A mechanistic dissection of the attentional modulation of pain

1. Research Objectives

(1) Identify the network of brain areas that interact with the spinal cord to generate attentional analgesia.
(2) Determine the role of opioids and noradrenaline in attentional analgesia.

2. Study Design

2.1 Design

This functional imaging study of healthy adult volunteers (n=40) will use concurrent application of discrete thermal stimuli (painful and innocuous) presented during attentional tasks of graded difficulty to study the brain regions involved in attentional analgesia. We will probe the neurochemical mechanism using two drugs and compare against placebo. The study will run over four sessions in a randomised, blinded, crossover study design. This builds on a previous study (Brooks, Davies, & Pickering, 2017) that used a similar design but with the addition of spinal cord imaging and the probe drugs.

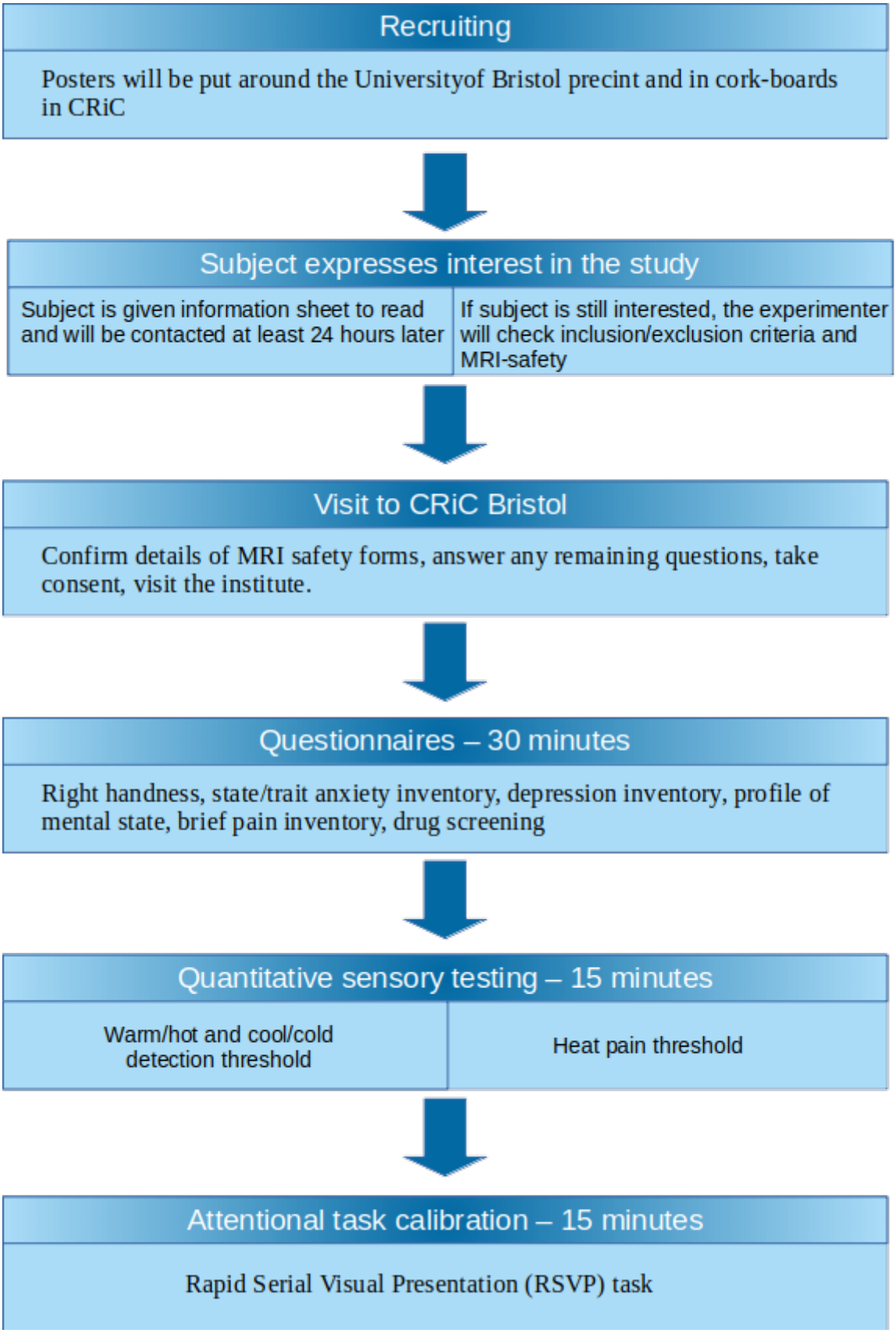

Figure 1: Initial session flow chart

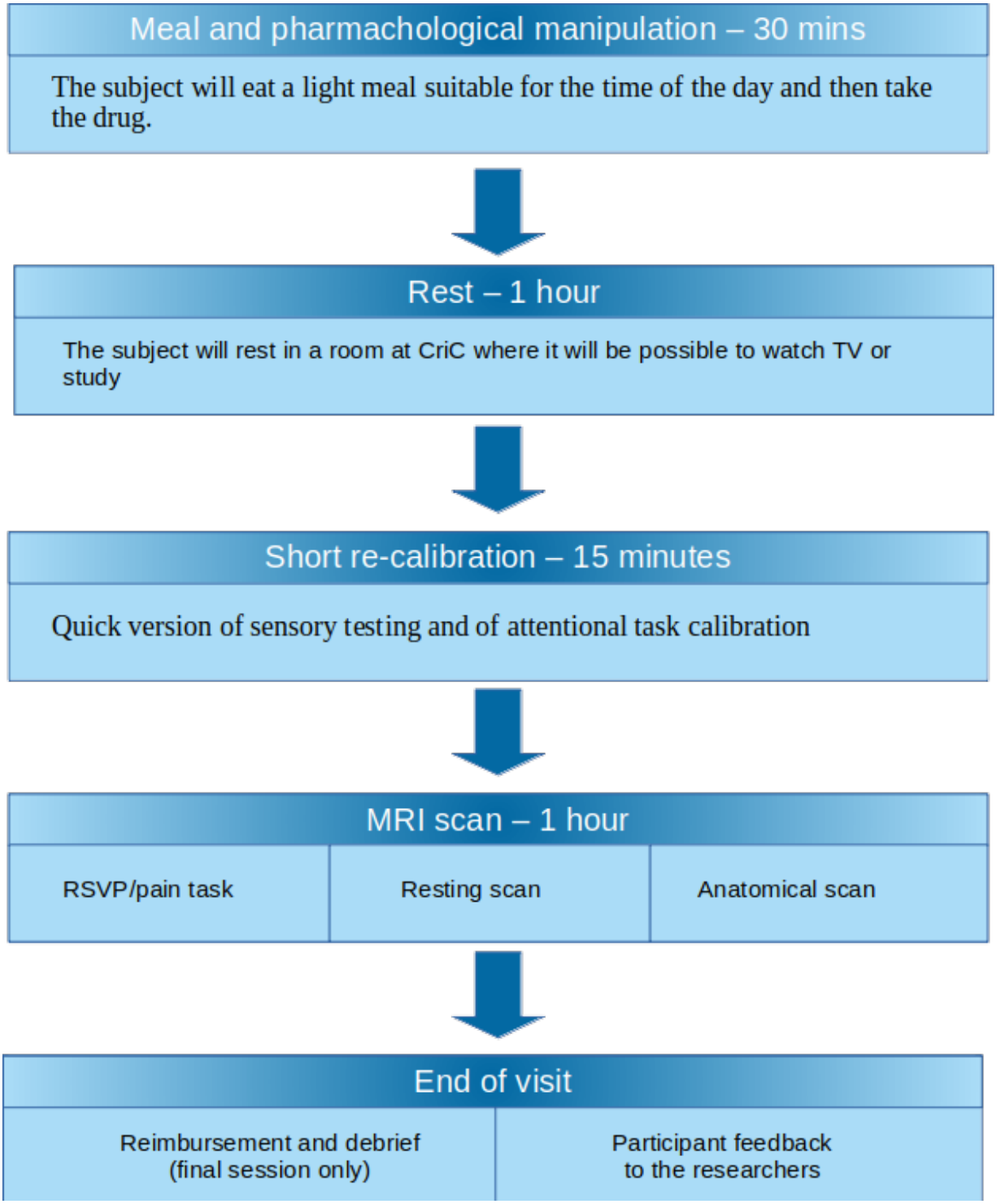

Figure 2. Imaging sessions flow chart.

The study design is outlined in the flowcharts above. All phases of the imaging sessions (Figure 2) will be repeated over the three sessions.

Visit to CRiCBristol: The details of the MRI safety form will be checked again to confirm that participants meet the MRI inclusion criteria. Participants will be encouraged to discuss any

questions or concerns they have about the project, and told that they are free to withdraw from the project at any time without providing a reason, and that this will not affect the standard of care they will receive. Written consent will be taken at this point and breakfast will be provided to the participant.

Questionnaires: We will ask participants to complete the right handedness, NIDA Drug Screening Tool, state/trait anxiety, depression, profile of mental state and brief pain inventory questionnaires. These will take approximately 30 minutes to complete.

Equipment: Thermal stimuli will be delivered by using a Pathway/CHEPS device (Medoc, Haifa, Israel). This investigational device is CE marked. The temperature of the device has an automatic cut-out set to 53°C, to avoid skin damage. Sensory testing will be performed by Ms Oliva and Dr Brooks, who has 10 years experience of using this equipment (Brooks et al., 2002; Bowsher et al., 2004; Brooks et al., 2005; Brooks et al., 2008; Brooks et al., 2012b; Brooks et al., 2017).

Quantitative Sensory Testing (QST): To determine the extent of nerve damage in each participant a series of warm/hot and cool/cold stimuli will be applied to the skin using the Pathway device and ATS thermode. We perform testing in a single site on the left forearm (C6 dermatome). The participant will be asked to press a button whenever they can feel a clear change in temperature, or when the stimulus becomes painful. We will be using the following testing procedures: thermal sensory limen, and the method of limits. This will take approximately 10 minutes to complete.

Pain thresholds: We will also measure participants' responses to a painful stimulus, which we will use during scanning to assess whether these signals are processed differently between the 2 participant groups. A series of hot thermal stimuli lasting 21 seconds will be delivered to the arm using the Pathway device and CHEPS thermode, and participants asked to rate how painful each one is using a numerical rating scale (i.e. 0 = not painful, 10 = most intense pain imaginable). By changing the temperature and repeating the stimulus (typically less than 6 times) we will determine the temperature required to reliably produce a pain rating of 6 out of 10. None of the temperatures used will be skin damaging. This will take approximately 10 minutes to complete.

Attention demanding task: participants will then be given a break from sensory testing, and asked to perform a simple recognition task, called a rapid serial visual presentation (RSVP) task. This task is used to provide a controlled cognitive challenge, which will then be used to investigate how much pain ratings are reduced when the participant is distracted from pain (see brain imaging below). The task is run on a computer running Presentation software (Neurobehavioral Systems Inc., Albany, CA, USA) using a mouse placed in their right hand, subjects watch a screen whilst a stream of letters and numbers appear at the centre. Their task is to press the left mouse button whenever they see the number "5". The task is repeated several times, with the speed of letter/number stream varied to make the task more difficult/easier. We do this to find a speed that each participant can reliably perform the task. This will take approximately 10 minutes to complete.

Brain imaging: participants will be asked to remove all metallic objects, jewellery, wallet, purse etc., and place these items into a locker, and will be reminded that they should make use of the toilet facilities before going into the scanner. Depending on their clothing we may ask participants to change into "pyjamas" (trousers & top) for their comfort during scanning. They will then be taken into the scanner room, and the scanning procedure explained to them. We will try to make the participant comfortable on the scanner bed, and provide them with the participant alarm button. When pressed this button generates an audible alarm in the scanner control room, and the operator will stop the scan immediately (within 5 seconds) and enter the scanner room to speak to the participant (typically within 15 seconds). During scanning the participant will have a rubber belt attached to their chest to monitor their breathing, and a pulse oximeter attached to their finger to

measure their heart rate – this way we can check on their welfare during the entire scan. The participant will have the MRI-compatible pain device (Pathway CHEPS device) placed on their left arm, and will hold a response unit (Cedrus Corporation, San Pedro, CA, USA) in their right hand. By using the buttons on the response unit they can respond to the RSVP task during scanning, and provide ratings of how painful each stimulus is. Imaging will be performed on CRiC Bristol's 3T MR system, using standard manufacturer provided MRI sequences.

The main experiment during scanning is the RSVP task. By simultaneously delivering painful thermal stimuli and the RSVP task, we can assess (1) how much participants' pain ratings are reduced when they are distracted from pain, (2) which brain, brainstem and spinal cord regions are active during pain reduction, (3) if naltrexone and/or reboxetine have an effect on this process. During the RSVP task subjects view a screen at the end of the magnet via a mirror placed above their eyes. If subjects have poor eyesight we can provide MR-compatible corrective lenses. Subjects will be asked to press a button whenever they see the number "5" on the screen, and will provide a rating of how painful each stimulus was using the buttons in their right hand to move a rating scale up and down. Subjects will be given time to practise this before going into the scanner. If at any time the thermal stimulation is too painful, participants can squeeze the alarm button in their left hand and the scanner/experiment will be stopped immediately. This part of the experiment lasts approximately 20 minutes, during which participants will receive 8 painful (hot) and 8 non-painful (warm) thermal stimuli, each lasting 21 seconds, separated by 45 seconds.

We will then acquire the remainder of the MRI scans, which do not involve any thermal stimulation or any specific participant involvement, other than they should try to stay as still as possible. The remaining scans consist of resting state blood flow measurement (duration 12 minutes) and finally high resolution anatomical scan (5 minutes). The high resolution anatomical scan will only be acquired on the first session.
We aim to have the total scan duration be no longer than 60 minutes for every session.

At any point during scanning if the participant is uncomfortable, needs the toilet, or no longer wants to continue they may press the patient alarm, and we will stop the scan immediately and check that they are okay.

The end of the visit: participants will be given £100, to cover their time, transport costs, drinks and food.

2.2 Inclusion/exclusion criteria

2.2.1 Inclusion:

(A) Adulthood
(B) Right hand dominant.

2.2.2 Exclusion:
(A) Chronic pain condition.
(B) History of neurological disease e.g. traumatic brain injury, Parkinson's disease, multiple sclerosis.
(C) Major psychiatric illness (e.g. clinical depression, schizophrenia).
(D) Regular or recent use of analgesic or psychoactive medications.
(E) Contraindication or allergy to study drugs (Reboxetine and Naltrexone).
(F) Inability to attend 3 visits or to comply with testing procedures.

(G) Weight greater than 150kg - a restriction of the MRI scanner bed
(H) Claustrophobia, to avoid including participants with brain abnormalities or at risk of anxiety, in particular, anxiety related to being in enclosed places.
(I) participants for whom it would not be safe to enter the MR environment. Contraindications for participation are:

1. Metal implants (internal defibrillator, cochlear implant, artificial heart valves, implanted drug infusion ports, metal dental work if not removable, cardiac pacemaker, artificial limbs or metallic joint prostheses, metal pins, screws plates or surgical staples, certain intrauterine device).
2. Tattoos with metallic ink or unremovable body piercing which might be attracted to the magnet used during fMRI scanning and to avoid image distortion.
3. Pregnancy, to avoid harm to the foetus.

See also MRI-safety screening forms and volunteer information sheet for MRI.

2.3 Recruitment

Participants will be recruited via posters and emails. If they accept to take part, we will confirm their suitability to participate (inclusion/exclusion criteria, MRI safety screening form), and answer any questions they may have about the study. Finally we will book them into the study and provide them with dates for their visits to the Clinical Research and Imaging Centre (CRiCBristol).

2.4 Intervention and randomisation

All participants will be randomly treated with placebo, Naltrexone and Reboxetine over the three sessions. Being a double blind experiment, neither the participant or the experimenter will know which of the three drugs will be used in a session.

3 Statistical considerations

3.1 Sample size

The fMRI-based power analysis employed novel methods developed (Mumford & Nichols, 2008) that are implemented in the fMRIpower software package (fmripower.org). This method estimates power for detecting significant activation within specific regions of interest, with the assumption that the planned studies will have the same number of runs per subject, runs of the same length, similar scanner noise characteristics, and data analysis with a comparable model. The effect sizes have been expressed in standard deviation (sd) units, which is analogous to the Cohens D measure. All power calculations are based on a region of interest (ROI) using the main effect of distraction mask of the PAG (obtained from (Brooks et al., 2017)with a p-value threshold of 0.005 for a 1-sided hypothesis test. With 40 subjects we will have at least
80% power to detect an effect of size of 0.4247 mean sd units in the PAG.

3.2 Analysis

3.2.1 Analysis of behavioural data
The cold, warm, hot pain and cold pain detection thresholds will be measured 3 times each and averaged.
To assess the optimal speed (70% of maximum d-prime score) for the RSVP task we will fit the d-prime score for each tested speed to a non-linear model (sigmoidal curve) using a least squares fitting routine (in Excel), and solve for the appropriate d-prime score.
Both pain ratings and task performance will be recorded and the effect of the experimental condition will be analysed using ANOVA in SPSS software.

3.2.2 Analysis of fMRI data

The fMRI data will be analysed with FSL (FMRIB Software Library). After appropriate pre-processing of the data (motion correction, spatial smoothing, high-pass temporal filtering and physiological noise correction), the fMRI data will be statistically compared within a scanning session using a general linear model (FEAT, FMRIB's Expert Analysis Tool); for example comparing BOLD contrast measurements between periods of rest and stimulation. The model will use pre-whitening (FILM, FMRIB's Improved Linear Modelling), and incorporate a physiological noise model (Brooks et al., 2008). Subsequently the estimated parameter maps can be compared at the group level using parametric statistics and a mixed effects model.

3.2.3 Analysis of resting state fMRI data

The resting fMRI data will be analysed with FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). After appropriate pre-processing of the data (motion correction, spatial smoothing, high-pass temporal filtering), independent components analysis (ICA) will be performed using MELODIC (part of FSL). This will identify temporally and spatially correlated activity in distinct regions of the brain and brainstem, which can then be compared across the group. The common components from these analyses can then be subjected to further analysis, to identify whether there are group differences (dual regression analysis, Zuo et al., 2010).

3.2.4 Analysis of relationships between the above outcome variables

Multivariate analysis of covariance and linear regressions will be used as appropriate to assess the relationship between behavioural variables and imaging data.

3.2.5 Psycho-physiological interaction (PPI) analysis

Changes in interactions between regions during the experiment will be analysed with a generalised PPI analysis, using FSL. Regions of interest will be defined in the previous analyses and will be used to investigate if other regions show an increased or decreased coupling during a specific experimental phase.

3.2.6 Dynamic Causal Modelling (DCM)

With the purpose of building a network of brain regions that underlies the attentional analgesia, we will use DCM in the Statistical Parametric Mapping (SPM) software (www.fil.ion.ucl.ac.uk/spm). We will build different possible DCMs and then see which one reproduce best the data thanks to Bayesian Model Comparison.