Supplementary Material

EMG Recording and TMS Procedure

Pairs of surface electrodes were placed in a belly-tendon montage over the ADM and the FDI right hand muscles. Electromyographic (EMG) signal was recorded using CED Power 1401 (Cambridge Electronic Design) connected to CED 1902 amplifier and interfaced with CED Spike software or using Biopac instrument (BIOPAC Systems). EMG signal was band-pass filtered (20 Hz-2.5 kHz), digitized (sampling rate 5 kHz) and stored for off-line analysis. A figure-of-8 coil connected to Magstim 200 Mono Pulse Transcranial Magnetic Stimulator (The Magstim Company, Carmarthenshire, Wales, UK) was placed over the left M1, contralateral to the recorded muscles. TMS coil was held by hand tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midline. This way the current induced in the neural tissue was directed approximately perpendicular to the line of the central sulcus, optimal for trans-synaptic activation of the corticospinal pathways (1). By using a slightly suprathreshold stimulus intensity, the coil was moved to determine the optimal position from which maximal amplitude MEPs were elicited in the FDI muscle. The optimal position of the coil was then marked on the scalp to ensure correct coil placement throughout the experiment. During video-clips presentation, magnetic pulse intensity was set at 120% of the resting motor threshold, defined as the minimal intensity able to evoke from both FDI and ADM muscles MEPs with amplitude of at least 50 μV with 50% probability (2).

Data Analysis

Neurophysiological data were processed off-line. Trials with EMG activity greater than 100 μV in the 150 ms prior to TMS were discarded from the analysis (less than 5%). MEP amplitudes were measured peak-to-peak (in mV) and were analyzed by two-mixed model ANOVAs (one for each muscle) with Group (two levels: AS vs C) as between subjects factor and Condition (4 levels: ‘Static’, ‘Pain’, ‘Touch’, ‘Tomato’) as within subjects factor. Post-hoc comparisons were carried out using the Newman-Keuls test.

Independent samples two tailed t-tests were used to show that the two groups were matched for age and IQ (Full scale, Verbal and Performance) and to assess group differences on several trait measures (AQ, SQ-R, EQ, IRI and TAS-20) and on subjective ratings of visual stimuli and pain attributes (VAS, McGill Pain Questionnaire and Hurts) (Table 2).

The Brown-Forsythe test was used to control for different variances between groups. Significant values were found for Intensity of Touch and Empathy during Touch videos ($p = 0.003$

and p = 0.013 respectively). Therefore, in these two conditions t-tests were corrected for unequal variances. Cohen's d [$d = (m_1 - m_2) / \sqrt{(\sigma_1^2 + \sigma_2^2)/2}$] (3) was calculated to address the effect size of the significant differences found between groups (Table 2).

In order to isolate the effect attributable to the observation of others' pain we computed a MEP amplitude change index by subtracting the response during the two dynamic control conditions 'Touch' and 'Tomato' from that evoked by the 'Pain' condition [Pain - (average of Touch and Tomato)] / [Pain + (average of Touch and Tomato)]. Two composite scores of subjective ratings of sensory and affective qualities of pain were created in order to combine slightly different aspects of a similar dimension and to facilitate understanding of results. The Sensory composite score includes MPQ Sensory, Hurt value and Pain Intensity, while the Affective composite score includes MPQ Affective and Pain Unpleasantness. Sensory and Affective composite scores were computed based on a factorial analysis performed on VAS and MPQ subscales during pain observation (4). Pearson correlation coefficients between MEP amplitude change index and self report questionnaires were calculated.

From a visual inspection of the correlation between MEP amplitude change and SQ-R (r = 0.29; p = 0.09) it was apparent that the two groups of participants had an opposite pattern (Figure 2 G). It was therefore decided to perform separate correlation analysis for each group. In fact separate correlation analysis highlighted that within the control group higher systemizers have reduced corticospinal inhibition during observation of others' pain (r = 0.49, p = 0.03); by contrast, in the group with AS this relation tended to go in the opposite direction (r = -0.51, p = 0.06). At this stage we have no explanation for this latter counterintuitive tendency.

Supplementary References

1. Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M (1992): Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *J Clin Neurophysiol* 9:132-136.

2. PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, *et al.* (1994): Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroenceph Clin Neurophysiol* 91: 79-92.

3. Cohen J (1992): Quantitative methods in psychology: a power primer. *Psychol Bull* 112: 155-159.

4. Avenanti A, Minio Paluello I, Bufalari I, Aglioti SM: The pain of a model in the personality of an onlooker: a single-pulse TMS investigation on embodied empathy for pain. *Neuroimage*, In Press.