**Edinburgh Pain empathy Study design**

**Research Questions and Hypotheses**

Nationality, is a real world market of group identity. However, to date there have been no studies investigating the effects of nationality on pain empathy, and whether or not in-group/out-group modulation is observed, as in studies of. Our central research question asks whether implicit presentation of a national flag modulates brain activation when processing the pain experience of in-group compared to out-group nationals. We hypothesise:

1. that implicit rather than explicit nationality cues will modulate neural activation when processing empathy for in-group and out-group nationals in pain. The triggering of a participant’s national identity (Scottish) by exposure to an implicit national (Scottish) flag, is expected to elicit greater activation in the neural circuitry associated with the processing of empathy for the pain of others when viewing in-group members in pain than when viewing out-group members in pain.
2. Specifically, covert presentation of the participant’s national flag is expected to elicit greater activation in the ACC and Anterior Insula when processing empathy for in-group members in pain than when processing empathy for out-group members in pain.

**Materials and Methods**

**Participants**

Button et al (2013) have noted the under-powered nature of many fMRI studies. These have are often been conducted on very small sample sizes. The most difficult aspect of estimating how many subjects are required is the challenge of estimating the critical parameters (Desmond and Glover 2002). Mumford and Nicholls (2008) have produced a method for power calculations but acknowledge its limitations as effect sizes are not normally known (Mumford 2012). Twenty to twenty five participants are required, at a minimum, for an fMRI study if it is to be repeatable and representative of the population (Friston, Holmes, & Worsley, 1999; Thirion, 2007). When effects are more variable or weaker, it has been argued that thirty participants are required (Seghier et al, 2008). Additional participants are required to replace scans that need to be excluded due to movement artifacts, neuroanatomical anomalies, and technical problems.

Thirty three healthy participants took part in this fMRI study. All participants in the study were healthy individuals recruited from community volunteers and were aged between 18 and 45 (15 females and 12 males).  Participants were free from mental illness, neurological disorders and previous brain injury, were right handed and were suitable for MRI scanning (for example, no metal implants or prostheses or claustrophobia). Suitability was confirmed by the MRI pre-screening form (Appendix 1) and by interview. Participants were paid £20 compensation for their participation in this experiment.

As the UK is a multi-national state, in which a resident of Scotland might identify as primarily Scottish or as British (or indeed as both) participants were pre-screened using the following question: ‘If you were forced to choose only one, you would identify yourself as: [Scottish/British/European/Other/English/Welsh/Northern Irish].’ Only those who self-identified primarily as Scottish were included in the study. Participants were not aware that this response was required for study inclusion, and relevant questions were embedded within a multi-focussed questionnaire designed to ascertain the suitability of participants for MR scanning. Six participants were excluded due to insufficient scan data and three participants were excluded due to incomplete questionnaire data. Analysis was conducted on the 24 remaining data sets.

**Ethics Statement**

This study recruited only healthy volunteers. The study did not involve clinical care or access to clinical records and was approved by the Research Ethics Committees of the School of Social and Political Sciences and the School of Psychology, Philosophy and Linguistics at the University of Edinburgh, in accordance with the Research Ethics procedures of the Clinical Research Imaging Centre, University of Edinburgh. All procedures, including written consent, were in accordance with APA guidelines and the Declaration of Helsinki.

**Procedure**

The study (E141333) was conducted at the Clinical Research Imaging Centre, and the Wellcome Trust Clinical Research Facility, at the University of Edinburgh, Queen Margaret’s Royal Infirmary, Edinburgh. On arrival at the scanning facility, participants were given a detailed overview of the study prior to their scanning appointment as well as a detailed participant information sheet (Appendix 2). Participants also completed all relevant MR safety questionnaires and consent forms (Appendix 3). Participants were reminded that they were free to withdraw from the study at any time without risk of personal consequence. They were then talked through the scanning procedure and allowed to change into appropriate clothing for their scan (for example, no metal elements). Participants were introduced to the CRIC radiographers. In the scanning room participants were allowed to familiarise themselves with the equipment and with the scanning environment. The procedures for communication with researchers and radiographers whilst in the scanner were explained and participants were reminded that they could ask to halt the study and to come out of the scanner at any point if they felt it necessary. Prior to scanning, participants were allowed to test and practice the experimental paradigm outside the scanner until they felt comfortable with the instructions and the response method. Participants were trained on how to rate pain intensity, and pain unpleasantness using a visual analogue scale (VAS). While the participants were receiving MRI scans, they were asked to view a series of 64 videos of individuals experiencing pain. Participants were asked to rate the intensity of the pain that the person being viewed was experiencing and also to rate how unpleasant that pain experience was for that person. Responses were recorded using MR compatible button presses.

All participants had previously been asked to complete a 60-item Empathy Quotient (EQ) questionnaire online (Baron-Cohen & Wheelwright, 2004) (Appendix 4). In addition, following their MRI scan, participants completed a further questionnaire. This provided demographic data and allowed us to quantify the salience of national attachment, in this case attachment to Scotland, for each participant. According to Sinnot (2005), the most effective available measure of sub-national, national or supra-national level attachment is a combination of categorical self-identification with a question that rates the importance or salience of the identification involved. Having been pre-screened as Scots identifiers, participants were asked to rate (a) how attached they feel to Scotland and (b) how proud they feel to be Scottish (Appendix 5, questions 9 (a) and 10). This combined measure allowed us to control for the extent to which their Scottish national identity was a more important or salient factor for some individuals than for others. This questionnaire was presented after the scanning session was completed, to avoid alerting the participants to the national identity-related focus of the experiment.

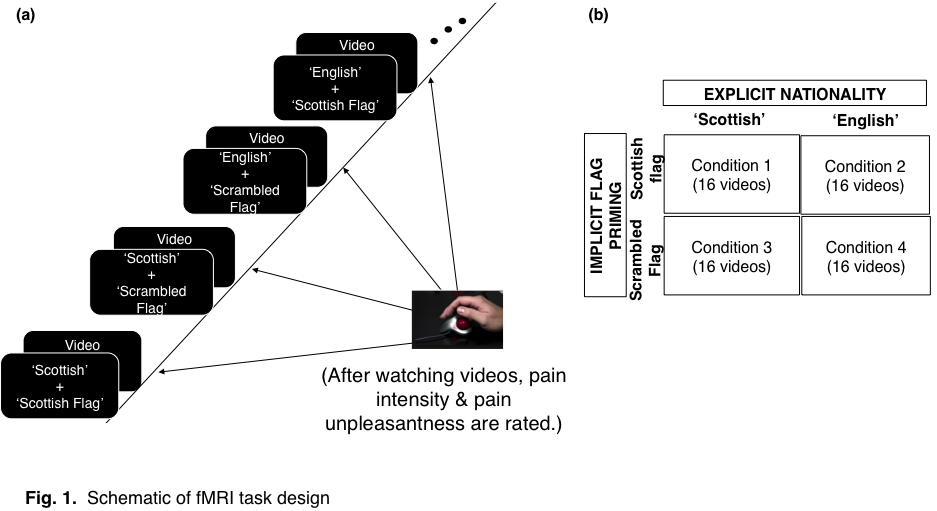
Following participation, participants were debriefed. At this point they were given more information about the study’s aims and were given the opportunity to hear more about the study results on completion, either by way of feedback meeting or written information sheets. Participants were also asked to comment on whether they were aware of the presence of the implicit prime. No participants reported awareness of the implicit prime.

**fMRI task**

The inferences to be drawn from this neuroimaging study relate to changes over a sequence of observations in a within subject design. These are based on neurophysiological indices of brain functions in an fMRI study. fMRI builds on the basic physical principles of Magnetic Resonance Imaging (MRI) and on our knowledge about the different magnetic qualities of oxygenated and deoxygenated blood. This difference forms the basis of what is know as the BOLD (Blood-Oxygenation Level Dependent) signal (Heuttel et al., 2014). This is used as an indirect measure of neural activity.

This study is based on forward inference. Participants took part in a well-established behavioural task that required them to consider and rate the previously-rated pain experience of others (Prkachin et al., 2008) whilst in the MRI scanner. Specific regions of interests were also identified on the basis of known associations between behavioural and neural responses drawn from earlier pain processing studies and meta-analyses (Poldrack, 2011). Regions of interest were defined anatomically, rather than being based on activation patterns in our data set. This helps to avoid circularity in our analysis or ‘double-dipping’ (Vul et al., 2009). Two key elements of experimental fMRI design relate to detection power (the ability to discern a signal) and estimation efficiency (the ability to distinguish the haemodynamic shape of the response to different stimuli, allowing comparison of amplitude and timing between stimuli). Block-designs typically maximize detection efficiency but have poorer estimation efficiency. Event-related designs, in contrast, suffer from weaker detection power but enjoy greater estimation efficiency (Ashby, 2011). This experiment employs a rapid event-related design with varying durations of stimuli presentation ensuring stimulus onset asynchrony (Friston et al., 2007).

During the fMRI sequence, participants viewed a series of videos of individuals experiencing shoulder pain (Prkachin & Solomon, 2008). Following each video, participants were asked to rate the level of the pain being expressed on a scale ranging from 0 (no pain) to 100 (most pain imaginable) (Price et al., 1994). Videos shown were sampled from the University of Northern BC-McMaster shoulder pain expression archive database (Lucy et al., 2011). This is a research database of videos of individuals expressing shoulder pain while performing movement tests. Videos are coded for the severity of the pain expressed by independent observers using the Facial Action Coding System (FACS) (Ekman et al., 2002) and by self-report. Videos sampled represented low and high levels of pain. Videos were sampled to represent individuals with a Northern European appearance. A total of 64 videos were shown to each participant, half of which showed individuals represented explicitly as ‘Scottish’ (same nationality) and half of which showed individuals represented explicitly as ‘English’ (different nationality). The explicit nationality cue was indicated as either “Scottish” or “English” appearing on screen before each video. (All individuals were, in fact, Canadian). In addition, half of the videos were preceded by an implicit prime of the Scottish national flag (Saltire), which was backward masked to ensure presentation below the threshold of conscious awareness, while the other half were preceded by a scrambled version of the flag. A scrambled version of the Scottish flag was created was created by randomising the blue and white colour of the Scottish flag using Presentation software (Neurobehavioural Systems, <https://www.neurobs.com>). The implicit prime was presented before half of the videos, counterbalanced across nationalities. Stimulus videos previously classified as either “high pain” or “low pain” using the facial action coding system (Ekman & Friesen 1978) were also counterbalanced across conditions. All stimuli were projected to participants via goggles in the MRI scanner and presented using Presentation software. To explore the impact of the implicit Scottish flag and the explicit nationality ‘Scottish’ or ‘English’ of the person in pain on participants’ behavioural and neural responses to viewing others in pain, we employed a 2 (Scottish flag prime v scrambled flag prime) X 2 (‘Scottish’ nationality v ‘English’ nationality) within subjects experiment design with high and low pain videos collapsed across all conditions (Fig. 1).



**MRI data acquisition**

Imaging data was acquired using a 3T Siemens Magnetom Verio Syngo whole-body MRI system (Siemens Medical Systems, Erlangen, Germany). Scanning consisted of a very short localiser scan to ensure head placement was optimal for subsequent scans. Structural images were acquired using a T1-weighted magnetisation-prepared rapid gradient echo (MPRAGE) sequence (TR 2300ms, TE 2.98ms, 256mm 93.8% FOV, 1mm slice thickness) and functional images were acquired using a T2\*-weighted gradient echo echo-planar imaging (EPI) (TR 2180ms, TE 27ms, 192mm 100% FOV, 3mm slice thickness, flip angle 90°) to acquire functional images. A total of 835 volumes were acquired for each participant in a single session (27 min 50s).

**Publication of results**

Results will be published in a peer-reviewed journal.