University of Reading

School of Psychology and Clinical Language Sciences
Whitenights
Reading RG6 6AL

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

Information Sheet

Supervisor:	Email:	Phone:
Prof Bhismadev Chakrabarti	b.chakrabarti@reading.ac.uk	+44 (0) 118 378 5551
Prof Glenn Gibson	g.r.gibson@reading.ac.uk	+44(0)118 378 8715

Experimenters:

Dr Anisha Wijeyesekara	a.wijeyesekera@reading.ac.uk
Dr Andrea Monteagudo	a.monteagudo@reading.ac.uk
Dr Valentina Fanti	valentina.fanti@pgr.reading.ac.uk
Dr Carolyn McNabb	c.b.mcnabb@reading.ac.uk

The gut microbiota is the assemblage of microorganisms that live inside and on the surface of our gastrointestinal tracts – It is estimated to outnumber our own cells by a factor of ten and aggregates 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, preventing growth of pathogens, etc. There is also evidence that some bacteria can produce neurotransmitters 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 on the human microbiome is still in its infancy and thus it is a very active field of research.

We are conducting an experiment to study how gut microbiota composition is associated with individual differences in brain functioning, behaviour and personality traits in the general population. We are also aiming to evaluate the impact of a dietary intervention (over-the-counter probiotics and/or prebiotics) on brain functioning, behavioural tasks and personality traits.

We would be grateful if you could assist us by participating in our study to test whether relative abundance of different gut microbial species at baseline and after a dietary intervention, influences brain functioning and behaviour.

Study design:

The study involves two different work packages: work package 1 and work package 2.

Work package 1 is a correlational study aimed at evaluating the baseline gut microbiota composition and how it is associated with individual differences in brain functioning, behaviour and personality traits.

Work package 2 involves a dietary intervention that may or may not contain living micro-organisms (e.g., on-the-shelf products such as Yakult, Multibionta capsules, Actimel).

This study has a set of inclusion and exclusion criteria in order to draw reliable inferences on the questions of interest. Specifically, we will recruit right-handed white males without any identified developmental/ psychiatric condition. All participants must be aged between 18 and 50 years, be resident and brought up in the UK, and have Body Mass Index between 18 and 28. We will exclude participants who have used PPI (protonic pump inhibitors i.e., omeprazole, lansoprazole) antibiotics, probiotics or prebiotics in the previous 3 months, participants who smoke, who regularly drink more than 14 units of alcohol/week, who use drugs acting on the brain for medicinal or recreational purposes and those who have a clinical diagnosis of psychiatric or neurological disorder or history or current diagnosis 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, use of alcohol or drugs acting on the brain, can have a significant impact on gut microbial composition and/or modify the performance in behavioural tasks, which can limit the statistical power of our study.

All volunteers who meet the inclusion criteria will be invited to take part in work package 1.
A randomly selected subset of volunteers will be invited to take part to work package 2 as well.
However, should you not be willing to take part to work package 2, you will still be welcome to be involved in work package 1 only.

Work package 1:
Before the experiment you will be asked to fill in a preliminary screening form to check that you meet our inclusion criteria, an MRI Contraindications Screening Sheet and Medical and Lifestyle questionnaire which will include information about your general health. These questionnaires will allow us to determine whether you meet the eligibility criteria for this study and that you can safely participate in this study (see above mentioned inclusion and exclusion criteria).

If deemed eligible, you will be asked to proceed to take part in the study, as summarised below.

In the week preceding the experiment we will invite you to the University of Reading to attend the **Screening visit** where you will be able to familiarise with the laboratories and researchers. You will be provided with detailed information about the procedures. If you agree to participate, you will be asked to sign the consent form.

During the screening visit you will also be provided with a kit to collect a faecal sample and instructed on how to use it. We will also instruct you to track your diet on a smartphone app (Enutri).

The day before the study visit, we will ask you to complete a Covid19 pre-visit questionnaire, to check if you have any symptoms of Covid.

On the day of the experiment **(Study Visit)**, we will ask you to deliver a faecal sample to the Food and Nutritional Sciences Department. You will also be asked to provide a urine and blood sample. The blood sample will be collected at the Hugh Sinclair Nutrition Unit by a trained phlebotomist and before accessing the unit you will be asked to complete a questionnaire to assess the risk related to Covid19.
The samples will be analysed in order to explore the relative abundance of all bacterial species in the colon, as well as their circulating metabolites.

Subsequently you will be invited to move to the Psychology department (Harry Pitt building) in University of Reading where the rest of the experiment will be run.

Your participation will take approximately 3 hours.
In the first part of the study visit, you will carry out some tasks on a computer and complete personality questionnaires which should take approximately 90 minutes. This part will take place in a lab in front of a desktop/laptop PC.
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 (e.g. similar to the vibration felt on a mobile phone on silent mode).

In the second part of the study visit we will ask you to undergo a neuroimaging battery that will include brain imaging in an MRI scanner. This part of the experiment will take place in the Centre for integrative Neuroscience and Neurodynamics and will last about 90 minutes.

Work package 2:

If you are invited, and choose to take part in work package 2, you will be asked to attend the University of Reading for 3 follow-up visits spread over 12 weeks (<3 months). The follow-up visits will be similar to those for Workpackage 1, and will take place on weeks 4, week 8 and week 12. The same brain imaging and behavioural test battery will be administered at each of these visits, and you will be asked to provide a faecal sample, as well as blood and urine samples.

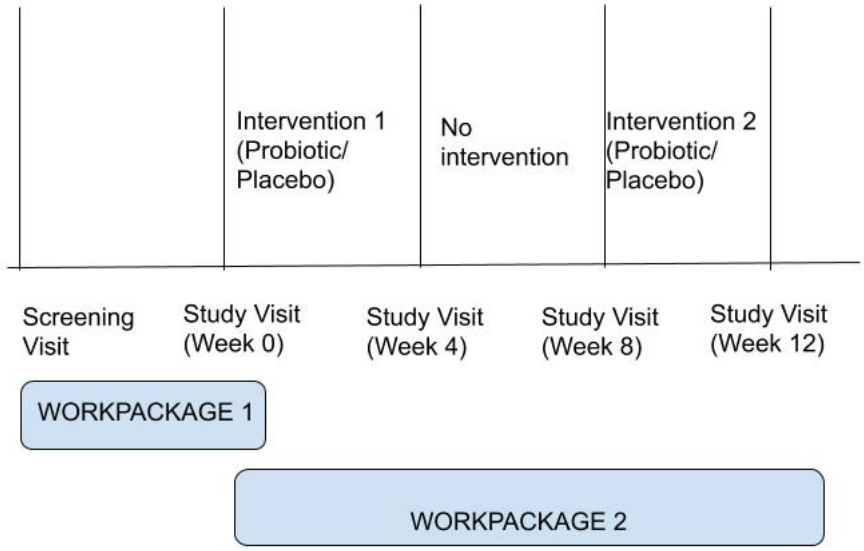
You will be randomly allocated to a group which will determine if you are going to start the trial with the placebo or the probiotics and/or prebiotics, and both you and the researcher will be blind about your allocation. Probiotics are live microorganisms that are beneficial to your health when administered 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). Prebiotics are non-digestible fibre which serve as ‘food’ for beneficial microbes that already live in your colon. They can be naturally found in some plants, such as onions, garlic, bananas, chicory root, and Jerusalem artichokes.

Depending on the group that you have been allocated to, you will be provided with the probiotic/prebiotic OR the placebo for 4 weeks, and will be asked to take these supplements daily.

At each **follow-up visit**, and you will be asked to provide faecal, blood and urine samples and will be tested with the above-mentioned neuroimaging and behavioural batteries and asked to fill in the questionnaires. Before each visit you will be screened for Covid19 symptoms with the above-mentioned questionnaire.

You will also be asked if you had any side effect from the dietary intervention, if you took it regularly and if you took any other medications.

The schematic diagram below illustrates the structure of both work packages.



Are there any risks for me by taking part in the 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 molecules 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 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 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 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 analysing blood and urine samples, will only be aimed at 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 work package 2, you will be asked to take an over-the-counter probiotic and/or probiotic for 4 weeks and a placebo (maltodextrin) for 4 weeks.
Both you and the researchers will not be aware of which dietary intervention you will be asked to take first.
Maltodextrin 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 most people. 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.
Prebiotics are safe for human consumption. However, 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 prebiotics at the same time as a meal.

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 acquired with Magnetic Resonance Imaging.

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 the General Data Protection Regulation in connection with the transfer of personal data from the UK and EU to the 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

Taking part in this study is completely voluntary; you may withdraw at any time without having to give a reason. Please feel free to ask any questions that you may have about this study at any point.

In consideration of the global pandemic of Coronavirus, specific measures will be taken for reducing the risks related to the attendance of the University of Reading for the study visits. All measures are listed in the Covid-19 procedure document.

This application has been reviewed by the University Research Ethics Committee and has been given a favourable ethical opinion for conduct.

Thank you for your help.

Prof Bhismadev Chakrabarti, on behalf of all investigators