 × 5 cm), was placed over the greater trochanter. The cathode, a ball electrode covered with damp foam, was placed over the femoral triangle (of Scarpa), 3 to 5 cm below the inguinal ligament. To determine the optimal location, the cathode was moved by small amounts while delivering pulses at 50 mA until the highest M-wave response was obtained over the vastus medialis. Markers were then set to maintain the anode position. A recruitment curve was performed at rest to determine which intensities to use during the protocol to elicit maximal M-waves (Mmax) and H-waves (Hmax). To determine the Mmax intensity, one pulse was delivered to the femoral nerve every 10 sec, with the intensity beginning at 50 mA and increasing by 10 mA until no further increase in twitch mechanical response and M-wave amplitude occurred. The maximal intensity at which maximal M-wave amplitude was reached was further increased by 10% (denoted supramaximal intensity) to ensure synchronous recruitment of all motor units. The supramaximal intensity was used to evoke M-wave at rest (Mmax) and during maximal voluntary contraction to deliver double-twitch pulses (doublet) at 100 Hz. Subsequent to Mmax intensity determination, the intensity at which the highest H-wave was observed was carefully sought (I_{Hmax}) using 2-mA increments. This intensity was used to evoke H-waves at rest (Hmax).

Transcranial magnetic stimulation was used to measure cortical voluntary activation (cortical VA) and corticospinal excitability. Single transcranial magnetic stimulation (TMS) pulses of 1-msec duration were delivered over the motor cortex using a Magstim 200 (Magstim Co., Whitland, UK). During the settings, TMS pulses were delivered during the course of an isometric submaximal voluntary contraction at 10% of the maximal quadriceps torque, because facilitation is often necessary to obtained a motor-evoked potential (MEP) on the lower limb.[1,2] The figure-of-8 coil was held over the contralateral motor cortex in the optimum scalp position to elicit MEP responses in the contralateral vastus medialis muscle. The contralateral motor cortex was first localized using the 10-10 electroencephalography (EEG) system (C3 point for right limb stimulation, C4 point for left limb stimulation). Then, the coil was moved by small amounts until the highest MEP response was obtained at 80% of the maximal stimulator power output. Markers were positioned over the scalp and over the coil to maintain the coil location. After that, a recruitment curve was performed during voluntary contraction at 10% of the maximal quadriceps torque in order to determine the maximal intensity.[3] One pulse was delivered every 10 sec with increasing intensity in steps of 2% until the highest response was obtained. A minimum of three pulses was delivered at each intensity to ensure reproducibility. The intensity at which the highest MEP was observed was then used during the protocol to assess cortical VA and corticospinal excitability.

References

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