Statistical Analysis Plan

Study Title: The effect of seven day prucalopride administration on emotional processing in healthy volunteers

CT.gov registration: NCT03572790
Ethics Ref: R57219/RE003

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Sponsor:
University of Oxford
Funder (if applicable):
Part sponsored by the Wellcome Trust (AdeC)

Last edited 16.12.2020

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Abbreviations:

5-HT	Serotonin
ANOVA	Analysis of Variance
AVLT	Auditory Verbal Learning Task
BDI	Beck Depression Inventory
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependent
BRC	Biomedical Research Centre
CUREC	Central University Research Ethics Committee
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECAT	Emotional Categorisation Task
EMEM	Emotional Recognition Task
EPQ	Eysenck Personality Questionnaire
EREC	Emotional Recall Task
ETB	Emotional Test Battery
FERT	Facial Expression Recognition Task
(f)MRI	(Functional) Magnetic Resonance Imaging
FSL	FMRIB Software Library
NART	National Adult Reading Test
OHBA	Oxford Centre for Human Brain Activity
PANAS	Positive and Negative Affective Schedule
PI	Principal Investigator
PILT	Probabilistic Instrumental Learning Task
PIS	Participant Information Sheet
ROI	Region of Interest
SAE	Serious Adverse Event
SCID-V	Structured Clinical Interview for DSM-V
SHAPS	Snaith-Hamilton Pleasure Scale
SPSS	Statistical Package for the Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitor
STAI	Spielberger State-Trait Anxiety Inventory
VAS	Visual Analogue Scales

1. Synopsis

Study Title	The effect of seven day prucalopride administration on emotional processing in healthy volunteers
Brief Title	7 day prucalopride and emotional processing
Study Design	Double-blind placebo-controlled experimental medicine study.
Study Participants	Healthy participants
Number of Participants	50 (anticipated 25 per group)
Planned Study Period	24 months
Non-CTIMP Study	This study does not fit the MHRA definition of a clinical trial as we are not investigating the efficacy of prucalopride, but rather using it as a probe to understand the role of 5-HT4 receptors in emotional processing.
Intervention (s)	Prucalopride (Resolor) 1mg for 7 days versus placebo for 7 days

2. Introduction

2.1 Background

Depression is a common condition associated with a substantial health disability (Moussavi et al., 2007). Selective Serotonin Reuptake Inhibitors (SSRIs) are the most commonly prescribed antidepressants. However, for many patients these drugs have limited efficacy, a poor side-effect profile, and a slow onset of therapeutic action (Barbui & Hotopf, 2001). Pharmacologically, SSRIs produce an indirect activation of several serotonergic receptor subtypes. It is possible that more specifically targeting particular receptor subtypes may be a potentially useful approach to the development of antidepressant treatment that is more effective, better tolerated and faster acting. Recent evidence from animal studies suggests that agonists of the serotonin receptor subtype 4 (5-HT4) have rapid effects on depression- and anxiety-related behaviours. For example, administration of the 5- HT4 agonists RS67333 and prucalopride reduces immobility in the forced swim test (Lucas et al., 2007). Consistent with this, in a mouse corticosterone model of anxiety/depression, RS67333 produced rapid anxiolytic and antidepressant effects in a battery of tests, including the open field test, the elevated plus maze, the tail suspension test and the novelty suppressed feeding test (Mendez-David et al., 2014). Importantly, these effects were seen after only three days of treatment (Lucas et al., 2007), whereas they are usually only observed after chronic (2-3 week) treatment with conventional SSRIs. This raises the intriguing possibility that 5-HT4 agonism might lead to more rapid and sustained activation of 5-HT neurons than SSRIs, and therefore potentially more rapid reductions in symptoms of depression when used clinically.

Work in our group has revealed that short-term treatment with conventional antidepressants such as SSRIs produces positive biases in the processing of emotional information in healthy volunteers (Harmer, Goodwin, & Cowen, 2009). For example, seven days treatment with the SSRI citalopram decreased recognition of negative facial expressions and recall of negative vs positive stimuli (Harmer, Shelley, Cowen, & Goodwin, 2004). There is also evidence showing that antidepressants have early effects on neural activity, acting on limbic-cortical brain regions that are known to be dysregulated in depression (Ma, 2015). Indeed, a key study by Godlewska and colleagues from our group showed that 7-day treatment with citalopram reduced activity in the anterior cingulate, insula, amygdala and thalamus in response to fearful versus happy facial expressions. Importantly, depressed patients with the greatest reduction in neural activity across these areas went on to respond better to SSRI treatment (Godlewska, Browning, Norbury, Cowen, & Harmer, 2016)

Such effects might be important neuropsychological mechanisms in the mediation of clinical antidepressant action and may also be useful as biomarkers to screen for potential antidepressant activity of novel compounds. It is currently unknown whether activating 5-HT4 receptors has similar effects on emotional processing and neural activity. Such findings would be consistent with the animal studies and important further demonstration that 5-HT_4 receptor agonism might be a potentially useful avenue for antidepressant drug development. A previous study in our group revealed acute prucalopride administration (1mg) in 40 healthy volunteers potentiated learning and memory, whereby significantly more words were remembered in a test of auditory verbal learning and memory, and fewer false alarms were

made in a delayed emotional recall task relative to placebo. A single dose of prucalopride did not, however, positively bias emotional processing.

This current study will therefore test the hypothesis that short-term, seven-day administration of prucalopride will have positive effects on emotional processing and neural activity. Prucalopride (“Resolor”) is a 5-HT4 receptor agonist which is currently licensed for the symptomatic treatment of chronic constipation in adults. The standard dose given is 2 mg once daily with or without food, at any time of the day. Healthy volunteers will be randomised to receive seven days of prucalopride (1 mg i.e. half the standard dose) or placebo. On the seventh day of drug administration, they will complete a battery of computer-based tasks that measure emotional processing (tasks of attention, interpretation, and memory), non-emotional cognition, and undergo fMRI scanning during an emotional processing task and while resting (no active task engagement).

2.2 Responsibility for Statistical Analyses

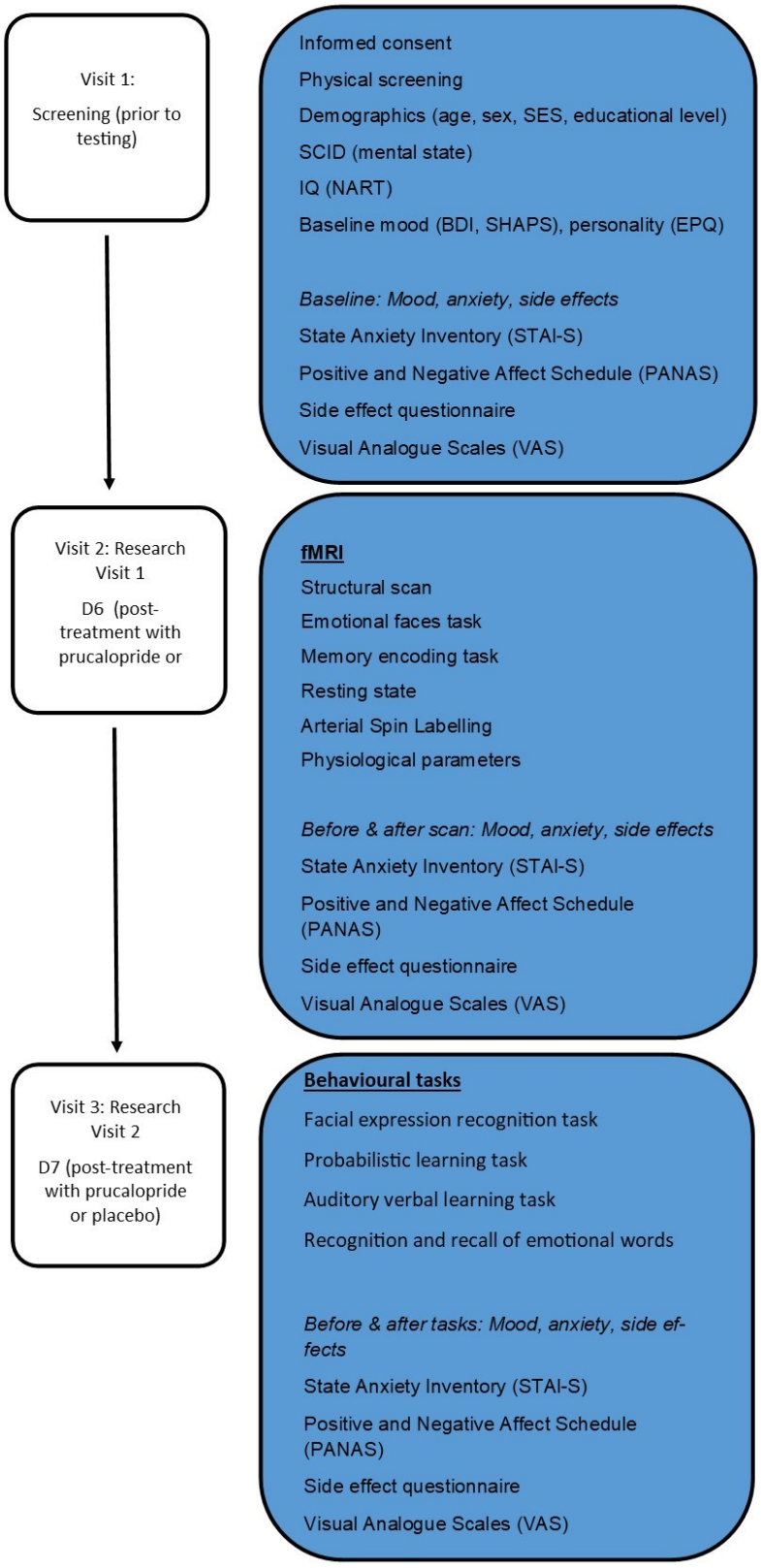
The study researchers will be responsible for performing and reporting the analyses of the cognitive task data, MRI data, and demographics and psychometrics data based on this SAP.

2.3 Reproducibility of Statistical Analyses

A copy of all raw research data (questionnaire, task data, MRI scan data) will be kept on secure university computers so that all data processing and analyses on the data can be replicated.

4. Study Design

4.1 Summary of study design



This is a double-blind placebo-controlled parallel group experimental medicine study, which will be conducted at the University of Oxford, Department of Psychiatry. The site will recruit 50 healthy volunteers between the ages of 18 and 40. Study staff will identify potentially eligible participants from the general public, who will be made aware of the trial through advertisements on local information websites (e.g. Daily Info, Oxford University Gazette), newspapers, local magazines, on the radio and on the lab webpage, Facebook page and Twitter account, and in universities and other public places. Each participant will attend 3 study visits in total which will be conducted at the Department of Psychiatry. After having confirmed initial study eligibility by phone or email (*Pre-Screening),* participants will be invited to the department for the *Screening Visit* (2 hours). If this visit qualifies them as eligible, participants will be recruited into the study and will be invited for visits 2 and 3 on two consecutive days, no later than 1 month after the Screening Visit. Visit 2 will be the *Research Visit 1* (~1.5 hours), and visit 3 will be the *Research Visit 2* (~3 hours), where behavioural and neural parameters will be obtained. Self-report clinical symptom severity (mood, anxiety and side effects) will be measured on all visits, and before and after testing on visit 2 and visit 3.

4.2 Participants

50 healthy volunteers aged between 18 and 40 years

Inclusion Criteria
• Participant is willing and able to give informed consent for participation in the study
• Not currently taking any medications (except the contraceptive pill)
• Male or female
• Aged 18-40 years
• Sufficiently fluent English to understand and complete the task
• Right handed
• Body Mass Index in the range of 18-30

Exclusion criteria
The participant may not enter the study if ANY of the following apply:
• Not fluent in English
• Any past or current Axis 1 DSM-V psychiatric disorder
• Current usage of psychoactive medication (except the contraceptive pill, the Depo-Provera injection or the progesterone implant)
• Current usage of any medication that will influence the MRI scan
• Current or past history of drug or alcohol dependency
• Currently pregnant or breastfeeding
• Study visits due to take place during the premenstrual week
• Not right handed
• Body Mass Index outside the range of 18-30
• History of cardiac, thyroid, or liver problems
• An autoimmune disorder
• Current, or a history of, gastrointestinal disorder or irritable bowel syndrome

• Epilepsy
• Known lactate deficiency or any other problem absorbing lactose, galactose, or glucose
• Participation in a study which uses the same computer tasks as those used in the present study
• Participation in a study that involves the use of a medication within the last three months
• Smoker > 5 cigarettes per day
• Typically drinks > 6 caffeinated drinks per day
• Any contraindication to MRI scanning (e.g. metal objects in your body, pacemakers, significant
claustrophobia)

4.3 Methods of assigning subjects to treatment groups

Eligible participants will be randomised to receive either seven-day prucalopride, or seven-day placebo administration. Randomisation will occur on the day of screening, or up to a maximum of four weeks after screening.

The randomization code will be drawn up by a researcher not involved in the study using an online randomization tool (www.sealedenvelope.com). Randomisation will be stratified for gender and blocked with a block size of 4.

4.4 Blinding

This is a double-blind study. Volunteers and investigators performing the assessments, scans and data analysis will remain blind to the identity of the treatment from the time of randomization until database lock and unblinding. The randomisation code will be kept confidential and will only be accessible to research team not involved directly in data collection and analysis prior to unblinding. Urgent unblinding of a particular participant would also occur in the case of emergency in the opinion of medically-trained personnel.

4.5 Determination of Sample Size

We will be using the Emotional Test Battery, which is a well validated set of emotional processing tasks that are sensitive to antidepressant effects. On the facial expression recognition task (FERT), one of the main outcome variables is accuracy at recognising fearful facial expressions and this is the variable used for power calculations. Based on data acquired in (Harmer et al., 2004) comparing citalopram to placebo, if we aim for 0.9 power and a 0.05 false positive rate, a suggested total sample size is 38 (G*power) to ensure determination of group level differences at this variable if they exist. As $5HT_4$ agonism is less well studied and the dose required of prucalopride for optimal agonism at the receptor is uncertain, we will aim for 25 individuals per group (total sample size of 50).

5. Changes in the Conduct of the Study or Planned Analyses

5.1 Changes in the Conduct of the Study

There were no changes to the planned conduct of the study.

5.2 Changes from the Analyses Planned in the Protocol

Following analysis of previous work in the research group using a single-dose of Prucalopride, we made minor amendments to the protocol in terms of the hierarchy of outcome measures. These changes were updated on clinicaltrials.gov (NCT03572790) and were resubmitted to the local ethics committee.

The planned analyses were amended in light of the unforeseen circumstance that it is not possible within FSL to add functional or structural masks to data that has been through an fmriprep pipeline due to current restrictions. Therefore, we reverted to our lab's existing fMRI analysis method where data is preprocessed using the FSL pipeline. Minor changes were made to analysis methodology to bring this in line with a typical FSL processing method.

There was also an error in the initial version of the SAP. We had planned to use data from Walsh et al 2018 to create a functional mask for the faces data. Unfortunately, this was not possible, as Walsh et al 2018 used a previous version of the faces task, and during an early stage of the analysis, we discovered that the old and new versions of the faces tasks worked very differently from each other. Therefore, we used an alternative method of creating a functional mask across both tasks for consistency, detailed in this SAP.

This amended version of the SAP was uploaded to clinicaltrials.gov (NCT03572790).

6. Outcome Variables

6.1 Schedule of Assessments

Procedures	Pre-screening Phone	Screening Visit VISIT 1	Scan Visit VISIT 2	Testing Visit VISIT 3
Send/ present PIS	**X**	**X**		
Eligibility assessment	**X**	**X**		
Written informed consent		**X**		
SCID		**X**		
Medical history		**X**		
Physical examination		**X**		
Sociodemographics		**X**		
Baseline mood (BDI, SHAPS), personality (EPQ) and cognitive (NART) trait measures		**X**		
Randomisation		**X**		
State and Trait Anxiety Questionnaire (State and Trait form of the State-Trait Anxiety Inventory STAIS)		**X**	**X (BEFORE AND AFTER)**	**X (BEFORE AND AFTER)**
Positive and Negative Affect Schedule (PANAS)		**X**	**X (BEFORE AND AFTER)**	**X (BEFORE AND AFTER)**
Side effect questionnaire		**X**	**X (BEFORE AND AFTER)**	**X (BEFORE AND AFTER)**
Stress test response (VAS)		**X**	**X (BEFORE AND AFTER)**	**X (BEFORE AND AFTER)**
Facial Expression Recognition Task (FERT)				**X**
Probabilistic learning task (PIL)				**X**
Auditory verbal learning task (AVLT)				**X**
Categorisation, recognition and recall of emotional words				**X**
Oxford Memory Test (OMT)				**X**
fMRI – Emotional Faces Task			**X**	
fMRI – Memory Task			**X**	
fMRI – Resting State			**X**	
MRI – ASL			**X**	
Physiological parameters, eye tracking			**X**	

*To be undertaken after baseline testing;

Abbreviations: PIS = Participant Information Sheet; SCID = Structured Clinical Interview for DSM diagnoses; BDI = Beck Depression Inventory; VAS = Visual Analogue Scale; EPQ = Eysenck Personality Scale; NART = National Adult Reading Test; (f)MRI = (functional) magnet resonance imaging; ASL = Arterial Spin Labelling; OMT = Oxford Memory Test

6.2 Objectives and Endpoints

These outcomes have been pre-registered on clinicaltrials.gov (NCT03572790). Primary outcomes are identified by bold typeface.

Objectives To investigate the effects of seven day prucalopride administration compared to placebo on:		Outcome measures / endpoints
Primary	Recognition of positive and negative facial expressions	**Accuracy to recognise computer-based positive and negative facial expressions (anger, disgust, fear, happy, sad, surprise)** Misclassifications (number of responses to each facial expression category incorrectly classified as another facial expression category) Reaction time to recognise facial expressions
	Performance on Auditory Verbal Learning Task (AVLT)	**Accuracy on AVLT (number of items correctly recalled across trials)** Number of intrusions (words incorrectly recalled) and number of repetitions (words repeated within the same trial) Number of hits and false alarms in the delayed recognition test
Secondary	Neural response to emotional faces	Blood Oxygen Level Dependent (BOLD) signal in areas including the amygdala, anterior cingulate cortex, and orbitofrontal cortex
	Neural response to novel vs. repeated scenes	Blood Oxygen Level Dependent (BOLD) signal to scenes that have previously been seen compared to novel scenes in areas including the hippocampus and parahippocampal regions
	Reward sensitivity as measured by the Probabilistic Instrumental Learning Task (PILT)	Amount won, amount lost, and total monetary amount earned Accuracy (correct or incorrect symbol choice) and proportion of group choosing correct symbol per trial

	Categorisation, recall, and recognition of emotional words on emotional memory tasks	Emotional Categorisation Task (ECAT): accuracy and reaction time to classify positive and negative descriptor words Emotional Recall Task (EREC): number of words correctly recalled (hits) and number of words incorrectly recalled (false alarms) Emotional Recognition Task (EMEM): accuracy and reaction time to correctly recognise positive and negative words (hits), and number of incorrectly recognised words (false alarms)
	Vigilance to fearful and happy faces on the Facial Dot Probe Task (FDOT)	Vigilance scores derived from reaction time
	Resting state connectivity	Resting state connectivity (using resting state fMRI) within and between networks including the default mode, salience, executive control, affective and limbic networks, identified via correlations between spontaneous BOLD activity in spatially independent regions while participants are not actively engaged in an experimental task
	Relative and global cerebral blood flow	Relative and global cerebral blood flow identified using Arterial Spin Labelling (ASL)
	Visual short term memory on the Oxford Memory Test (OMT)	Accuracy: proportion of correct probe selections, absolute error for probe location, proportion of misbinding errors Reaction time

7. Statistical Methods

7.1 Overview

The statistical analysis will be conducted on the behavioural, MRI, resting state, and psychometric data after completion of recruitment and data collection. The data will be statistically compared between the two groups (prucalopride and placebo).

7.2 Data Cleaning

Data cleaning of the behavioural data and fMRI data will be performed prior to unblinding. Cleaning of behavioural data (i.e. accuracy and reaction times) from the emotional processing tasks will be checked to ensure the tasks have been completed appropriately by

a clinical data analyst at P1vital Products. Specifically, trials where participants show unusually low response times (< 200ms) or high response times (> 6000ms) will be excluded. Similarly, participants who show abnormally low accuracy on the emotional tasks will be excluded (<50% correct responses on the FERT and FDOT). Cleaning of behavioural data from the non-emotional cognition tasks will be performed by the research team, coordinated by the Principal Investigator. Extreme outliers will be excluded from each group on a per task basis. The fMRI data cleaning will be performed by the research team, with guidance from BRC Imaging Support. fMRI data will be visually checked for excessive movement and other artefacts such as signal drop out, as well as to ensure all required data files are present. Formal MRI quality assessment will be undertaken using the MRIQC package (https://mriqc.readthedocs.io/en/stable/index.html), with data considered for rejection if falls outside the normal range of values in the derived image quality metrics. Following collection of all data to be analysed, a final data inclusion meeting will be held with the Principal Investigator prior to unblinding. This will review data quality and confirm exclusion of data from specific tasks. Data will be excluded on a per task basis—that is, a participant may have data excluded for one task, but have all other data available for analysis. Analyses of each task will then be performed on all randomised subjects who have data available for that task.

7.3 Software

Behavioural Acquisition:
Qualtrics.XM (https://www.qualtrics.com) - screening and research visit questionnaires
P1vital® Limited Products – Emotional Test Battery
Neurobehavioral Systems Presentation software (https://www.neurobs.com) - PILT, memory encoding task
Oxford Memory Test application “Short_Fractals1” – modified from “What was where task” (Pertzov et al., 2013) running on iOS 12.3.1

Behavioural Analysis:
IBM SPSS Statistics 25 or latest version
Mathworks MATLAB_R2018a

MRI stimulus presentation: Psychopy version 1.84.2 (http://www.psychopy.org)
MRI physiological measurement acquisition: Biopac MP150 system, software level 3.0.0.4 (https://www.biopac.com), Eyelink ® (https://www.sr-research.com)
MRI analysis:

heudiconv 0.5.4 (https://github.com/nipy/heudiconv);
MRIQC (latest version available) (https://mriqc.readthedocs.io);
FSL 6.0 or latest version available (https://process.innovation.ox.ac.uk/software/p/9564/fslv5/1);
BASIL (latest version available) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL);
Quantiphyse (latest version available) (https://process.innovation.ox.ac.uk/software/p/14419/quantiphyse-academic-workflow/1)

All final versions of software and computing environments will be fully documented for computational reproducibility of analysis.

8. Statistical Analysis

8.1 Analysis Populations

The population used for the analysis of data in this study will be:

- All randomised subjects for non-fMRI data, except for withdrawn participants who will not be included in the analysis.
- As per protocol set for fMRI data (criteria: fMRI data available). fMRI data will not be available for all participants per se depending on need to exclude / withdrawal.

8.2 Handling missing data

Withdrawn participants' data will not be included in the analysis.

8.3 Measurements of treatment compliance

Participants were given a pill bottle containing 9 capsules of prucalopride or placebo: seven for daily administration over the study period, and two spare in case participants dropped or lost a capsule. Participants were sent daily text or email reminders to take their capsule. On day 7 (Research Visit 2), participants were asked their adherence to the seven day schedule. Participants were asked to return their pill bottle and the researchers recorded how many pills remained, along with any reasons for greater or fewer than two pills remaining.

8.4 Analysis of outcome parameters

Details of the study endpoints can be found in section 6. All key endpoints will be summarized (mean, standard deviation) in tables and bar charts (mean ±SEM).

8.4.1 Demographic data and psychometric data

To assess changes in subjective mood and anxiety before and after prucalopride/placebo administration, mixed model ANOVAs will be performed with time as a within-subjects factor and group as a between-subjects factor on STAI-S, PANAS, and VAS ratings. Post hoc analyses using independent samples t tests will follow up any significant interactions. A generalised linear model will be used to analyse side effects.

8.4.2 Behavioural data

When conducting t tests, degrees of freedom will be corrected where the assumption of equal variances between groups is violated (i.e. Levene's Test is significant). When conducting ANOVAs, the Greenhouse-Geisser procedure will be used to correct the degrees

of freedom where assumptions of equality of variance are violated. The within-subjects factors for each task, and the contrasts tested in the ANOVA, are included in a table detailing the planned repeated measure ANOVA comparisons. Post hoc independent samples t tests will be performed to follow up any significant interactions found in ANOVAs. If there is a significant group x condition interaction found, we will not use the Bonferroni correction for multiple comparisons.

8.4.2.1 Primary outcomes

Facial Expression Recognition Task (FERT):
As in previous work (Harmer et al., 2004, Harmer et al., 2009), data will be analysed using repeated measures analyses of variance (ANOVAs). Group (prucalopride or placebo) will be the between-subject factor, and emotion (6 levels) will be the within-subject factor.

Auditory Verbal Learning Task (AVLT):
The number of correctly recalled words on the following trials will be calculated: List A immediate recall trials 1-5. A repeated measures ANOVA will be used to examine if there is an effect of prucalopride administration on word recall (between subject factor: group; within-subject factors: List A acquisition trial). Independent samples t-tests will be conducted to test if there is a group difference in the number of words accurately recalled after a short or long delay (List A short and long delay trials respectively, and the number of intrusions (incorrect words not present in list) and repetitions (correct words recalled more than once in the same acquisition trial) across List A acquisition trials. Independent samples t-tests will also be used to compare List B immediate recall and delayed recognition performance (number of hits and false alarms) between the groups.

8.4.2.2 Secondary outcomes

Probabilistic Instrumental Learning Task (PILT):
In line with previous work (Walsh, Browning, Drevets, Furey, & Harmer, 2018), independent samples t-tests will be used to compare the amount won, the amount lost, and the total monetary amount earned during the task between prucalopride and placebo groups. The proportion of each group (prucalopride/placebo) who choose the correct symbol in the win condition of the PILT (associated with high probability win) and the loss condition (associated with low probability loss) will be calculated, and plotted on a learning curve to determine where learning plateaus. A mixed model ANOVA will be carried out on the proportion of participants choosing the correct symbol over the trials where learning has plateaued (between subjects factor of group [placebo/prucalopride], within subjects factor of condition [win/loss]). Computational analyses will be conducted on the PILT by fitting a reinforcement learning model to participant choice data. Learning rate and decision temperature parameters will be calculated for the win and loss outcomes. Mixed model ANOVAs with within-subjects factor of learning rate or decision temperature parameter, and between-subjects factor of group, will be used to further characterize the effect of the drug on the task, and complement the non-model based analysis (Pulcu & Browning, 2017).

Categorisation, recognition, and recall of emotional words:

Similar to previous work (Harmer et al., 2009), data will be analysed using mixed model analyses of variance (ANOVAs). Group (prucalopride or placebo) is the between-subject factor, and emotion (positive or negative) and recall or recognition accuracy measures (hits or false alarms) are within-subjects factors.

Vigilance to fearful and happy faces (FDOT):
Data will analysed using mixed models analyses of variance (ANOVAs). Group (prucalopride or placebo) is the between subjects factor and emotion (positive or negative) and probe duration (masked or unmasked) are the within subjects factors.

Visual short term memory as measured by the Oxford Memory Test (OMT)
Data will analysed using mixed models analyses of variance (ANOVAs). Group (prucalopride or placebo) is the between subjects factor and trial condition (1 or 3 memory probes) is the within subjects factors. Analyses will be conducted for accuracy and reaction time.

Planned repeated measure ANOVA comparisons:

Task	**Within Subject Factors**	**Number of Levels**	**Outcomes Used**	**Critical Contrasts Tested in the Analyses**
FERT	Face Emotion	6 (Fear, anger, happy, surprise, disgust, sad)	1. % Accuracy 2. Reaction time 3. Misclassifications	1. Face emotion x treatment group
ECAT	Word Valence	2 (Positive, negative)	1. Accuracy 2. Reaction time	1. Word Valence x treatment group
EREC	Word Valence Accuracy	2 (Positive, negative) 2 (Hits, false alarms)	1. Number of words recalled (correctly and incorrectly)	1. Word valence x treatment group 2. Word valence x accuracy x treatment group
EMEM	Word valence Accuracy	2 (Positive, negative) 2 (Hits, false alarms)	1. Number of words recognised (correctly and incorrectly) 2. Reaction time	1. Word Valence x treatment group 2. Word valence x accuracy x treatment group

FDOT	Face Emotion	2 (Fearful, happy)	1. Vigilance scores derived from reaction time	1. Face emotion x treatment group 2. Face emotion x probe duration x treatment group
	Probe Duration	2 (Masked, unmasked)		
AVLT	Trial	5 (List A acquisition trials 1-5)	1. Number of words recalled 2. Number of intrusions 3. Number of repetitions	1. Trial x treatment group
PILT	Trial type	2 (Win, loss)	1.Proportion of people choosing correct symbol	1. Trial type x treatment group
OMT	Trial Condition	2 (1 probe, 3 probes)	1.Proportion of correct probe selections 2. Absolute error for probe location 3. Reaction Time 4. Proportion of misbinding errors	1.Trial condition x treatment group

8.4.3 MRI data

fMRI analysis will be performed using open source and custom analysis tools.

8.4.3.1.1 Data acquisition and transfer

Data will be acquired on a 3 Tesla Siemens Prisma using the sequence acquisition parameters stored with this SAP on OSF. Data will be transferred from the scanner server to the high performance computing analysis cluster in Digital Imaging and Communications in Medicine (DICOM) format. Data will then be converted to Brain Imaging Data Structure (BIDS) format nifti files using heudiconv. Formal quality assessments will be undertaken prior to unblinding with MRIQC.

Ancillary physiological data will be collected during MRI scanning and may be used to remove noise in the brain data. These data include pulse oximetry, respiratory activity and eye gaze location.

8.4.3.1.2 Pre-processing

Pre-processing will be carried out using the FSL pipeline, using FEAT (both FLIRT and FNIRT). This will include:

- Brain extraction
- Spatial smoothing
- High pass temporal filtering
- B0 unwarping using fieldmap images
- Motion correction MCFLIRT

- Registration of EPI images to T1w images and to MNI standard space

fMRI resting state analysis will be undertaken using FSL Melodic for noise component removal.

Perfusion: The ASL data will be processed using the BASIL tools from the FSL suite. This procedure results in a statistical map estimate of local cerebral blood flow for each participant.

8.4.3.2 First level analysis

8.4.3.2.1 Task data

Mass Univariate Analysis: First level analysis will be conducted using FSL to investigate whether brain activity varies during a task within individual participants. For each participant, task specific regressors describing the onset and duration of task relevant events will be defined for each participant. These regressors will be convolved with a gamma haemodynamic response function (HRF) and the temporal derivative to account for variations in HRF across volume slices or brain regions. A high pass filter is then calculated by FSL to define the highest frequency which would be expected in the data given the temporal structure of the task (calculated using the 'cutoffcalc' algorithm to retain 90% of the expected signal after filtering), and applied to both the convolved task structure and the pre-processed brain data. The pre-processed data is also smoothed with a 4-5 mm full width half maximum (FWHM) kernel which is appropriate to detect effects in small structures such as the amygdala. A general linear model (GLM) is then constructed containing the convolved task structure. We will consider including nuisance regressors representing the sources of physiological noise in the fMRI signal. These regressors will be created using the PNM tool of the FSL package for pulse oximetry and respiratory bellows data collected during the tasks, and custom scripts eye gaze direction. Gaze information may be used to remove trials where participants were not correctly fixating on the task, or account for variance in activation between participants or groups where systematic variations in gaze location are observed. The purpose of these regressors is to account for noise in the data and thus increase the sensitivity of the analyses. The model is regressed against the smoothed, pre-processed BOLD data to generate maps (betas) of how well the data in each voxel is explained by the model (after the effects of the nuisance regressors are removed if these are being used). Explicit task effects are determined by the beta weights of each individual task relevant event, which are contrasted between conditions to address the experimental questions. Testing of the GLM will employ FMRIB's Improved Linear Model (FILM) prewhitening of the BOLD data to allow robust estimation of task relevant betas with consideration of autocorrelation in the voxel time series.

The tasks specific regressors will be:

Emotional Faces Task: Two regressors coding for (1) blocks of fearful faces and (2) blocks of happy faces (the rest period between blocks of images will be modelled explicitly). Planned contrasts are: i) all faces (the mean activation) [0.5 0.5 -1]; ii) fear only [1 0 -1]; iii) happy only [0 1 -1]; iv) fear > happy [1 -1 0]; v) fear < happy [-1 1 0]. These contrasts will

identify regions which are related to the processing of emotional content in faces and specific to each emotion. The experimental question will be addressed by the contrast fear > happy.

Memory Encoding Task: Two regressors coding for (1) blocks of novel scenes and (2) blocks of repeated ("familiar") scenes (the rest period between blocks of images will be modelled implicitly). Planned contrasts are: i) all images (the mean activation [1 1]; ii) novel only [1 0]; iii) familiar only [0 1]; iv) novel > familiar [1 -1]; v) novel < familiar [-1 1]. These contrasts will identify regions related to memory encoding. The experimental question will be addressed by the contrast novel > familiar.

8.4.3.2.2 Resting state data

For the resting state fMRI, an independent component analysis (ICA) is performed on the concatenated first level data from all subjects. This analysis will yield statistical maps of the brain which show the correlation of each voxel with a maximally independent temporal signature if that component. Each independent component is thus characterized by a map and the time-series (prototypical time series of the component) associated with that map. The components representing meaningful resting state networks will be identified and used in further analyses, such as a network analysis.

8.4.3.3 Second level analysis

Data will be compared across all participants using a random effects "second/higher level analysis" to determine whether brain activity is significantly altered across participants or differs between groups of participants. For all fMRI tasks and the ASL data the following second level analyses will be run: a) whole brain analyses (i.e. looking at all voxels in the brain); b) small volume corrected analyses (i.e. image based analyses limited to the prespecified regions of interest listed section 8.4.3.5 below; c) a region of interest analysis (i.e. using the mean activity within the prespecified regions of interest listed in section 8.4.3.5 below). We will also consider adding grey matter and / or ASL maps and / or gender to the confounder variables as a confirmatory analysis.

For fMRI analyses the inputs are statistical maps of model fit derived from the first level analyses. For region of interest analyses (see section 8.4.3.5) summary estimates of activity (model beta parameter estimates) within predefined regions are extracted from the first level statistical maps and analysed as a dependant variable using standard statistical software (SPSS). For analyses looking at the change in activity induced by prucalopride the inputs will be differences in beta weight contrast images activity between placebo and prucalopride groups, calculated using a random effect, between subject analysis. Summary estimates derived from individual regions of interest (ROIs) will be treated as repeated measures.

For the ASL data the input to the second level analysis will be the first level maps.
For resting state analyses, we will focus on the networks, which have been identified as showing significant differences in resting state connectivity between patients with depression and healthy controls: the default mode network; the salience network; the executive control

network; the affective network; the limbic network. We will also conduct exploratory analysis of all commonly identified resting state networks.

8.4.3.4 Correction for multiple comparison in image data

Image based statistical analyses will be corrected for multiple comparisons within the region of brain analysed. For whole brain analyses this will be all voxels within the brain, for analyses limited to a prespecified anatomical location (i.e. “small volume corrected analyses”) this will be across the voxels within the prespecified mask (prespecified regions of interest). Multiple comparison correction will be achieved using threshold free cluster enhancement (TFCE) or cluster-based analysis while controlling for family wise error rate with a z threshold of 3.1 and a corrected p value of < 0.05. If randomise is used, we will interrogate for group differences by non-parametric permutation testing (5000 permutations) with FSL randomise (TFCE, $p<0.05$ corrected).

8.4.3.5 Prespecified regions of interest

For both the faces and memory encoding tasks we will conduct 1) whole brain analysis; 2) small volume corrected analysis within functionally defined masks; 3) ROI analysis within predefined anatomical structures.

For the faces functional masks, we will use a mask for each contrast of interest created by multiplying mean activation for all participants for this contrast by a Harvard-Oxford Histological atlas anatomical mask at a 50% threshold.

Hippocampal functional masks will be constructed as for the faces functional masks. Region of interest analysis will be applied to higher-level analyses of the faces and memory encoding tasks using anatomically defined ROIs described below:

- Left and right amygdala (faces task only)
- Left and right hippocampus (faces and memory encoding task)
- Medial prefrontal cortex including the anterior cingulate cortex (faces task only)
- Orbitofrontal cortex (faces task only)

Anatomical ROIs will be initially defined using the Harvard-Oxford Histological Atlas, using a high probabilistic inclusion threshold (50-90%) for subcortical areas. The goodness of fit of these ROIs will be assessed against individual high resolution structural images after transformation into normalised (MNI) space. Segmentation algorithms (e.g. the Freesurfer “Segmentation of hippocampal subfields and nuclei of the amygdala” routine (Saygin et al., 2017) or FSL FIRST (Patenaude, Smith, Kennedy, & Jenkinson, 2011)) may be employed if there is found to be generally poor fit between individual structure and the Harvard-Oxford probabilistic histological atlas, such that parameter estimates can be extracted from individually defined and segmented regions. If segmentation is applied, we will use equivalence testing to demonstrate that there are no volumetric differences in the size of the segmented area between groups, as volumetric differences may bias the magnitude of the

extracted parameter estimates. If the volumes are not equivalent across the groups, we will include the volume of the segmented area as a regressor in all further analysis to remove variance associated with volumetric differences.

9. References

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