# Making tools to facilitate the study of the gut-brain axis

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## 1 Introduction

### 1.1 Motivation and context

The human body comprises an intricate complex of biochemical relationships between individual components, and the balance of those components is crucial for optimal health. Among these components, the gut microbiota, the live bacteria living in our gut, has been found to play a vital role in maintaining a healthy body. Imbalance in the intestinal microbiota has been related to various disorders, including obesity, diabetes, inflammatory bowel, cancer, and even psychiatric conditions such as autism or depression.[1][2] Recent research has shown that gut microbiota is associated with eating behavior and brain activity. Thus, understanding the relationship between the gut microbiome and the brain could lead to new solutions for improving human health.[3] To that end, a project is underway to study the connection between the gut microbiome and the brain through both in-vivo and in-vitro trials.

In vivo trials have been applied to further investigate the relationship between the gut microbiome and brain function. Some authors utilized functional magnetic resonance imaging (fMRI) to acquire brain information while subjects' taste (palate) the basic tastes, including sweet, sour, salty, bitter, and umami. The fMRI technique allows for the measurement of blood flow changes in different brain regions, providing insights into the neural pathways involved in taste perception and preference.[4] Most of the authors use a gustometer to assist in these trials, a gustometer is a device used in sensory science to deliver precise amounts of taste stimuli to human or animal subjects in a controlled manner, allowing researchers to investigate taste perception and preferences. By combining these techniques with the measurement of in vivo gut microbiome composition and activity, this project seeks to uncover how the gut microbiota influences taste perception and preference and how this information can be used to develop targeted dietary interventions to improve human health.

### 1.2 Objectives

This project aims to develop a gustometer to be used during neurofunctional assays in humans (the Brain), in order to assist further developments in the comprehension of the gut and brain axis.

## 2 State of the art

### 2.1 The Gut-Brain Axis

The bidirectional communication system between the digestive and central nervous systems is called the "gut-brain axis." This network enables the integration of gut function with brain function and behavior through a complex system of neuronal, hormonal, and immunological communication channels. New research indicates that the gut microbiota, or population of bacteria that live in the gastrointestinal system, is essential for controlling brain functions.

Studies have shown that the gut microbiota can influence the production and release of neurotransmitters, such as serotonin and dopamine, which are critical for regulating mood, cognition, and behavior.[5] Additionally, the gut microbiota has been shown to regulate the hypothalamic-pituitary-adrenal (HPA) axis, which plays a significant role in the body's stress response. Numerous psychiatric and digestive illnesses, including depression, anxiety, and inflammatory bowel disease, have been linked to dysregulation of the HPA axis.[2]

Recent research has also emphasized the importance of the gut-brain axis in the regulation of appetite and metabolism. For instance, studies have demonstrated that the gut microbiota can influence the production of hormones, such as ghrelin and leptin, which regulate hunger and satiety. Furthermore, type 2 diabetes, insulin resistance, and obesity have all been linked to the gut microbiome.[6]

Age-related disorders including Alzheimer's and Parkinson's disease may be influenced by the disruption of gut-brain communication as we become older. According to studies, these illnesses may occur as a result of changes in the gut microbiota, inflammation, and oxidative stress. For instance, changes in the composition of the gut microbiota have been connected to the buildup of beta-amyloid plaques in the brain, a defining feature of Alzheimer's disease. Moreover, persistent intestinal inflammation can result in the creation of reactive oxygen species, which can harm brain cells through oxidative stress.[7][8] Overall, research on the gut-brain axis in aging points to the possibility that therapies aiming at enhancing gut health may be effective in treating diseases associated with aging.

### 2.2 Taste and Brain

A crucial element of human experience that affects dietary preferences and food preferences is the feeling of flavor. The five basic tastes—sweet, sour, salty, bitter, and umami—are detected by particular receptors on the tongue and other areas of the mouth. The gustatory cortex and the insula are just two of the many brain areas that are involved in the intricate neuronal processing of taste.

Recent findings suggest that the neural representation of taste is not just dependent on actual sensory input, and that the brain is capable of developing a neuronal code for imagined and inferred tastes. Functional magnetic resonance imaging (fMRI) was utilized in a study by Avery et al. (2023)[9] to look at how the brain reacts to both imagined and inferred tastes in human volunteers. The

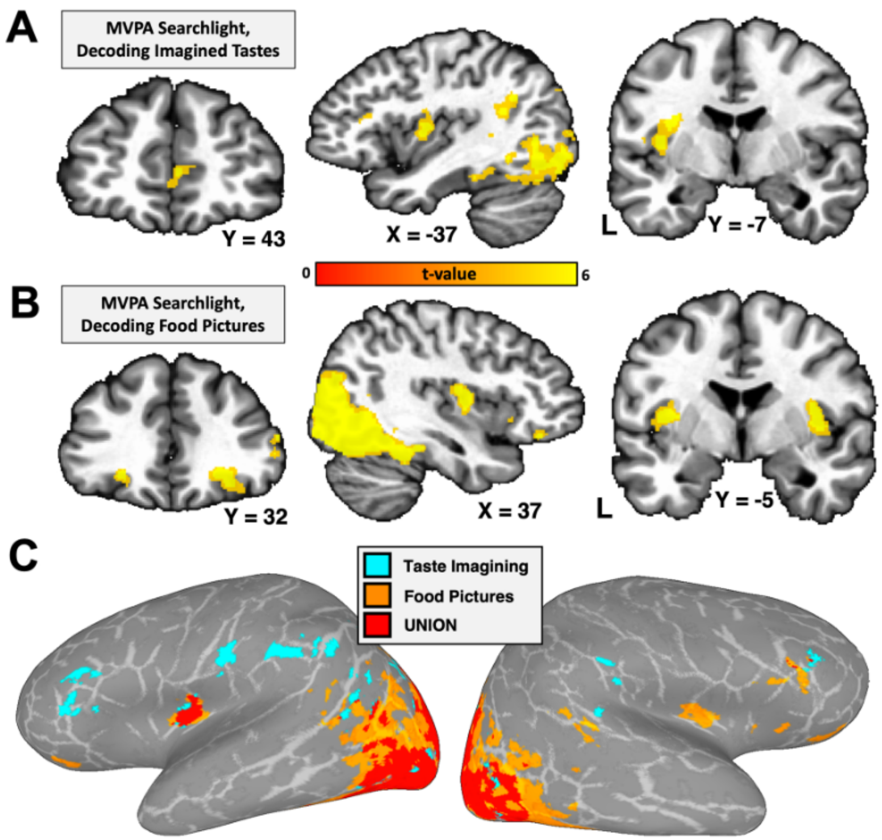


**Fig. 1.** Multivariate Pattern Analysis (MVPA) Searchlight results. A) MVPA searchlights reliably classify imagined tastes (sugar, salt, lemon juice) within brain regions including the left mid-insula and oral somatosensory cortex; B) Food picture category was reliably classified within bilateral dorsal mid-insula and orbitofrontal cortex; and C) A conjunction of both searchlight maps (projected onto an inflated cortical surface model) identifies a set of regions, including the left dorsal mid-insula and ventral occipito-temporal cortex, which reliably discriminate between imagined tastes as well as food picture category. Statistical maps were thresholded at p¡0.001 voxelwise, with a cluster-size correction for multiple comparisons at p-FWE ¡0.05.[9]

study discovered that the same brain areas, including the left oral somatosensory cortex and the bilateral dorsal mid-insula, were engaged for both real and simulated tastes, indicating that a single neural code is employed to describe taste information, as it can be seen in figure 1. Moreover, individual differences in brain activity in response to different tastes have been found to predict food choices and dietary habits, suggesting that brain responses to taste may be a potential target for interventions aimed at promoting healthy eating habits.

Additionally, elements like context, expectation, and prior experience might have an impact on how something tastes. For instance, the perception of sweetness can be enhanced by the presence of other sweet flavors, while bitterness can be suppressed by the addition of sweetness. This phenomenon is known as taste modulation and is thought to be mediated by complex interactions between sensory, cognitive, and affective processes.[10]

Last but not least, it is important to note that a scientific study has recently refuted the commonly believed belief in a ”tongue map,” which holds that distinct parts of the tongue are specialized for detecting particular flavors. Although it is possible that some tongue regions are more sensitive to some tastes than others, all tongue regions are capable of detecting all basic tastes.[11]

In conclusion, the gustatory cortex and the insula are just two of the many brain areas that are involved in the intricate neuronal processing of taste. Current findings indicate that regardless of whether a taste is true, imagined, or inferred, it is represented by a common neural code. Furthermore, context and prior experience are just two more aspects that have an impact on how we perceive taste, suggesting that programs targeted at encouraging healthy eating habits could be using brain reactions to taste as a potential target.

### 2.3 Gustometer and Computational Developments

Gustometers are devices used to deliver precise amounts of taste stimuli to human subjects, allowing for accurate measurements of taste perception and preferences. In comparison to conventional taste testing, the use of gustometers has several benefits, including improved precision, uniformity, and stimulus delivery flexibility. Gustometers can also be used in conjunction with other methods to provide a more complete understanding of how taste is processed in the brain, including electrophysiology and neuroimaging. The current work uses a gustometer to precisely deliver taste stimuli to human individuals to examine taste perception using functional magnetic resonance imaging (fMRI). Python code will be used to sync the gustometer's delivery of various tastes with the subject's chosen flavors, ensuring exact timing and control of the stimulus administration.

More sophisticated and adaptable gustometers have been created as a result of recent developments in technology and computational tools. Commercial gustometers are extensively used and provide benefits including dependability, uniformity, and usability. They are often designed for use in research and food industry settings and can be costly. On the other hand, self-made gustometers offer the advantage of flexibility and customization, allowing researchers to design and build a device tailored to their specific research needs at a lower cost.

A novel modular, scalable, and affordable device for rapid injection of small volumes of taste solutions during fMRI experiments was proposed by Canna et al. [3], which also gathers the possibility of flexibly increase the number of channels, allowing complex multi-dimensional taste experiments. Another study by Andersen et al. (2019) [12] used a custom-built gustometer with a low-pressure syringe pump system to deliver taste stimuli with high temporal precision, they also added a mouthpiece with a spray head attached because it atomizes the liquid and evenly distributes it to a large surface area of the tongue.

The first objective of this project is to equip the gustometer with a microprocessor, such as an Arduino, to regulate the flow of water and flavor stimuli. This will make it possible to automate some components of the experiment as well as to provide stimuli at precisely the right time and under perfect control. Several methods have been used in previous studies to control the delivery of taste stimuli, including other microprocessors, such as Raspberry Pi and BeagleBone Black. [13]

Overall, the integration of microprocessors and computational methods into taste research has the potential to greatly enhance our understanding and control of these complex systems.

## 3 Experimental Design

### 3.1 Participants

To ensure that the sample for this study was representative and covered a range of ages and body weights, the participants were carefully chosen. For the study, volunteers between the ages of 18 and 45 were recruited. It is significant to emphasize that all subjects were in good health and had no known histories of gastrointestinal, neurological, or psychological disorders.

In order to investigate the potential effects of obesity on the neural processing of taste stimuli, a subgroup of participants was specifically recruited to include individuals with obesity. This approach allowed for a comparison between participants with varying body weights, shedding light on potential differences in brain responses to taste perception across the weight spectrum.

To ensure the safety and validity of the MRI data, participants were screened for MRI contraindications and excluding criteria. The existence of pacemakers or other metallic implants, as well as any prior history of gastrointestinal conditions that would confound the results, were excluded factors. These requirements were crucial for ensuring the security and quality of the MRI data gathering, avoiding any artifacts that could compromise the accuracy of the findings.

### 3.2 Paradigm

In this study, we used an outcome-devaluation paradigm to examine the brain responses and goal-directed behavior related to various food outcomes. Using a method known as selective satiation, the goal was to selectively reduce the

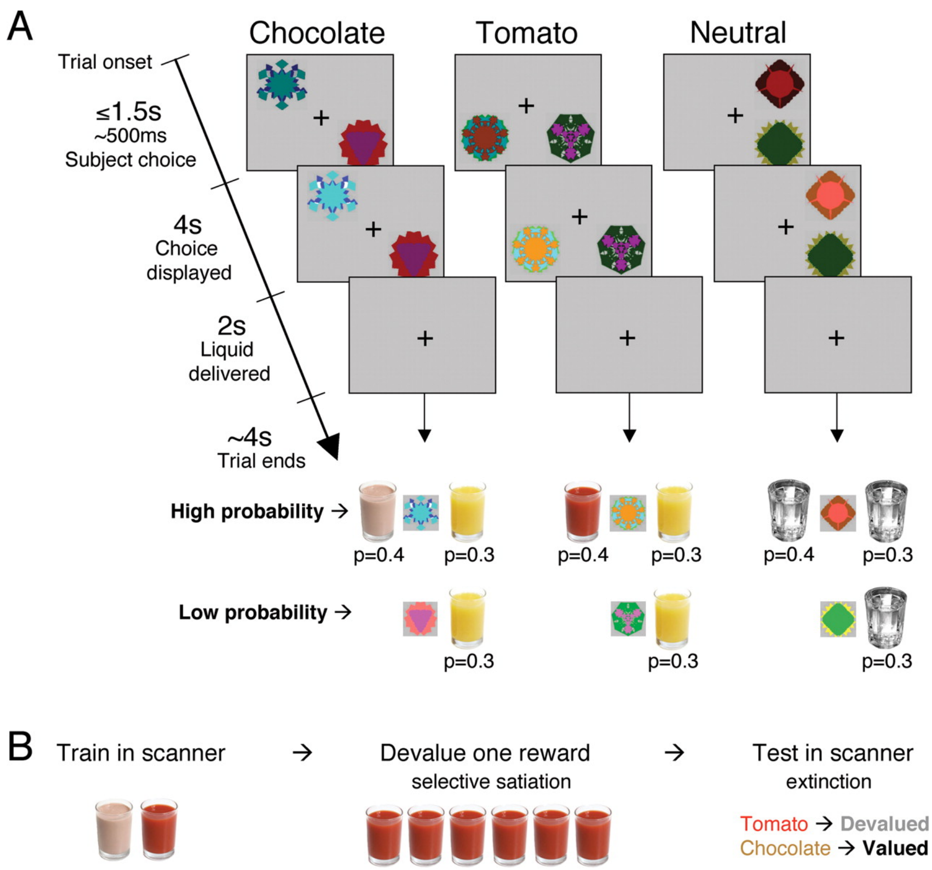


**Fig. 2.** A, Instrumental task illustration. The different actions available in the tomato, chocolate, and neutral conditions were signified by different arbitrary stimuli placed in one of four different locations. On each trial, subjects had to choose between two possible actions, one leading to a high probability of a food outcome ( p=0.7) and the other a low probability ( p=0.3). Depending on the condition, the high-probability action yielded tomato juice or chocolate milk with p=0.4, a common outcome (orange juice) with p=0.3, or else nothing. The low-probability action yielded the common orange juice outcome with p=0.3. Once an action was chosen, the stimulus signifying that action was illuminated, and 4 s later the outcome was delivered. B, Illustration of the experimental design. Subjects were trained in the scanner to choose two high-probability actions, one that led to chocolate milk, and one that led to tomato juice. They were then removed from the scanner and invited to consume either tomato (illustrated here) or chocolate to satiety, resulting in a selective decrease in the pleasantness of that food (selective satiation). They then underwent the same instrumental choice procedure in the scanner in extinction (the tomato or chocolate outcomes were no longer delivered, although the orange juice outcome continued to be delivered to maintain some degree of responding on both actions). At test, the condition involving the food eaten (in this example tomato) is designated "devalued" and the other is called "valued."[14]

value of one taste result while retaining the value of another. This paradigm was previously applied by Valentin et al. in a related experiment in 2007, illustrated in figure 2.[14]

Participants completed a fasting period of at least 6 hours before beginning the experimental task to guarantee hunger. On a scale from very unpleasant to very pleasant, we recorded behavioral judgments of the liquid meals' pleasantness and hunger levels.

Two 30-minute scanning sessions—one for training and the other for testing—made up the experiment. There were 50 trials in each of the three conditions—chocolate, tomato, and neutral—in each session's 150 total trials. Throughout both sessions, pseudorandom intermixing of the trial types was used.

One pair of the three fractal pattern pairs was shown to participants in each trial. They were given the task of selecting one of the various acts without being informed of the connection between those actions and the results. They were aware that each pair of actions had one action that had a larger likelihood of producing a result than the other.

Participants in the training learnt to choose behaviors that are more likely to result in enjoyable liquid items, such as chocolate milk and tomato juice. Each participant received an equal amount of the specific meal used for selective satiation (devaluation). Following instructions, participants were taken out of the scanner and encouraged to eat the discounted item (such as chocolate ice cream or tomato soup) until they were satisfied. Selective satiation was predicted to result in a decline in the food's pleasantness rating.

Participants were sent back inside the scanner for the test session after the devaluation procedure. They went back to deciding between activities that might result in various food outcomes. The devalued and non-devalued results, however, were no longer displayed during the exam. On the two possible outcomes, only the non-devalued orange juice result was offered with an equal chance. By avoiding the confounding aspect of having to learn new connections again, this made sure that participants relied on their already acquired linkages between outcomes and behaviors.

By comparing the difference in response to the action associated with the valued outcome and the action associated with the devalued consequence, goal-directed performance was assessed. Following the test session, further behavioral assessments of the liquid food's palatability and degree of hunger were gathered.

## 4 Methods

### 4.1 Gustometer Setup

The gustometer configuration included a custom-designed device and an Arduino ESP32S3 microcontroller. The gustometer shown in Figure 3 was created using white PLA filament that was 3D printed. It has six pumps and silicone tubes (intern diameter/ extern diameter, 3/5 mm) for precisely delivering flavor stimuli.

Sterilized lines measuring 30 cm were attached to the gustometer and changed before each session to ensure cleanliness. The incorporation of ”rewashing” amounts and separate routes for each flavor efficiently prevented fluid contamination, enabling a distinct perception of each taste.

Prior to the fMRI experiment, the tubes were pre-filled to prevent any fluid drip during inactivity. The injection of 2 mL of solution at a flow rate of 200 mL/min was ensured by using a set delivery time of 1200 ms. The time required for the fluid to propagate through the tubes and reach the subject's mouth was considered negligible due to the pre-filled nature of the tubes.

The gustometer's architecture comprised two main sections: the control and interfacing system, driven by the Arduino ESP32S3 microcontroller, and the system of peristaltic pumps responsible for solution administration. The gustometer prototype was built using white PLA filament that was 3D printed.

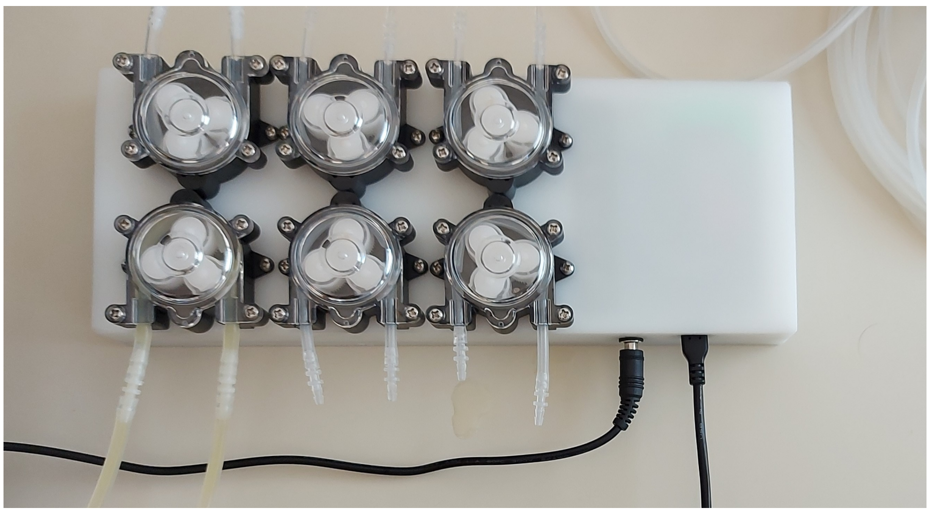

**Fig. 3.** The 3D printed gustometer, constructed using PLA material, equipped with six pumps and controlled by an Arduino ESP32S3 microcontroller.

### 4.2 MRI Acquisition

The MRI acquisition for this study utilized a Siemens Healthineers Magnetom Skyra Fit scanner operating at a field strength of 3 Tesla. This state-of-the-art scanner offered exceptional imaging capabilities, enabling high-resolution and detailed visualization of the targeted anatomical regions. The field strength of 3 Tesla provided an enhanced signal-to-noise ratio and improved image quality, allowing for more accurate and reliable data acquisition.

In conjunction with the scanner, the study employed the New syngo MR XA software (Win10). This advanced software platform offered a range of innovative features and tools for image acquisition, processing, and analysis. The software's comprehensive functionality and user-friendly interface facilitated efficient data management and manipulation, ensuring the researchers could optimize their workflow and extract meaningful insights from the acquired MRI data.

### 4.3 Data Preprocessing

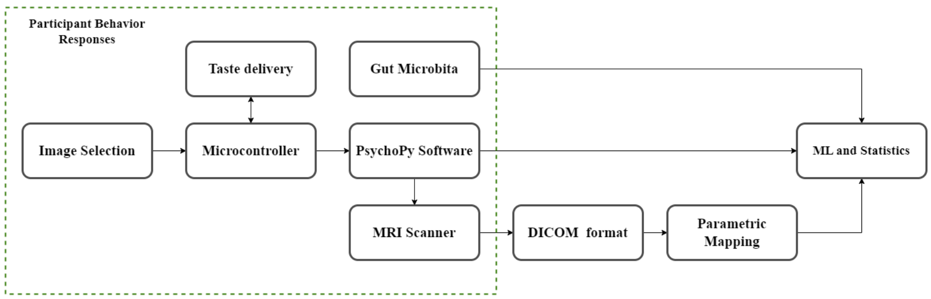


**Fig. 4.** Workflow illustrating the data processing and analysis pipeline, from patient responses to preprocessing, feature extraction, and the application of machine learning (ML) and statistical analysis techniques.

In the present study, data preprocessing was a crucial step in integrating the various data sources to gain comprehensive insights into the gut-brain axis and taste perception. Multiple data streams were obtained, including liquid delivery participant reactions using PsychoPy paradigm software, MRI data in DICOM format, and gut microbiota data. Each of these data types needs particular preprocessing procedures to ensure compatibility and facilitate meaningful analysis.

The PsychoPy paradigm software and microcontroller interactions provided important information regarding flavor perception and participant behavioral responses. These responses were obtained in a randomized manner to prevent bias and assure fair data collecting. To validate that the taste stimuli and participant reactions were precisely aligned, accurate matching and synchronization were used to merge these responses with the liquid delivery data. This matching procedure enabled us to link subjective taste perception with objective liquid delivery, allowing us to conduct a thorough examination of taste-related brain responses.

The MRI data, acquired in DICOM format, required additional preprocessing steps to transform the 3D images into numerical matrices suitable for analysis. The conversion to numerical matrices enabled uniform data representation and additional analysis utilizing computational statistics and machine learning techniques. Aligning the MRI data with the paradigm data was crucial in demonstrating linkages between brain responses and taste perception. By matching the temporal dynamics of the MRI data with the timing of taste stimuli, we could investigate the neural correlates of taste perception and explore the activation patterns within specific brain regions.

In addition to taste-related data, the study collected gut microbiota data from each participant. This data provides insight into the composition and diversity of the gut microbiota, offering a new perspective on the link between gut health and taste perception. By connecting gut microbiome data with taste perception results, we may look at possible connections between different microbial profiles and flavor preferences. By merging these several data modalities, including the gut microbiota, we can find unique linkages and get a greater knowledge of the gut-brain axis and its effect on taste perception.

## 5 Main Findings

Studies utilizing a gustometer and MRI have provided important new information on the brain processes underpinning gustatory processing. These investigations have shown that the representation of taste attributes and the steering of goal-directed instrumental decisions depend critically on the activation of particular brain areas, such as the insular cortex and orbitofrontal cortex. Researchers have been able to study the brain's reactions to taste stimuli, interpret taste attributes, and examine the effects of taste-related elements on decision-making processes by combining gustometer devices with MRI methods. These novel methods have improved our comprehension of the neurological underpinnings of gustatory perception and have the potential to guide further investigation into topics including taste disorders, dietary preferences, and therapeutic uses of taste perception.

Valentin et al. (2007)[14] employed a gustometer, functional magnetic resonance imaging, and a paradigm resembling ours to provide light on the neural mechanisms underlying goal-directed instrumental choice in humans. The results demonstrate that the orbitofrontal cortex plays a crucial role in controlling these choices. The neuronal activity in this region of the brain significantly changed while choosing a devalued action as contrasted to an undervalued action. This suggests that the orbitofrontal cortex contributes significantly to the processes that lead to instrumental actions and reward outcomes. Additionally, goal-directed learning throughout the training session activated the orbital and medial prefrontal cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, ventral and dorsal striatum, and amygdala. These findings highlight the involvement of these brain regions in the cognitive and emotional aspects of goal-directed behavior.

In more recent work, Avery et al. (2023)[9], used high-field fMRI to examine the brain processes related to imagining tastes and viewing food images. The results shed light on how taste characteristics are represented in the brain. One of the key cortical areas known to be sensitive to the perception of taste, the bilateral dorsal mid-insula, was shown to be activated by imagined flavors in the research. The study also showed that the patterns of those imagined tastes might be used to decode within the mid-insula area the prevailing taste quality (sweet, sour, or salty) linked with food photos. This shows that regardless of whether it is explicitly imagined or automatically inferred while observing food, there is a similar code for describing taste quality. These findings contribute to our understanding of how the brain processes and represents taste information.

Canna et al.[3] introduced a novel open-architecture, low-cost flavor delivery system for brain-computer interface and gustatory fMRI research in 2019. The effectiveness of this device in creating significant clusters of fMRI activity was evaluated, particularly in the insular cortex and other regions connected to taste processing. According to the study, specific brain areas were engaged by certain basic taste qualities and intensities, such as sweet and bitter sensations. Notably, extra-insular activations were found in the putamen, caudate nuclei, cingulate cortex, temporal and parietal regions, as well as the pre-and post-central gyrus. The device's construction in accordance with a scalable and open architecture makes it viable for future research in gustatory fMRI trials. The device's ability to control taste stimuli delivery with different concentrations and the wide range of brain regions activated by taste further enhances our understanding of gustatory processing in the brain.

In a mouse model of schizophrenia, Ward et al. (2012)[15] looked at the neurobiological and psychological factors behind decreased incentive motivation. The study concentrated on the function of overexpressed striatal D2 receptors and how it affected motivational impairments. The taste-reactivity paradigm and the effort-related choice paradigm were used by the researchers to evaluate the foundation of the motivational deficiency through the use of a gustometer. This research varies slightly from others because flavored powder rather than liquid is administered via the gustometer in this one. The findings showed that in mice with overexpressed striatal D2 receptors (D2R-OE), hedonic responses to appetitive stimuli were not impaired. However, these mice showed reduced incentive motivation, which suggests distorted cost-benefit analyses. The inability to accurately depict the worth of potential rewards may be the cause of this lack of motivating behavior. The results highlight the significance of dopamine signaling in motivating behavior and offer prospective targets for the creation of novel medications to address the negative symptoms of schizophrenia.

The combination of the four studies mentioned above, which used gustometers as an essential part of their experimental design, contributes to a thorough understanding of the various neural mechanisms underlying incentive motivation, taste perception, and goal-directed instrumental choice. Various studies illuminate the critical roles played by particular brain areas, including the striatum, orbitofrontal cortex, and dorsal mid-insula, in various cognitive processes. Our understanding of decision-making, taste representation, and the neuroscience of motivational impairments has been greatly improved by the findings taken together. These findings are significant because they may have implications for the creation of focused therapy approaches to deal with the unpleasant symptoms of mental diseases. They also open the door for future investigations aimed at elucidating the complexities of these events and enhancing our understanding of these fields.

## 6 Discussion

The current study developed a customized tasting paradigm employing an Arduino-based gustometer to enable the examination of the gut-brain axis. We aimed to improve our comprehension of the neural mechanisms underlying taste perception and its impact on eating behavior by incorporating computational statistics and machine learning techniques, which have gained significant traction in health studies, particularly in the analysis of MRI data.

We may predict likely neuroimaging results of our paradigm by extrapolating findings from prior studies that employed comparable approaches. The complicated relationships between the stomach and the brain can be uncovered using a gustometer in conjunction with neuroimaging methods like fMRI. Using our tasting paradigm in conjunction with neuroimaging techniques, we expect to see activations in important brain areas related to taste processing, such as the orbitofrontal cortex, insula, and striatum. Combining computational statistics and machine learning algorithms will allow us to extract relevant information from the MRI data, showing patterns and correlations that may not be readily apparent using traditional analytic methods.

The significance of this work lies in its potential to support the development of customized therapies and treatments intended to improve eating habits. Understanding the neural mechanisms behind taste perception and its connection to the gut-brain axis would enable us to better comprehend why individuals have varying dietary and nutritional preferences. By utilizing the skills of computational statistics and machine learning, we may discover patterns and biomarkers within the MRI data that may serve as predictors of eating behavior and assist in the development of specialized therapies for individuals with particular food problems.

Furthermore, using the Arduino ESP32S3 microcontroller for the gustometer implementation proved to be a valuable decision. Because of its versatility, reliability, and ease of integration, it has allowed for seamless interactions with the taste paradigm, allowing for exact control and assessment of taste stimuli. The Arduino platform, coupled with the ESP32S3, offers flexibility for future advancements and modifications, opening avenues for innovative research in the field of taste perception and the gut-brain axis.

## 7 Conclusion

This study contributes significantly to our understanding of the gut-brain axis and its influence on eating behavior. By combining an Arduino-based gustometer with computational statistics, machine learning techniques, and neuroimaging methodologies, we aim to gather meaningful neuroimaging data that will shed light on the neural mechanisms behind taste perception. The use of computational tools improves our capacity to evaluate complicated MRI data, discover relevant patterns, and relate brain responses to taste preferences. The study's findings have the potential to inspire the creation of tailored therapies that take advantage of the interplay between the gut-brain axis, taste perception, and eating behavior. The utilization of the Arduino ESP32S3 microcontroller demonstrates its suitability for precise and reliable taste stimulus control. Moving forward, further exploration and refinement of these methodologies will advance our understanding of the gut-brain axis and pave the way for targeted interventions to improve eating behavior and promote overall health and well-being.

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