

# The Effects of Selective Serotonin Reuptake Inhibitors on the Human Gut Microbiome

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## ABSTRACT

The front-line treatment for depression are SSRIs (selective serotonin reuptake inhibitors). They prevent the removal of serotonin, a neurotransmitter, from the synapse by inhibiting pre-synaptic reuptake proteins. Many patients taking SSRIs often report significant weight fluctuation and changes in appetite, which can be linked to disturbances of the gut microbiota. For instance, SSRIs may be responsible for depleting weight regulating bacteria, increasing the chances of dysbiosis, or imbalance of the gut microbial community. Aside from weight fluctuations, SSRIs might be having a direct impact on gut microbial community structure, a change that results in a signal via the gut-brain axis that alleviates depressive episodes.

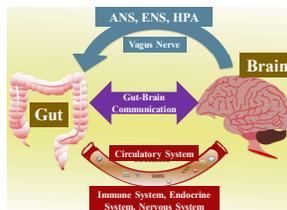
The goal of this ongoing project is to investigate if SSRIs have a direct significant effect on human gut microbial populations. This can be accomplished through in vitro drug administration provided to isolated human gut microbial communities in an anaerobic environment.

## INTRODUCTION

SSRIs block the reuptake of serotonin via transport proteins on the axon terminals of serotonergic neurons, allowing more serotonin to stay in the synaptic cleft and continue to bind to the post-synaptic cells.



The term 'microbiome' refers to the essential microbes that live in and on the human body<sup>8</sup>. The gut microbial community serves many functions, including regulation of body weight and maintaining a balanced environment<sup>3</sup>. Contemporary research has established a two-way connection between the nervous system and the gut microbiota, referred to as the microbiota gut-brain axis (MGB axis)<sup>2,5</sup>. Recent studies have found that the gut microbiota can have profound effects on the brain in terms of physiology, behavior, and cognition<sup>7</sup>.

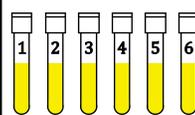


SSRIs may have unspecified effects in the intestinal tract. This project seeks to determine if SSRIs are directly causing any changes to gut microbial populations, and if so, how these changes could impact patients with depression who take SSRIs.

## METHODS

- Collection of microbes
  - Microbes were obtained from stool samples of healthy male donors not taking SSRIs.
- Preparation of SSRIs: 3 SSRIs- Paroxetine (Paxil), Fluoxetine (Prozac) and Citalopram (Lexapro)- were dissolved in solvent DMSO to yield 1000x stock solutions.
- Drug administration to gut microbiota in vitro
  - 6 conditions per drug, outlined in Figure 1.
  - Contents of tubes were incubated in an anaerobic chamber over 5 days.
  - Samples from each tube were aliquoted at 24-hour increments.
  - After samples were taken, 1.25 mL BHI was added back to each culture tube to replace the volume lost. Tube 3 received 12.5  $\mu$ L DMSO and tubes 4-6 received half of the amount of drug initially provided.
  - All samples were placed in a -80 degrees Celsius freezer for later use.
  - All drugs were tested in triplicate with Figure 1 set-up.
- DNA extraction (current stage)
  - Samples are thawed and DNA extraction protocol is used with a Homebrew Kit to extract DNA
  - DNA concentration is assessed via a NanoDrop spectrophotometer.
- DNA sequencing and bioinformatic analysis (future steps)
  - DNA samples will be sent to the Loyola Genomics Facility for 16S rRNA gene amplification.
  - Changes to the microbial communities with each set of trials will be assessed.

## Figure 1: Breakdown of Culture Tube Contents For Each SSRI



- Negative Control: BHI
- Positive Control: BHI, bacteria
- Solvent Control: BHI, bacteria, DMSO
- 0.1x: BHI, bacteria, 0.1x drug
- 1x: BHI, bacteria, 1x drug
- 10x: BHI, bacteria, 10x drug

At t=0, all tubes received 2.5 mL BHI growth media. Additionally, tubes 2-6 received 50  $\mu$ L of the same microbial community. As the solvent control, tube 3 received 25  $\mu$ L DMSO. Tubes 4, 5, and 6 received 0.25  $\mu$ L, 2.5  $\mu$ L, and 25  $\mu$ L SSRI, respectively.

## MATERIALS/NOTABLE EQUIPMENT



Coy Anaerobic Chamber



NanoDrop Spectrophotometer

## PREDICTED RESULTS

Others who have studied the effects of Fluoxetine (Prozac) on murine gut microbial communities found increases in bacterial populations that are involved in dysbiosis. They also found decreases in populations of *Lactobacillus johnsonii* and *Bacteroidales S24-7*, bacteria belonging to phyla that play an important role in regulating body mass<sup>3</sup>. It is likely that the data gathered from these sets of experiments will yield similar results. Although I have used drugs of the same class, it may or may not be the case that administration of each of the SSRIs yields unique fluctuations.

## REFERENCES

- Brain-Gut-Microbiome image. [https://www.mdpi.com/ijms/ijms-21-07551/article\\_deploy/html/images/ijms-21-07551-g001.png](https://www.mdpi.com/ijms/ijms-21-07551/article_deploy/html/images/ijms-21-07551-g001.png)
- Klancic, T. & Reimer, R. A. Gut microbiota and obesity: Impact of antibiotics and prebiotics and potential for musculoskeletal health. *J Sport Health Sci* **9**, 110–118 (2020).
- Lyte, M., Daniels, K. M. & Schmitz-Esser, S. Fluoxetine-induced alteration of murine gut microbial community structure: evidence for a microbial endocrinology-based mechanism of action responsible for fluoxetine-induced side effects. *PeerJ* **7**, e6199 (2019).
- NIMH » Major Depression. <https://www.nimh.nih.gov/health/statistics/major-depression.shtml>.
- Quigley, E. M. M. Microbiota-Brain-Gut Axis and Neurodegenerative Diseases. *Curr. Neurol. Neurosci. Rep.* **17**, 94 (2017).
- Vojvodic, J. *et al.* The Impact of Immunological Factors on Depression Treatment - Relation Between Antidepressants and Immunomodulation Agents. *Open Access Maced J Med Sci* **7**, 3064–3069 (2019).
- Wang, H.-X. & Wang, Y.-P. Gut Microbiota-brain Axis. *Chin. Med. J.* **129**, 2373–2380 (2016).
- Xavier, J. B. *et al.* The Cancer Microbiome: Distinguishing Direct and Indirect Effects Requires a Systemic View. *Trends Cancer Res.* **6**, 192–204 (2020)