Supplemental Data

Methods of neurophysiological investigations.

Large nerve fibre assessment was based on conventional nerve conduction studies. Recordings were made at the four limbs, using a Keypoint machine (Dantec, Skovlunde, Denmark). The studied parameters were as follows.

1. Upper limbs:
   - Compound muscle action potentials (CMAPs) were recorded in hand muscles to median and ulnar nerve stimulation at wrist and elbow, with measurement of CMAP amplitude (peak-to-peak), terminal motor latency and minimal F-wave latency to wrist stimulation, conduction velocity at forearm between wrist and elbow stimulation.
   - Sensory nerve action potentials (SNAPs) were recorded at wrist to median and ulnar nerve stimulation, with measurement of SNAP amplitude (peak-to-peak) and distal conduction velocity.

2. Lower limbs:
   - CMAPs were recorded in foot muscles to peroneal and tibial nerve stimulation at ankle and knee, with measurement of CMAP amplitude (peak-to-peak), terminal motor latency and minimal F-wave latency to ankle stimulation, conduction velocity at leg between ankle and knee stimulation.
   - SNAPs were recorded at ankle to superficial peroneal and sural nerve stimulation, with measurement of SNAP amplitude (peak-to-peak) and distal conduction velocity.
   - H reflexes (HRs) were recorded over the soleus muscles to tibial nerve stimulation at the popliteal fossa, with measurement of HR amplitude and latency.

Regarding small nerve fibre assessment, four neurophysiological tests were performed at four limbs as follows. Electrophysiological recordings were made using a Phasis II machine (EsaOte Biomedica, Florence, Italy).

1. Sensitivity to cold and warm stimuli was measured at the dorsum of the hands and feet by using a 16cm² Peltier probe connected to a TSA 2001 thermal sensory analyzer (Medoc, Ramat Yishai, Israël). Quantitative sensory testing (QST) consisted of cold and warm detection thresholds (CDT, WDT, in °C), which were determined using the method of limits. After an adaptation period at a neutral temperature of 32°C, temperature decreased (cooling down to 0°C) or increased (heating up to 50°C) at a linear rate of 1°C/sec, until the patient pressed a signal-button at the first perception of thermal sensation. Thresholds were determined as the average value from three trials.
2. Laser evoked potentials (LEPs) were recorded to laser stimulation of the dorsum of the hands and feet by using a CO2 laser (Neurolas, Electronic Engineering, Florence, Italy). Each stimulus consisted of a brief radiant heat pulse (wavelength: 10.6 mm, power: 9 W, beam diameter: 3.5 mm). Pulse duration was individually adjusted to evoke a reproducible painful sensation ranging from 60 to 70 on a 0-100 visual analogue scale. Habituation, sensitization, and nociceptor fatigue were minimized by slightly moving the laser beam between stimuli. LEPs were recorded using a scalp electrode placed at Cz, according to the International 10-20 EEG System, referenced to linked earlobes. A Velcro bracelet strapped around the left forearm served as ground electrode. The electro-oculogram was recorded with pre-gelled surface adhesive electrodes placed at the right infra-orbital lateral margin. Two blocks of 10 to 12 trials were performed and averaged off-line. Signal was bandpass filtered at 0.5-30 Hz and trials contaminated by eye-blinks, ocular movements (saccades), or any raw signal exceeding 70 µV were excluded from averaging. The N2-P2 complex was studied, with measurement of N2-P2 amplitude and N2 latency.

3. Sympathetic skin responses (SSRs) were recorded at the palms and soles following electrical stimulation of the trigeminal nerve at the supraorbital fossa, by using pre-gelled self-adhesive disposable surface electrodes (#9013S0241, Dantec, Skovlunde, Denmark). Signal was bandpass filtered at 0.1-100 Hz. The amplitude (peak-to-peak) and latency of SSRs were measured.

4. Heart rate was recorded with surface electrodes placed at both wrists. R-R interval variation (RRIV) was measured at rest and during deep breathing.