University of Reading
School of Psychology and Clinical Language Sciences
Whiteknights
Reading RG6 6AL

Title of Study: "Individual differences in gut microbiota, brain, and behaviour"

Participant Information Sheet

Before you decide whether to take part in this study, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish.

If anything is not clear, or if you would like more information, please talk to a member of our study team via email at **gutbrain@reading.ac.uk** or any of the contact details provided below. Further information about the study is also available at: **www.gutbrain.bhismalab.org**.

Thank you for taking the time to consider taking part in our study.

Prof Bhismadev Chakrabarti, on behalf of all investigators

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Why are we doing this study?

- The gut microbiota is the group of microorganisms (very small living things, such as bacteria and fungi) that live inside and on the surface of our digestive system (e.g. gut, colon, intestines).
- It is estimated to outnumber our own cells by 10 times and combines over a hundred times more genes than the human genome.
- These microorganisms are known to perform useful functions, such as fermenting food material, producing vitamins, and preventing growth of pathogens (harmful organisms).
- There is also evidence that some bacteria can produce neurotransmitters (chemical messengers) such as serotonin and GABA (Gamma Amino Butyric Acid).
- Some studies have associated abnormalities of the microbiota composition with metabolic disorders, such as inflammatory bowel disease and obesity, as well as psychiatric conditions, such as depression and anxiety.
- The research in this field is still in its early days and is therefore very active.

We are conducting an experiment to study how gut microbiota composition is related to brain functioning, behaviour, and personality. We will also assess how a dietary intervention consisting of a probiotic may impact these.

Who can participate in this study?

This study has a set of inclusion and exclusion criteria to help us draw reliable inferences on the questions of interest.

You are eligible to take part in the study if you:

- Are male,
- Are aged between 18 and 50,
- Are White Caucasian,
- Grew up in the UK (United Kingdom) or other European country,
- Have a body mass index (BMI) between 18.5 and 30,
- Are right-handed.

You are not eligible to take part in the study if you:

- Have taken antibiotics in the last 3 months,
- Have used a protonic pump inhibitor (PPI), (e.g., omeprazole, lansoprazole) in the last 3 months,
- Use probiotic/prebiotic supplements,
- Are a current smoker,
- Regularly drink more than 14 units of alcohol per week,
- Have a current clinical diagnosis of a neurological, developmental, or psychiatric condition,
- Have a current diagnosis or history of gut microbiota related conditions, such as inflammatory bowel disease or irritable bowel syndrome.

The reason for these inclusion and exclusion criteria is that several factors, such as gender, ethnicity, or the use of alcohol or drugs acting on the brain, can have a big effect on gut microbial composition and can change performance in the computer tasks you will be asked to do.

All volunteers who meet the inclusion criteria will be invited to indicate a preference for work package 1 or work package 2 (see below for explanations).

What does taking part in this study involve?

Initial screening questionnaire

- Before the experiment you will be asked to complete a questionnaire to check whether you meet our inclusion criteria. You will be asked questions relating to magnetic resonance imaging (MRI) safety, and medical and lifestyle questions about your general health.
- This questionnaire will allow us to determine whether you meet the eligibility criteria for this study and that you can safely participate in this study (see inclusion and exclusion criteria above).

Screening visit

- In the week before the study appointment we will invite you to the University of Reading to attend a screening visit where you will be able to familiarise yourself with the university campus, laboratories and researchers.
- Alternatively, if you are unable to travel to the university for the screening visit, we can organise a meeting online using Microsoft Teams with one, or more, of our researchers present.
- You will be provided with detailed information about the study procedures.
- If you agree to participate, you will be asked to sign a consent form.
- During the screening visit, you will also be provided with a kit to collect a faecal and urine sample and instructed on how to use it.
    - By 'faecal', we refer to a sample of your poo/stool.
    - By 'urine', we refer to a sample of your pee.
- We will also instruct you to track your diet on a smartphone app (eNutri). This app asks you to answer questions regarding the types and quantities of food you have eaten over the last few weeks.
- The screening visit will last approximately 30 minutes.

Following the initial screening questionnaire and screening visit, you will be invited to proceed to take part in the study. The study involves two different work packages: work package 1 and work package 2. You can choose which work package you would prefer, or which would suit your schedule better. The contents of these work packages are explained below:

- **Work package 1** is aimed at evaluating the relationship between baseline gut microbiota composition and individual differences in brain functioning, behaviour and personality traits.
- **Work package 2** involves a dietary intervention that will contain probiotics and a placebo.
    - **Probiotics** are live microorganisms that are beneficial to your health when administered in adequate amounts. Probiotic bacteria are found in a variety of different products, including foods, dietary supplements, infant formulas and pharmaceuticals (e.g., Yakult yogurt).
    - A **placebo** has no therapeutic value and is used so that we can test the effectiveness of the probiotic.

Work package 1 (WP1)

- On the day of the study appointment, we will ask you to deliver your faecal and urine samples to the Food and Nutritional Sciences Department, in the Harry Nursten Building.
- You will also be asked to provide a blood sample. The blood sample will be collected at the Hugh Sinclair Nutrition Unit by a trained phlebotomist. It will be a small blood sample of 9 millilitres (ml) collected in a single tube.
- The samples will be analysed in order to explore the compositions of different bacterial species in your gut, and also any metabolites (biological products) that they may have produced.

- Next, you will be invited to move to the Psychology department (Harry Pitt building) where the rest of the experiment will be run.
- At this stage, we will ask you to have an MRI scan.

- Finally, you will carry out some tasks on a computer and complete personality questionnaires.
- The tasks that you will be invited to carry out will include watching stimuli (e.g., emotional faces or patterns) on a computer screen and making simple judgments on them using the computer keyboard.
- It will also involve a test of sensory abilities, where you will be asked to make simple judgments on tactile stimuli in the form of a vibration (e.g., feels similar to the vibration felt on a mobile phone on silent mode).

The overall study appointment should last approximately 3 hours.

Work package 2 (WP2)

- If you choose to take part in WP2, you will be asked to attend the University of Reading for 3 follow-up appointments spread over 12 weeks (i.e. 3 months).
- The follow-up appointments will be like those for WP1, and will take place on weeks 4, week 8 and week 12.
- The same brain imaging and behavioural tests will take place at each of these appointments, and you will be asked to provide a faecal sample, as well as blood and urine samples for each appointment.
- You will also be asked if you had any side effects from the dietary intervention, if you took it regularly and if you took any other medications.
- You will be allocated to a group which will determine if you are going to start the trial with the placebo or the probiotics, and both you and the researcher will be blind about which supplement is which.
- Depending on the group that you have been allocated to, you will be provided with the probiotic OR the placebo for 4 weeks and will be asked to take these

supplements daily. At your third study appointment, you will be provided with the opposite supplement and will be asked to take that daily for 4 weeks.

The schematic diagram on the last page of this document illustrates the structure of both work packages.

Are there any benefits for me in joining the study?

- We are happy to pay you for your time. You will be paid £50 for participating in WP1. For WP2, you will be paid £50 per study appointment, i.e., £200. We will also offer a £50 bonus to participants who complete all four WP2 study appointments.
- Any travel expenses you incur getting to and from your screening visit and study appointment(s) will be reimbursed.
    - Please hold on to any physical receipts (e.g., train tickets) and give these to the study researchers to create copies. Alternatively, if you have any electronic receipts, please forward them to gutbrain@reading.ac.uk.
    - If you drive, we will need some information about your car to reimburse you for fuel. This includes your car engine size, fuel type (petrol or diesel), and how many miles you have covered getting to and from your appointment.
- At the end of your participation, we will also send you a high-resolution image of a structural scan of your brain.

What are the risks of taking part in this study?

- MRI is an imaging technique that uses a strong magnetic field and radiofrequency (RF) electromagnetic waves (similar to radio and television waves) to “excite” hydrogen atoms in the body. The MRI scanner measures small changes in magnetic fields produced in your brain and uses these to generate different images.
- You will be asked to lie down on the scanner bed and a plastic coil will be placed around the top part of your head. Foam pads will be placed between your head and the coil to limit head movement during the study. You will then be moved into the bore (centre) of the magnet and asked to lie still for approximately 60 minutes, during which time MRI images will be acquired.
- There are minimal risks from MRI, and MRI scanning itself is painless.

- Nevertheless, you may experience some discomfort, including from mechanical noise made when the scanner is collecting measurements. You will be provided with earplugs to minimise the impact of this noise.
- The bore (centre) of the MRI scanner is large enough to fit an adult but may feel small to some participants. Individuals with a history of claustrophobia should not take part in the study.
- If you wish to interrupt the MRI scan at any time due to feelings of distress, you will have the ability to do so.
- This study is part of a research protocol and is not intended to provide a comprehensive clinical MRI examination of the brain. Your MRI scan will not be read by a radiologist. However, if a potential abnormality is identified on your MRI scan, a copy of the image will be sent first to a radiologist for advice, then, if advised by the radiologist, to your General Practitioner (GP). By signing the consent form, you authorise us to do this. If you are not willing to authorise this, please do not volunteer for the study.

- The results obtained by analysing the faecal samples may help detect deviations of your gut microbiome from the population average or even indicate potential associations to particular neural characteristics. However, these correlations are still preliminary and currently do not have proven health relevance.
- The results obtained from analysing blood and urine samples will only be used for assessing the quantity of specific metabolites relevant to our study and will not have any health relevance.
- This research study has no medical or diagnostic purposes and is not aimed to produce medical information or to provide medical or health-related advice.

- If you agree to be involved in WP2, you will be asked to take an over-the-counter probiotic for 4 weeks and a placebo for 4 weeks.
- Both you and the researchers will not be aware of which dietary intervention you will be asked to take first.
- The placebo is a standard placebo product for use in human studies involving prebiotics, and has been used safely at this dose, in many trials over the last 20 years.
- The probiotics, and possible associated prebiotics, will be selected from available off-the-shelf supplements, which will already have been deemed acceptable for the market and are therefore safe to take regularly.
- Probiotics are safe for human consumption. These microorganisms have been previously tested and shown to provide a health benefit. However, seeking a doctor’s approval is recommended for people who suffer from immune disorders, short bowel syndrome or a serious illness.
- In addition, some people could report an increase in gas production, with slight bloating, flatulence and mild diarrhoea. Such cases are very rare. As

such, to minimise symptoms it will be recommended that volunteers take probiotics at the same time as a meal.

- In consideration of the global COVID-19 pandemic, the applicable local measures will be taken to reduce the risks related to the attendance of the University of Reading for the study appointments.

What data will you collect from me and how will my data be kept?

We will always protect your privacy and ensure that your data is handled in accordance with the General Data Protection Regulation (GDPR) (EU) 2016/679.

- If you agree to take part in the study, we will collect some data about you. Specifically, we will collect some information about your age, diet, ethnicity, physical and mental health to evaluate if you meet the inclusion criteria for our study.
- We will also collect some identifying information (name, telephone and email contacts). This information will allow us to get in contact with you when needed (i.e., organising study visits).
- During the study we will collect and analyse your faecal, blood and urine samples, and we will collect data about your performance in the above-mentioned behavioural tasks.
- We will also collect and analyse images of your brain from the MRI scans.

- Until the end of the study, all data entered on eNutri will be temporarily stored by the password- protected Google Firebase technology (a reliable and widely-used platform developed by Google for creating mobile and web applications).
- It is certified under major privacy and security standards and complies with requirements under GDPR in connection with the transfer of personal data from the United Kingdom (UK) and European Union (EU) to the United States (US) (data may be processed in other countries including the US).
- Data transferred between your web browser and Google Firebase is encrypted (i.e., it is hidden from or inaccessible to anyone outside of the research team).
- When you use eNutri, your data is pseudonymised (i.e., linked to your unique eNutri ID code, not your name so you will not be personally identifiable).
- Your eNutri data will be accessible to the University of Reading's eNutri Research Team (led by Professor Julie Lovegrove), who will use this data for further research.
- Since data sharing is important within the scientific community, your fully anonymised/unidentifiable eNutri data (without names or ID codes) will be made available on a data repository for other researchers to conduct further research.

- Your personal data will be kept confidential and securely stored for five years, in a coded form, whereby data and observations will be linked to the personally identifiable information only through a code available only to the Principal Investigator of the study.

- Information linking that number to your name will be stored securely and separately from the data you provide us.
- All personally identifiable information collected for the project will be stored for five years and destroyed thereafter.

- The anonymised data collected during the study will be analysed and the results of our study will appear in scientific publications.
- At the end of the project, all the data will be preserved and made available in anonymised form (on e.g., University of Reading's Research Data Archive and OpenNeuro), so that they can be consulted and re-used by others.
- Should you have questions about how your personal data is managed, you can contact the University Data Protection Officer at: **imps@reading.ac.uk**.

Do I have to take part?

- No, taking part in this study is completely voluntary.
- You are free to withdraw from the study at any time, without giving a reason.

Who has approved the study?

This project has been reviewed and was given a favourable opinion by the NHS Wales Research Ethics Committee 4 Wrexham (23/WA/0042).

Who do I contact if I have any concerns?

- If you have any concerns or complaints about anything to do with the study, please reach out to us via email at **gutbrain@reading.ac.uk**.
- You can also contact any of the study researchers more directly using any of the contact details provided on the first page of this document.
- Alternatively, if you would like to write to the principal investigator, please send your letter to:

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Schematic diagram of both work packages