PROTOCOL

Does 5-HT$_4$ receptor agonism have an acute procognitive effect in young adults with psychotic-like experiences: proof-of-concept study

Short title: The SERENE study: **SE**rotonin **R**eceptor 4 **E**ffect on **NE**urocognition

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SPONSOR: University of Birmingham

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1.4 Funder and reference:

Pump Priming funding (£39,998.20) agreed by the Mental Health Mission Translational Research Collaboration (MH-TRC) Pump Priming Funding Scheme 2024.

Reference: NIHR203316

1.5 Compliance with Good Clinical Practice and Other Regulatory Requirements:

The study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

1.6 Confidentiality Statement:

This document contains scientific information that are privileged or confidential and may not be disclosed unless such disclosure is required by law or regulations. In any event, persons to whom the information is disclosed must be informed that the information is privileged or confidential and may not be further disclosed by them. These restrictions on disclosure will apply equally to all future information supplied to you that is indicated as privileged or confidential.

2. List of Abbreviations

5-HT_4R	Fourth serotonin receptor / Serotonin-4 receptor
AVLT	Auditory Verbal Learning Task
BDNF	Brain Derived Neurotrophic Factor
BOLD	Blood Oxygenation Level Dependent signalling
CTIMP	Clinical Trials of Investigative Medical Product
FERT	Facial Expression Recognition Task
GAD-7	Generalised Anxiety Disorder Assessment anxiety scale
GP	General Practitioner
HRA	Health Research Authority
HBRC	Human Biomaterials Resource Centre
IL-1b	Interleukin-1beta
IL-6	Interleukin-6
IL-10	Interleukin-10
ICF	Informed Consent Form
IFN-g	Interferon-gamma
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic Resonance Imaging
N-back	Working memory task
PDQ	Perceived Deficits Questionnaire subjective cognition scale
PHQ-9	Patient Health Questionnaire depression scale
PI	Principal Investigator
PILT	Probabilistic Instrumental Learning Task
PLE	Psychotic Like Experiences
PPIE	Patient and Public Involvement and Engagement
PRESCIENT	Trajectories and Predictors in the Clinical High Risk for Psychosis Population: Prediction Scientific Global Consortium study
PROGRESS	PRucalopride and cOGnition in REcovered depression study
QCD	Quality Control Document
RA	Research Assistant
S100B	S100 calcium binding protein B
SAP	Statistical Analysis Plan
SCID-5	Structured Clinical Interview for DSM-5

SOP	Standard Operating Procedure
SuPAR	Soluble Urokinase Plasminogen Activator Receptor
TNFa	Tumour Necrosis Factor alpha
UoB	University of Birmingham
YAG	Youth Advisory Group

3. Study Overview / Synopsis

Study Type	Experimental medicine proof of concept
Study Population	Young adults ages 18 to 40 inclusive who have recent (in last 12 months) psychotic-like experiences
Sample Size	N = 36
Recruitment Population	Community healthy volunteers
Study Design	Experimental medicine proof of concept study examining whether 5-HT_4R agonism may affect cognition in healthy volunteers with psychotic-like experiences. 50% of the study population will be randomised to receive a highly-selective 5-HT_4 R agonist (prucalopride) and 50% will receive placebo for 7 days. Baseline and follow-up measures will include neurocognitive tasks and resting state fMRI; changes in mood, anxiety, subjective cognition, and blood biomarkers and prucalopride levels, will also be assessed
Primary Objectives	To determine if pharmacologically activating the 5-HT_4R compared to placebo has an effect on acute neurocognition in healthy volunteers with psychotic-like experiences
Study Endpoints	Data will be collected at the end of 7-10 days' prucalopride or placebo for each participant
Study Duration	2 years

4. Lay Summary

5. Background and Rationale

5.1 Context for the research study:

Psychosis is a disabling mental disorder associated with poor physical health and daily functioning[1]. Cognitive impairments are also common, likely contributing to suboptimal functioning and disease progression[2]. Unfortunately, evidence-based treatment options during early and preventative stages of psychotic disorders are limited, with antipsychotic medication indicated only after psychosis develops[3-5]. Targeting cognitive impairments in early psychosis could offer a novel treatment approach helping prevent progression to frank psychosis.

Studying non-impactful psychotic-like experiences (PLEs) is a well-established method for exploring mechanisms underlying the more severe psychosis[6]. PLEs are subclinical, not associated with the distress or significant cognitive impairment of psychosis[7, 8], and affect around 5-10% of the healthy population(UK[9]; World[10]). However, compared to those without, individuals with PLEs often have subtle cognitive differences[11] making them a useful non-clinical model for screening potential early psychosis treatments.

Preclinical data suggest that selectively targeting the 5-HT_4 receptor (5-HT_4R) could improve cognition by enhancing acetylcholine/serotonin neurotransmission and boosting neurotrophic factors (BDNF/CREB)[12]. Our pilot work shows that 5-HT_4R activation, using prucalopride, enhances human cognition and leads to cognition-related neural changes [13-15], including increased functional connectivity within cognitive networks using resting-state fMRI, and involving both emotional/non-emotional stimuli. The effect of 5-HT_4R agonism has never been assessed in those with psychosis-related symptoms. However, our emulated target trial using electronic health records found that prucalopride treatment (5-HT_4R agonist) was associated with >70% lower risk of psychosis compared to other anti-constipation treatments[16].

Experimental medicine models allow rapid assessment of medications that probe specific targets (i.e. prucalopride on 5-HT_4Rs) using proxy measures (i.e. effect on acute neurocognition) to establish proof-of-concept in a model of the clinical population of interest (i.e. PLE population as a model of early psychosis). These are not CTIMPs as they occur after clinical trials evaluating safety and prior to assessing clinical efficacy.

5.2 Health Research Authority (HRA) classification

Prucalopride is a highly-selective, safe 5-HT_4R agonist licensed for constipation with excellent brain penetration. Experimental medicine models allow rapid assessment of medications that probe specific targets (i.e. prucalopride on 5-HT_4R) using proxy measures (i.e. acute neurocognition) to establish a proof-of-concept for further assessment. These are not clinical trials of investigative medical products (CTIMPs) as they occur after clinical trials evaluating safety and prior to assessing clinical efficacy.

Therefore, in line with guidance from the Health Research Authority (HRA) we propose that this experimental medicine study using prucalopride would be a basic science experimental medicine study involving procedures with healthy human participants, *not* a clinical trial of investigational medicinal product. This is because the proposed study does not aim to ascertain or verify/compare the safety of the medicine. Safety of prucalopride in humans is already established, and prucalopride is licensed in the UK for treatment of constipation. The study also does not aim to ascertain or verify/compare the efficacy of the medicine in the clinical treatment of early psychosis. To assess efficacy in relation to psychosis would not be valid or possible with a seven-day-dose study; assessment of efficacy would instead require chronic dosing and inclusion of clinically-accepted efficacy outcome measures, neither of which is the case for this protocol (see below). The key outcome measures for this seven-day-dose experimental medicine study will be intermediate markers of response with regards to neurocognitive outcomes (behavioural measures and neuroimaging).

5.3 Why the research is important:

People with lived experience of psychosis from the Birmingham Youth Advisory Group (YAG) highlighted that improving cognition and future outcomes was a research priority for people with early psychosis.

Answering this question with this mechanistic study will:

1. Potentially establish novel proof-of-principle evidence for acute behavioural and neural neurocognitive effects of 5-HT_4R agonism in people with PLEs
2. Yield an estimate of effect size to aid future design of next stage clinical trials where clinical impact on cognition and other outcomes in early psychosis can be explored.

People from the Birmingham Youth Advisory Group (YAG) including those with lived experience of psychosis highlighted that investigating potential therapies to improve cognition/future outcomes was a research priority in early psychosis (20.2.2024); 3 members have volunteered to join our study-specific PPIE group.

6. Study Design and Research Questions

Study design: A double-blind placebo-controlled experimental medicine study of a 5-HT4 receptor agonist (prucalopride) or placebo in healthy young adults with psychotic-like experiences (PLEs).

Research question: Does pharmacologically activating the 5HT_4R compared to placebo have an effect on acute neurocognition (behavioural and / or neural) in healthy volunteers with PLEs?

7. Participant Population

7.1 Description

36 healthy participants (18-40y) experiencing PLEs in last 12 months. Screening with 7-item PLE Questionnaire[17] will identify potential participants for full assessment with the Questionnaire for Psychotic Experiences[18]. Mild anxiety/mood experiences will be allowed, but mental disorder, antidepressant/antipsychotic treatment or prucalopride/mental health safety concerns identified on assessment (SCID-5) will lead to exclusion.

7.2 Sample size

The study will be complete when there is full data for 36 participants meeting inclusion criteria.

7.3 Recruitment

Public advertisement: poster advertising in the local community and around the University of Birmingham will be used to promote the study and provide an email address for interested persons who have recent PLEs. Following email / other contact from such persons, the study team will give potential participants a Participant

Information Sheet (PIS) and the 7-item questionnaire regarding psychotic-like experiences (PLEs) [17] to give participants information regarding the study and allow them to determine whether or not they are likely to meet the eligibility criteria. A similar method has been used with success by the PRESCIENT study. At the full screening session, we will use this 7-item psychotic-like experiences questionnaire to identify potential participants for full screening assessment including assessment for PLEs with the Questionnaire for Psychotic Experiences[18].

Appropriate tools will be used to recruit potential trial participants from the community. These will include advertisements on websites or newspapers, attendance at recruitment events, and social media advertising. These tools will be co-produced with individuals from the Birmingham YAG recruited specifically to support this study (with payment for their time and expertise).

Participants will also receive reimbursement for their time and necessary travel expenses (see 8.6 Participant Remuneration). These will be clearly outlined in the PIS as per Standard Operating Procedure (UoB SOP: PARTICIPANT ENGAGEMENT AND INFORMED CONSENT (UoB-PEI-SOP-001).

7.4 Inclusion Criteria

A participant will be considered eligible for inclusion in this study if they:

[1] Are aged 18-40 years inclusive

[2] Are able to consent to the study

[3] Have recent psychotic-like experiences

[4] Are fluent in English

7.5 Exclusion Criteria

If any of the following apply potential participants would not be able to take part in the study:

[1] Current antipsychotic medication

[2] Current antidepressant medication

[3] Documented history of intellectual disability

[4] Past or current clinically relevant central nervous system disorder

[5] Current significant medical disorder

[6] Current or past treated or untreated psychotic episode

[7] Pregnancy, breastfeeding, or actively trying to become pregnant. Participants will be asked to avoid becoming pregnant.

[8] Individuals with contraindications for MRI, including those with non-MRI-safe metallic or electronic implants, incompatible medical devices, severe claustrophobia, or exceeding scanner size limits.

[9] Recent (in last 3 months) involvement in a study that uses an experimental drug or device

[10] Recent (in last 6 months) involvement in a study using similar thinking or emotional tasks

7.6 Withdrawal

Participants are free to withdraw at any time up to the end of data analysis, without affecting their clinical care or association with the University, and do not need to give a reason for this to the investigators.

Rarely, it may be necessary for participants to withdraw from the study if they feel unwell or there is a concern from one of the study team. If this happens, the PI will discuss with the participant the reasons for withdrawal and any future plans to ensure that the participant's wellbeing is maintained.

7.7 Replacement of Participants

Participants who discontinued from the study may be replaced.

8. Visits and Assessments including Outcomes

8.1 Overview of study procedure

Participant information prior to screening

If potential participants express an interest in the study we will send the PIS and the 7-item PLE Questionnaire(20). This will allow participants themselves to undertake a preliminary check if they have had any of the unusual experiences we are considering for this study. If they confirm that they think they meet inclusion criteria they will then proceed to organising a full screening session. If participants would rather skip this step and proceed to attending the screening interview without receiving the 7-item PLE Questionnaire this option will be open to them.

Screening visit (before randomisation) – Study Visit 1:

- **Informed consent procedure**
- **Demographic data collection:** age, sex, gender, race / ethnicity, socioeconomic status, employment, educational attainment, age and education of biological parents
- **Medical assessment:** number of cigarettes smoked per day, units of alcohol consumed per week, caffeinated drinks consumed per day, pre-existing medical and psychiatric history (including family history of psychiatric illness), current medication and in three months prior to study, previous use of psychotropic medication, allergies and intolerances, MRI screening form to ensure no contraindications to MRI scanning
- **Psychiatric assessment:** Questionnaire for Psychotic Experiences[18], SCID-5 (to assess for psychiatric comorbidities, and to confirm that no psychotic disorder present), NART (National Adult Reading Test to give a measure of verbal intelligence)

Study Visit 2 (baseline and randomisation) & Study Visit 3 (Follow-up after 7-10 days medication):

All procedures are the same for visit 2 and 3 unless otherwise specified

1) **Questionnaires:**

-7-item Psychotic Like Experiences (PLE) questionnaire [17]

-Perceived Deficits Questionnaire (PDQ) subjective cognition scale

-Patient Health Questionnaire (PHQ-9) depression scale

-Generalised Anxiety Disorder Assessment (GAD-7) anxiety scale

-Snaith-Hamilton Pleasure Scale (SHAPS) anhedonia scale

-Side effects checklist

2) Neurocognitive battery suitable for repeated measures known to be sensitive to 5-HT_4 agonism in previous studies

-Auditory Verbal Learning Task (AVLT)

-Working memory (N-back)

-Facial Expression Recognition Task (FERT)

-Probabilistic Instrumental Learning Task (PILT)

3) **MRI / MRS scan** (as in (17)) including physiological assessment (heart rate variability)

-Structural (T1) (Baseline visit only)

-Resting state fMRI scan

-Magnetic Resonance Spectroscopy (MRS)

5) **Blood samples** (pre- and on-drug/placebo samples): for preparation/storage and analysis of immune cell phenotypes/biomarkers and prucalopride levels

- A blood sample of approximately 20ml (1.25 tablespoons) will be collected to measure: IL-1b, IL-6, IL-10, TNFa, IFNg, BDNF, S100B and SuPAR and prucalopride levels

- Blood samples will be collected by a qualified phlebotomist / trained clinician at two separate study time points (baseline and after 7-10 days' of medication).

- The intention behind taking the blood samples is to process them for the purpose of analysis of subcellular components (all biomarkers and drug levels will be analysed from plasma / serum).

- After collection, blood samples will be transported to Professor Nick Barnes' Laboratory group based in the College of Medical and Dental Sciences, Institute for Clinical Sciences at the University of Birmingham on the same day for preprocessing and storage as per their Standard Operating Procedures. This will include the use of the HBRC for storage of cellular material for analysis in future studies. Pre-processing to cellular / acellular components and transfer of cellular material to the HBRC will occur within one week of collection. After pre-processing, plasma and serum will be stored in Nicholas Barnes' Laboratory until required for analysis.

6) Optional Microbiome analysis (collection to take place at home):

- Proof of concept measuring uptake of optional microbiome analysis (i.e. acceptance rate). Using Standardised Operating Procedures, we will offer home collection of samples using bio safety boxes. Standard stool collection containers are used that contain stabilisation buffer for transport, including via Royal Mail. Instructions for data collection will be explained and given to participants. Collection boxes will be given to participants during the Visit 2 (baseline visit), to obtain one sample prior to receiving study medication and one sample after receiving study medication. Alternatively, we can post collection boxes directly to their home if this is preferred. Collected samples will either be collected at Visit 3 by investigators, or be posted by

participants, and delivered to Professor Lindsay Hall's Laboratory group, based in the Institute of Microbiology and Infection at the University of Birmingham.

6) **Physical observations**:

At baseline and the follow up visits, physical observations including height, weight, blood pressure, pulse will also be assessed. A urine sample will also be taken at the baseline visit only to exclude pregnancy in people with a uterus of child-bearing potential. The urine sample will be analysed using a rapid point-of-care pregnancy test, which detects the presence of human chorionic gonadotropin (hCG), a hormone produced during pregnancy. This test provides results within a few minutes. Once the analysis is completed and the results are recorded, the urine sample will be disposed of immediately. This component will take approximately 10 minutes to complete. For further details relating to this overview, please see section 7.3.

8.2 Table 1: Schedule of assessments

Visit	Consent	Demographics	Medical	Psychiatric	PLE	PDQ	PHQ-9	GAD-7	SHAPS	VAS	Side effects	Cognitive testing	sMRI	fMRI	MRS	Bloods & Phys Obs.	Microbiome
Screening	✓	✓	✓	✓													
Baseline					✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓ (boxes given)
Follow-up					✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓ (boxes collected)

8.3 Specific timings, details, and rationale for study procedures

8.3.1. Informed consent (10 minutes) – SCREENING

Undertaken just prior to the screening interview, this ensures that participants have another opportunity to ask questions about the study (they will have been sent the Participant Information Sheet prior to being invited for screening and been able to ask questions via email / verbally). Where consent is being taken electronically, the Joint statement on seeking consent by electronic methods (PDF - 209 KB) advice issued by the HRA and MHRA will be followed (see 14.3 Informed Consent).

8.3.2 Screening interview (50 minutes) – SCREENING

Demographic data will be collected to allow assessment of whether we have recruited an appropriately diverse population for the study. If it is found that we are missing a key part of the population in our study we will discuss as a study team at our regular team meetings including our PPI group whether we should consider targeted recruitment (for example a particular ethnic group).

Medical history and psychiatric assessment ensures that any potential issues can be raised with clinical investigators prior to randomisation. These might include past medical or family history.

8.3.3 Physical observations (5 minutes) – BASELINE AND FOLLOW-UP

Establishing blood pressures, pulse, and BMI as a baseline and at follow up confirms that participants are in good medical health on the study days.

A urine pregnancy test where relevant allows us to identify those who are currently pregnant and not aware of this, as it is an exclusion criterion for this study (only to be done at baseline).

Any concerns can be raised with medically-qualified investigators. Undertaking this on the first study day rather than screening is important as there may be up to 4 weeks between screening (Study Visit 1) and the baseline study visit (Study Visit 2) to suit participant schedules.

8.3.4 Questionnaires (15 minutes) – BASELINE AND FOLLOW-UP

Questionnaires ensure before and after medication validated assessment of mood and affect symptoms, anxiety symptoms, subjective cognition, psychotic experiences, and side effects using brief validated scales.

8.3.5 Neurocognitive tests (40 minutes) – BASELINE AND FOLLOW-UP

We will undertake three different neurocognitive tasks that have previously been sensitive to change with 5-HT_4R agonism – an auditory verbal learning task (AVLT), a working memory task (N-back), a facial expression recognition task (FERT), and a reward learning task (PILT). These will be delivered using electronic equipment where necessary. For the AVLT, participants are read a list of 15 words 5 times, after each reading they are asked to recall as many of the words as necessary. They are then asked to recall these words again after a short (3 minutes) delay – where they are read a different list of 15 words – and long (20-30 minutes) delay. For the N-back, participants are asked to recall the letter / number shown in a sequence either just before the current letter / number, or 1 before (1-back), 2 before (2-back), or 3 before (3-back). During the FERT, participants are shown a face on a computer screen briefly (circa 0.5s) and then asked to identify the emotion shown from happy / sad / fear / anger / disgust / surprise / neutral. Facial expressions are graded in terms of proximity to neutral (from 10% to 100%). In the PILT, for each trial, the participant is presented with 2 stimuli that have reciprocal probabilities (0.7 vs. 0.3) of a win outcome (plus 20 points) versus a no-win outcome (0 points) in reward condition trials or a loss outcome (minus 20 points) versus a no-loss outcome (0 points) in loss-

condition trials. Participants chose one of the two stimuli, following which they received visual feedback on the trial outcome and their current total earnings. Each block of the PILT consists of 30 reward trials and 30 loss trials. Participants performed 3 blocks of the PILT in each behavioural testing session. Different task stimuli are used in each block. The behavioural measure of interest is choice accuracy.

8.3.6 3T MRI / MRS scan (up to 40 minutes including preparation time) – BASELINE AND FOLLOW-UP

T1 (5 min) – ONCE ONLY

A Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) with high spatial resolution (1mm or better) that provides an excellent contrast between grey and white matter. This measure is required for anatomical segmentation of the brain.

Resting-state functional MRI (10 min) – BOTH VISITS 2 AND 3

A multi-band gradient echo acquisition sensitive to blood oxygen levels (BOLD) most robustly in grey matter. This measure provides an estimate of functional connectivity within and between particular brain networks. Participants will be asked to have eyes closed and eyes open at specific points of the scan for consistency. After the visit, participants will be asked to verbally recall what they were thinking during the scan (warning of this question will be given).

Magnetic resonance spectroscopy (MRS) (10 minutes) – BOTH VISITS 2 AND 3

sLASER MRS to focus on the hippocampus (initial scoping to ensure practical already completed). Magnetic resonance spectroscopy (MRS) is a non-invasive neuroimaging technique that measures the chemical composition and metabolism of tissues, most commonly used to assess brain metabolites such as choline and glutamate / glutamine. MRS does not represent any additional risks to participants compared to MRI.

8.3.6 Blood samples (5 minutes) – BASELINE AND FOLLOW-UP

Biomarkers that can be quantified from peripheral tissue (such as plasma, blood cells or hair) are promising indicators of the pathophysiological processes that underlie mental disorders. For this study, we selected blood biomarkers with an established association with psychotic symptoms and / or 5-HT_4R agonism whilst also minimising participant burden, and limiting the invasiveness of specimen collection.

8.3.7 Optional Microbiome analysis (collection to take place at home) (5 minutes) – BASELINE AND FOLLOW-UP

Proof of concept to measure uptake and acceptability of optional microbiome analysis. 5-HT_4 agonists, as drugs with action of gut receptors, may impact on the human microbiome.

8.3.7 Discussion re study and intervention (5 minutes) – BASELINE AND FOLLOW-UP

At baseline / Study Visit 2, participants will receive their study medication and confirmation of instructions on how to take this. There will be an opportunity to ask questions.

At follow up / Study Visit 3, there will be an opportunity for participants to ask questions about the study and to give feedback on the process.

8.4 Duration of assessments

Screening (may be conducted online or in person): lasts 1 hour

Baseline assessment: lasts approximately 2 hours (includes MRI scan time with preparation)

Follow-up assessment: lasts approximately 2 hours (includes MRI scan time with preparation)

8.5 Outcomes

Primary: The effect of 7-10 days' prucalopride versus placebo on neurocognitive task performance.

Secondary include: Prucalopride's effects on subjective cognition, other measures (including mood/anxiety symptoms), task and resting state functional connectivity (cognitive versus affective networks). Blood samples will allow subsequent association of responders with biomarkers and drug levels.

8.6 Participant remuneration

Participants will receive £100 for completing the study, and up to £25 for travel expenses throughout the study. Ineligible participants at screening or participant withdrawals before the end of the study will receive £10 remuneration.

9. Randomisation and Blinding

Drug procurement, & encapsulation will be carried out by an approved external provider sourced through the University of Birmingham: the NHS Scotland Pharmaceutical 'Specials' Service.

The Encapsulation Lead / Regulatory Manager from the Oxford Health Biomedical Research Centre will generate a randomisation list using Sealed Envelope; https://www.sealedenvelope.com (an online randomisation tool established since 2001; ISO 27001 compliant and accredited by UKAS). The University of Birmingham is a partner of the OHBRC and therefore as such is able to make use of their facilities and resources. Sealed Envelope is a well established web-based randomisation tool that has been used and cited in numerous peer-reviewed papers, including a published experimental medicine study by this proposed study's PI (A N de Cates et al., The effect of the 5-HT4 agonist, Prucalopride, on functional magnetic resonance imaging faces task in the healthy human brain. Frontiers Psychiatry (2022) 13:859123). The list will be randomised against 2 treatment groups (placebo vs drug) and stratified according to gender (male vs female). Randomisation will be blocked in design (Blocks of 4) to ensure even distribution of treatment allocations for every 4 stratified participants.

Drug & placebo encapsulation and labelling will subsequently be carried out using this randomisation list according to the NHS Scotland Pharmaceutical 'Specials' Service SOP signed by a Qualified Person (QP). In summary, drug tablets and placebo are over-encapsulated in matched capsules. This is led by their Encapsulation Lead and carried out by 2 people such that each part of the encapsulation process is double checked. Encapsulation is carried out on 'clean' benches in designated rooms, by personnel wearing PPE to prevent contamination of the encapsulated drugs. Correct number of either prucalopride or placebo capsules are counted into labelled bottles according to the blinded randomisation list. Bottles are labelled numerically with gender identifiers e.g. F01, F02 for the first two female participants, M01, M02 for the first male

participants. Thus labelling with these unique identifiers enable the capsule contents to remain blinded to the participants and study personnel.

The unblinded randomisation code list will be kept securely in a password protected file on a secure server at Oxford University (Encapsulation Lead has access). In the event of an emergency where unblinding is necessary, an emergency medic has been identified at Birmingham who will hold a password protected copy of this list. This document will be emailed from the Encapsulation Lead to the emergency medic before recruitment starts. The Encapsulation Lead will also hold a copy of the unblinded list at Oxford and study personnel will have been notified ahead of recruitment about her contact details. The Study PI (de Cates) will ensure that emergency contacts for participants are available at the time participants are taking the treatments.

Blinded study staff at Birmingham will receive the labelled bottles of drug/placebo capsules securely from the external encapsulation provider, and these will be stored in a locked medicine cabinet in Birmingham and Solihull Mental Health Trust Pharmacy. Bottles will be allocated to participants in numerical order by a study clinician and a record kept.

Experimental medicine studies using drugs that are not CTIMPs, such as this one, do not need to adhere to clinical trial regulations including those associated with treatment/drug preparation. However, the Encapsulation Lead has also carried out Good Manufacturer training, and all staff at NHS Scotland Pharmaceutical 'Specials' Service adhere to Good Manufacturing Practice.

The encapsulation group at Oxford has a number of years experience with preparing randomisation lists for experimental medicine studies that have been approved by University of Oxford Ethics committees. The BRC Depression Therapeutics Theme will fully support Angharad de Cates with this study and offer any support and advice throughout the study as needed. Integrity of the blind and code break will be confirmed at the end of the study by asking for participant feedback on randomisation guesses. All documentation relating to randomisation and blinding, will be archived along with the rest of the TMF at the end of the research project. See 16.3.6 Data Archiving for further information.

10. Intervention

9.1 Prucalopride

Name of drug/substance to be used	Prucalopride
Formulation and route of administration for study	1mg prucalopride tablets will be encapsulated in opaque capsules.
Dose and route of administration for study	1mg oral dose daily for 2 days, then 2x1mg oral dose daily for 5-8 days
Duration of treatment for study	Seven to ten days
Licence status of this drug/substance	Prucalopride is a licensed drug.
Usual Indication	Prucalopride is indicated for the symptomatic treatment of chronic constipation in adults.
Usual Dose	The usual dose given is 2 mg once daily with or without food, at any time of the day.

Usual duration of treatment	Prucalopride can be administered long- term, although the efficacy of the drug in the treatment of constipation has only been established up to 3 months.
Where will drug/substance be sourced from?	The drug will be sourced from an approved supplier in the form of 1mg film-coated tablets.
Where will drug/substance be stored?	Prucalopride will be stored in a locked drug cabinet in Birmingham and Solihull Mental Health Trust Pharmacy. It will be stored at room temperature in this locked cupboard, which is suitable for drug storage.
How will drug/substance be dispensed?	Prucalopride will be dispensed by a study clinician.
How will the drug/substance be prepared by the researchers for use in this study?	Prucalopride will be encapsulated by trained support staff using the Standard Operating Procedure of NHS Scotland Pharmaceutical 'Specials' Service

9.2 Placebo

Name of drug/substance to be used	Lactose Placebo
Formulation and route of administration for study	Placebo will be encapsulated in opaque capsules.
Dose and route of administration for study	One capsule taken orally, daily for 2 days; 2 capsules for remaining 5-8 days
Duration of treatment for study	Seven to ten days
Licence status of this drug/substance	N/A
Usual Indication	N/A
Usual Dose	N/A
Usual duration of treatment	N/A
Where will drug/substance be sourced from?	Placebo will be sourced from an approved supplier of NHS Scotland Pharmaceutical 'Specials' Service
Where will drug/substance be stored?	Placebo will be stored in a locked drug cabinet in the Birmingham and Solihull Mental Health Trust (BSMHFT) Pharmacy. It will be stored at room temperature in this locked cupboard, which is suitable for drug storage.
How will drug/substance be dispensed?	Placebo will be dispensed by a study clinician.
How will the drug/substance be prepared by the researchers for use in this study?	Placebo will be encapsulated by trained staff of NHS Scotland Pharmaceutical 'Specials' Service according to their SOP.

11. Safety Measures and Contacts During the Study

Participants will be closely monitored for serious mental illness when in the study. Symptomatology of participants will be tracked using questionnaires for psychosis, mood and anxiety (PLE, PHQ-9, GAD-7). Dr de Cates and Professors Broome and Upthegrove have experience in the management of acute mental illness including transition to psychosis. Participants will also be asked to provide the contact details of their GP or other health care professional who can be informed about their participation in the study.

Similarly, relevant medical history will be deemed as that reported during the baseline screening assessment.

The dose of prucalopride will be titrated with 1mg for 2 days, followed by 2x1mg for the remainder or the study. All participants will received a check-in from the study team at day 2 and 4. If participants are experiencing side effects at the day 2 check-in (before potential dose escalation) or day 4 check-in (after potential dose escalation), a clinician on the study team will be contacted to discuss the best management plan for the participant. Dose escalation for prucalopride in this manner has been found to be very well tolerated, including in participants who are antidepressant – naïve (as determined by the PROGRESS study). Side effects will be formally checked at baseline and study end using the side effects checklist.

Reports of adverse events which are reported by the participant and/or study team member(s) as possibly having been caused by the study activities (e.g. distress during interview, or because of interaction with study team) will include separate individual assessment of seriousness and causality. We will also report these as required using the Yellow Card Scheme: www.mhra.gov.uk/yellowcard.

Adverse events and serious adverse events will be recorded, investigated, and reported as per the Standard Operating Procedure (UoB SOP: ADVERSE EVENT REPORTING, V2.0, 20.1.2023).

12. Risk Mitigation

A project-specific risk assessment has been performed and is documented below as per Standard Operating Procedure (UoB SOP PROJECT OVERSIGHT & QUALITY MANAGEMENT: UoB-POS-SOP-001, V3.0, 8.1.2024).

1. Risk: Potential for insufficient recruitment from the community
   Mitigation: We have allowed additional time (3 months) for recruitment in our study plan to allow for slower recruitment in some months (estimated 1-2 participants per months after data collection starts). We are also co-producing recruitment materials with specifically recruited Birmingham YAG members to ensure that our participant-facing materials are as inclusive as possible and are attractive to the community.

2. Risk: It is possible that some participants may find it difficult to understand instructions regarding study processes.
   Mitigation: We will give these both verbally and in written form, and will check understanding prior to study procedures.

3. Risk: It is possible that participants may have varying sub-clinical cognitive and mood differences at recruitment, which may impair our ability to detect signal in neurocognitive measures.

Mitigation: We will assess baseline mood and subjective cognitive difficulties, and we can incorporate these into analytical models as necessary to account for subclinical differences in cognition and mood.

4. Risk: Participants may deteriorate in terms of mental state during the course of the study.
   Mitigation: As the study is short, and involves only healthy volunteers, this is unlikely to occur. However, we will monitor the results of the SCID at screening, and the PHQ-9, GAD-7 and PLE during each study visit. Any concerns will be discussed with the PI and / or supervising medical investigators, all of whom are psychiatrists with experience in managing patients with serious mental illness and suicidal thoughts and behaviours. We will also ask all participants to provide the details of a GP or other health professional so that the study team can advise them of their participation in the study

5. Risk: Bias from accidental unblinding
   Mitigation: To preserve the blind, prucalopride and placebo will be encapsulated to appear identical and labelled blinded. The randomisation list will be generated by an independent pharmacist and access to the unblinded code list will be restricted to the randomisation service provider.

6. Risk: Participant becomes pregnant during the study
   Mitigation: Given the short duration of the medication course and the requirement for a negative pregnancy test at screening, the likelihood of a pregnancy occurring during this study is very low. However, participants will be advised to avoid becoming pregnant during this time. This is because, although there are no known risks of prucalopride in pregnancy, it is not currently recommended during pregnancy. If a participant does become pregnant, they will be instructed to inform a member of the research team and consult their healthcare provider or General Practitioner for further guidance and to stop taking the medication if the study is still in progress.

A health and safety risk assessment and a biological risk assessment will be completed prior to commencement of the study, and updated during the study where necessary as per the SOP above.

13. Public Involvement (PPIE)

We will involve lived experience advisors throughout the research as per standard practice for the Institute for Mental Health, UoB and the Standard Operating Procedure PARTICIPANT ENGAGEMENT AND INFORMED CONSENT (UoB-PEI-SOP-001). The University of Birmingham Institute for Mental Health's PPI Youth Advisory Group (IMH YAG) provided detailed in-person feedback on the study proposal when it was presented at their 20th February 2024 meeting. This directly fed into funding proposals and the protocol including the amount of remuneration to offer participants, and we were able to confirm with them that in their opinion participants would be happy to undertake minimally invasive blood tests and would like to be offered daily SMS text reminders to take their study medication.

Similar to other studies, the Institute for Mental Health PPIE lead (Niyah Campbell) and his team are supporting us to recruit three individuals from the YAG to co-produce this project via a study-specific lived experience advisory group (LEAG). They will be reimbursed for their time to participate in discussion groups (meetings every 3 months to provide ongoing PPIE feedback and oversight), read research documentation (such as the participant information sheet (PIS)) and provide comments, and be involved in wider publicity and dissemination of research findings. Costs also include travel for face-to-face consultation where this is required. Based on previous experimental trials and NIHR-guidance, this is costed at £25/hour per person.

14. Ethical Considerations

14.1 Ethical approval

Prior to the commencement of the study, the protocol and any subsequent amendment(s), Participant Information and Consent Form, other information provided to participants (including advertising) and product information, will be submitted for NHS ethical approval via IRAS (Integrated Research Application System). The approval letter should refer to the study by title, protocol number and version and dates of documentation reviewed and approved. A copy of the signed and dated letter of approval (on institutional letterhead) will be provided before study commencement.

During the course of the study, the Principal Investigator / Sponsor is required to submit the following to the ethics committee: any amendments to the protocol; progress reports according to regulations and guidelines; Final Study Report as required.

Protocol amendments will be agreed upon by the PI, supervising medical investigator, and the Sponsor and submitted to the ethics committee for approval prior to implementation.

14.2 Ethical practice during study

The Investigator will ensure that the study is performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP).

14.3 Informed consent procedure

Prior to participation in the research study, each participant will undergo a complete consenting interview with the delegated study team members and provide consent on the ethically-approved form. The contents and process of obtaining informed consent will be in accordance with all applicable regulatory requirements. All delegated study team members taking part in informed consent will have appropriate training.

All eligible participants will have the study explained by the PI or the delegated assistant / other study member. They will receive a full explanation, in lay terms of the aims of the study, the discomfort, risks and benefits in taking part as well as insurance and other procedures for compensation in case of injury. It will be explained that the study is for research purposes and may not provide benefit to the individual. It will be pointed out that they can withdraw from the study at any time without prejudice. Each participant will acknowledge receipt of this information by giving informed consent for participation in the study. Informed consent may also be provided electronically. The participant will be given a copy of the signed or electronically acknowledged Participant Informed Consent Form (ICF) to retain. All study documents will be fully approved by the ethics committee prior to being used for consenting purposes. The Joint statement on seeking consent by electronic methods (PDF - 209 KB) advice issued by the HRA and MHRA will be followed when consent is taken electronically (see 14.3 Informed Consent).

14.4 Participant data protection

The ICF will explain that study data will be safely stored in computer databases. Participants in this database are identified by a unique participant identification number and their initials. Adherence to the Data Protection Act and General Data Protection Regulation Act 2018 is applicable.

15. Study Management & Monitoring

15.1 Study Oversight

A formal Data Safety and Monitoring Committee will not be established due to the size of the project and the low-risk nature of the intervention (use of a licensed drug in medically healthy individuals, where previous studies [13] have demonstrated excellent tolerability).

Safety signals (adverse events) will be regularly reviewed by the PI and other medically trained personnel, and discussed with other investigators during Study Management Group meetings (occurring 3-4 monthly during the study), and the Sponsor if required, as per the Standard Operating Procedure (UoB SOP: ADVERSE EVENT REPORTING, V2.0, 20.1.2023).

The Study Management Group will consist of the PI, the Supervising and Co-Supervising Medical Leads, the Laboratory Lead, and any Study-specific Research Assistants and Lived Experience Consultants.

15.2 Monitoring and auditing

Study monitoring will be performed in accordance with applicable regulations, ICH-GCP, and University of Birmingham Standard Operating Procedures (SOPs). An audit is a systematic and independent examination of study-related activities and documents to determine whether the approved study-related activities were conducted, and the data were recorded, analysed and accurately reported according to the protocol, SOPs and any applicable institutional requirements.

The Investigators will ensure that direct access to source data / documents for the purposes of monitoring, audits, ethics review and regulatory inspections is available throughout the study and during the record retention period. In addition, the PI will ensure that each study participant has consented, in writing, to their records being available for monitoring audits and regulatory inspections.

Considering the small sample size over a single site, it will be appropriate to monitor the study as follows: At each three-four monthly Study Management Group meetings the following aspects of the study will be reviewed and minuted.

- Numbers and completeness of case report form (eCRF)
- Compliance with the protocol's treatment according to participant report
- Any adverse event report form completed
- Any notification of protocol deviations
- Any concerns regarding quality of data

15.3 Staff training

As per GCP, each individual involved in the conduct of a study will be qualified by education, training and experience to perform his or her respective tasks. The PI will maintain a record of all individuals involved in the study. The PI will ensure that appropriate training relevant to the study is given to staff, and that they will receive any new information of relevance to the performance of this study.

15.4 Study amendments

Study procedures will not be changed without the mutual agreement of the PI, the Sponsor and ethics committee. If it is necessary for the study protocol to be amended, the amendment or a new version of the study protocol must be approved by the ethics committee. If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining ethical approval, the deviation or change will be submitted to the ethics committee for review and approval.

15.5 Study completion

The end of the study will be the date of the final study visit for the final participant.

16. Data Management

16.1 Sample size calculation

Previous studies investigating the effect of prucalopride / other serotonergic drugs on acute cognition assessed with behavioural measures indicate an effect size of 0.8-0.9 may be expected. As relatively little is known about 5-HT_4R stimulation in healthy participants we PLEs, we conservatively estimated a smaller effect size (0.5-0.7) for sample size calculation. This indicates 15-17 participants/group would give 85-90% power to detect a significant group difference at a 0.05 false positive rate. We aim to recruit up to 36 (18 in each group) to allow for withdrawals and exclusions for data quality reasons.

16.2 Trial master file

All essential documents relating to the study will be kept in the Trial Master File (TMF) as per the UoB Essential Documents Checklist (UoB-ESD-QCD-001). This will be maintained and stored securely during the study. The TMF will be archived appropriately as below (see 15.3.6 Data Archiving).

16.3 Data management plan

Data management will be conducted in line with UoB Standard Operating Procedures (UoB SOP: DATA MANAGEMENT, V1.0, 3.1.2023 (UoB-DMA-SOP-001), adhering to the UoB Principles of GCP for Clinical Research (UoB-GCP-POL-001). All investigators and site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

16.3.1 Data collection methods

Data collected will consist of:

- Demographic, medical and psychiatric interview and physical observations data (collected and stored electronically using an electronic case report form (eCRF))
- Questionnaire data (collected and stored electronically)
- Neurocognitive task data (collected and stored electronically)
- MRI scan data (collected and stored using MRI secure servers) and contemporaneous physiological measurement data (collected and stored electronically)
- Blood samples. These will be collected, handled, and transported securely for pre-processing, storage of acellular material, and analysis under Professor Barnes' team at the University of Birmingham (data from analysis will be stored electronically). Storage of Peripheral Blood Mononuclear Cells (PBMCs) will be via the HBRC for at least 5 years.
- Microbiome samples. Samples will be collected at participants homes and either transported by post or via study researchers to Professor Lindsay Hall's Laboratory group, based in the Institute of Microbiology and Infection at the University of Birmingham.

Consent forms (ICF) will be stored electronically with participants receiving a paper copy for their own records.

The eCRF will be created according to UoB Standard Operating Procedures (UoB SOP: CASE REPORT FORM DEVELOPMENT (UoB-CRT-CRF-SOP-001).

Coding and analysis of data will be documented in advance using the Statistical Analysis Plan.

16.3.2 Participant log and link key

A screening log of all potential participants will be maintained. This will include potential participants who were considered but later deemed ineligible due to meeting one of the exclusion criteria or withdrew. The reasons for exclusion or refusal will also be recorded against their ineligibility / refusal status. This log will be stored securely on University of Birmingham servers. For participants who withdraw up to the end of data analysis, their data will be withdrawn. It will not be possible to withdraw data, once data analysis has completed. This will be communicated in the PIS.

All participants who are considered for or enrolled in the study will receive an individual identification number (Participant ID). Documentation regarding potential participants and participants enrolled in the study will only be accessible to study team members who need this information for conducting the study. All data collection will use the Participant ID to link the data to one individual. The key that links Participant ID and participant names will be stored on secure University of Birmingham servers and will be kept until publication of the study.

16.3.3 Safeguards for data collection and storage:

- Source data are documents where the study data are first recorded. This will include the eCRF for demographic and interview data, neurocognitive computer task responses, MRI data, and blood sample data. As data collected will be personal and potentially sensitive data will be retained in a secure location at or by the recruitment site, accessible only to delegated study team members and relevant site staff.
- Demographic and interview data (collected on the eCRF) will be stored using secure servers at the University of Birmingham – supported logistically by University of Birmingham (UoB).
- Questionnaire data will be stored using an electronic platform (REDCap). There is an agreement with REDCap that they will meet and provide us with a database which is specific to the requirements of this study and as per CTOC. This data will be downloaded onto university secure servers- supported logistically by University of Birmingham (UoB).
- Neurocognitive task and MRI data will be stored and analysed using university secure servers.
- Blood samples will be handled and transported within UoB by study team staff and received, pre-processed, and acellular material stored securely by Professor Barnes' lab, using Standard Operating Procedures and in accordance with the Human Tissue Act 2004 (UoB SOPS: LABORATORY SET UP AND MANAGEMENT SOP, V3.0, 14.3.2022 (UoB-CRL-SOP-001) & LABORATORY FACILITIES SOP, V3.0, 14.3.2022 (UoB-CRL-SOP-002) & SAMPLE MANAGEMENT SOP, V3.0, 14.3.2022 (UoB-CRL-SOP-003); UoB QCD: LABORATORY ROLES AND DUTIES (UoB-CRL-QCD-002). PBMCs will be stored using the HBRC. Following analysis, the acquired data will be stored on university secure servers as per UoB SOP: LABORATORY ANALYSIS SOP (UoB-CRL-SOP-004).
- Participant IDs will pseudoanonymise data, the link key for which will be stored on the University's secure server until publication.
- Anonymised electronic and blood sample and microbiome data is held for 10 years on the University's secure server (see 15.3.6 Data Archiving) – supported logistically/financially by UoB.

- Data will only be accessible to the study team with capacity for full audit trail.
- Data stored on University secure servers will have appropriate back-up and security measures in place. The study data system will be recorded on the University's data asset register, amended accordingly should any amendments to the system be required. Relevant staff will be appropriately trained in using the system prior to dealing with the research data and of the need for any modifications to the data to be logged (i.e. there is an audit trail).

16.3.4 Data Sharing

Analysis code, and pseudoanonymised quantitative raw and MRI data, along with the protocol and analysis plans will be shared where practicable using repositories such as Open Science Framework and Neurovault. PBMCs will be stored anonymously on the University of Birmingham HBRC archive to serve as an ongoing resource for future research. This will ensure long-term curation of this data, and will be specified in the consent form and Participant Information Sheet.

Personal information will not be shared. The act of pseudoanonymising information will ensure that all personal information about research participants is removed and replaced with a code number. During the study, the research team will have the link key between the participant's personal identifying information (Participant ID) and the coded data should the need to re-identify the participant arises. The PI and study team will keep this link key in a securely protected database.

16.3.5 Protocol Deviations and Suspected/Serious Breaches

Deviations are any (minor or major) breach, divergence or departure from the requirements of Good Clinical Practice or the clinical trial protocol. A protocol deviation is a less serious non-compliance with the approved study protocol. Examples of protocol deviations may include visits / assessments performed early or later than scheduled date. A suspected breach is a possible serious breach of GCP or the protocol which has been identified by a third party. An example of suspected breaches include missing consent forms. A serious breach is a breach of GCP or the protocol that is likely to affect to a significant degree the safety or rights of a study participant, or the reliability and robustness of the data generated in the study. An example of a serious breach is confirmation that informed consent was not obtained.

Any deviations to the data management process will be appropriately managed (according to the UoB SOP: DEVIATIONS AND SERIOUS BREACH REPORTING SOP (UoB-DSB-SOP-001). A serious breach should be notified to Dr Birgit Whitman, Head of Research Governance & Integrity Email: researchgovernance@contacts.bham.ac.uk.

16.3.6 Data Archiving

All paper and electronic data including the Trial Master File and trial database will be retained and archived in accordance with University of Birmingham Standard Operating Procedures (UoB QCD: GUIDE TO RETENTION TIMES, v2.0, 21.8.23 (UoB-ARC-QCD-003); UoB SOPs: ESSENTIAL DOCUMENTS DEVELOPMENT AND MAINTENANCE SOP (UoB-ESD-SOP-001) & ARCHIVING, v2.0, 21.8.23) which are compliant with the UK GDPR. This indicates a retention period of 10 years minimum is to be expected.

17. Statistical Methods

Data analysis will be outlined in the Statistical Analysis Plan (SAP), which will be approved by the study team and uploaded to clinicaltrials.gov prior to analysis. The CI (or their delegate) will lock the data from any further changes once data collection has been completed and the SAP approved to allow for statistical analyses to begin (as per the UoB SOP: Statistics (UoB-STA-SOP-001)).

An outline of planned analysis is given below.

17.1 Analysis plan outline:

Questionnaire data

Comparison of ANOVA or linear mixed models to compare groups (prucalopride versus placebo) over two time points (baseline versus follow up) on:

1) Questionnaires of psychotic-like experiences (PLE), perceived cognitive deficits (PDQ), mood and anxiety (PHQ-9, GAD-7, SHAPS, visual analogue scale of state affect)

2) Side effect checklist

Behavioural neurocognitive testing (excluding FERT)

Comparison of ANCOVA or linear mixed models to compare groups (prucalopride versus placebo) over two time points (baseline versus follow up).

FERT

As in previous work [21, 22], FERT 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.

fMRI

fMRI data will be preprocessed prior to analysis.

Resting state fMRI: (i) Network Analysis

The preprocessed cleaned functional data are temporally concatenated across subjects and decomposed into independent components using FSL MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components). Dimensionality estimation for group maps will be set to a number of independent component maps and then identified as either being analogous to the most frequently reported major resting-state networks (RSNs) or reflecting noise (physiological, scanner, movement). Dual regression is then used to generate subject-specific versions of spatial maps and the associated time series from group-average spatial maps. Subsequently, we will test for statistically significant differences between the groups across all identified networks using FSL's randomize permutation testing tool. The RSNs of interest for this analysis are from the triple network model, comprising the DMN, SN, and CEN. Other RSNs (i.e., visual, auditory) were analyzed as control regions where we did not expect to see changes with prucalopride. Statistics will be assessed using a familywise error–corrected cluster significance threshold of $p < .05$ applied to the suprathreshold clusters to correct for multiple comparisons at the voxel level. The general linear model will include the groups of interest for comparison: placebo > prucalopride and prucalopride > placebo.

Resting state fMRI: (ii) Seed Analysis

For the seed analysis, predetermined region-specific masks are chosen based on the previous literature. Masks for these areas are binarized and thresholded before creating a standard- to high-resolution matrix, which is applied to each mask for each participant in turn to register the mask into each individual's functional (echo-planar imaging) space. We then extract the time series for each mask for each participant. First-level connectivity is calculated as the correlation (both positive and negative) of time series of the seed with all other voxels in the brain using FSL FEAT (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT). A white matter and cerebral spinal fluid (CSF) mask are also created in standard space and then registered into the individual's functional (echo-planar imaging) space before being included as covariates of no interest. We then use FSL FEAT to perform group-level analysis with 2 explanatory variables (prucalopride vs. placebo) testing for the contrasts placebo > prucalopride and prucalopride > placebo. Cluster-based thresholding ($z > 3.1$, familywise error $p < .05$) will be used to identify significant clusters for each seed analysis.

Blood

The exact path of blood samples during the study will be as follows:

- Whole blood collection at the University of Birmingham School of Psychology –> same day to Nicholas Barnes' Laboratory at the University of Birmingham – > storage short term in Nicholas Barnes' Laboratory –> pre-processing to cellular / acellular -> transfer of PBMCs from Nicholas Barnes' Laboratory to University of Birmingham HBRC within one week from collection date + longer-term storage of plasma and serum in Nicholas Barnes' Laboratory prior to analysis.

Short term storage of any cellular material will not exceed one week before being processed to acellular material; if cellular material exceeds one week, this will be reported, and cellular material will be destroyed.

Isolated PBMC will be collected and stored in liquid nitrogen, allowing future studies to investigate the function of individual cell subsets in the PBMC mix and also derivation of induced pluripotent stem cells for downstream functional studies aimed at understanding genetic versus epigenetic impact upon function, and assays of immune cell phenotypes (e.g. enumerated cell subsets based on immuno-phenotyping, single cell sequencing technologies). PBMCs will be stored by the University of Birmingham Human Biomaterials Resource Centre (HBRC) for at least 5 years.

Serum and plasma (acellular) will both be stored at -80°C. These samples will be used to quantify a range of biomarkers including inflammatory markers (e.g. IL-1β, IL-6, IL-10, TNFα, IFNγ, BDNF, S100B and SuPAR) and prucalopride drug levels to assess patient compliance.

18. Dissemination Plan

To facilitate academic impact after study end:

1) At 3-6 months, we will aim to present initial findings locally and / or with potential collaborators for feedback and to minimise duplication of data, as well as to national and international psychopharmacology networks (i.e. BAP/ECNP)

2) At 6-9 months, we will submit articles for peer-reviewed publication, including press releases to public and clinical networks.

3) We will make findings available to pharmaceutical organisation representatives (i.e. Takeda and Pfizer) via the Mental Health Mission (MHMTC).

19. Finance and Insurance

This study is funded by the Mental Health Mission Translational Research Collaboration (MH-TRC) Pump Priming Funding Scheme 2024.

Indemnity for the study will be provided by the University of Birmingham, acting as sponsor.

20. Amendment History

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2		Angharad de Cates	Added cognitive task, MRS, optional microbiome analysis. Updated pharmacy and encapsulation provider. Updated timings of visits.

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