Neural indicators of human gut feelings

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Abstract

Understanding the neural processes that govern the human gut-brain connection has been challenging due to the inaccessibility of the body's interior. In this study, we aimed to identify neural responses to gastrointestinal sensation (i.e., the neural basis of 'gut feelings') in healthy humans using a minimally invasive mechanosensory probe. Combining electroencephalography and electrogastrography with signal detection theory measures, we quantified brain, stomach, and perceptual (button-press) responses following the ingestion of a vibrating capsule. The relationship between vibration strength and perceptual sensitivity was evaluated using two stimulation conditions (normal and enhanced). Most individuals successfully perceived capsule stimulation in both conditions, as evidenced by above chance accuracy scores. Perceptual accuracy improved significantly during the enhanced relative to normal stimulation, which was associated with faster reaction time and reduced reaction time variability. Stomach stimulation induced responses in a cluster of parieto-occipital leads near the midline via a late positive potential emerging 300-600 milliseconds after stimulation onset. Moreover, these 'gastric evoked potentials' showed dose-dependent increases in amplitude and were significantly correlated with perceptual accuracy. Our findings are consistent with recent neurogastric and optogenetic studies demonstrating a role for posteromedial cortices in gastrointestinal interoception and body dissociation and highlight a unique form of enterically-focused sensory monitoring within the human brain. Overall, these results show that this minimally invasive approach could serve as a useful tool for understanding gut-brain interactions in healthy and clinical populations.

Significance Statement

The human brain continuously receives input from the stomach and intestines. These sensations are intuitively incorporated as ‘gut feelings’ into decision-making during daily life, yet we still know very little about how the brain processes gut signals. Here, we developed a minimally invasive approach to studying human gut feelings. In healthy individuals we found that an ingestible vibrating capsule produced reliable changes in both stomach sensation and gastric-evoked brain activity. These changes were significantly associated, in a dose-dependent fashion. We propose that this approach provides an opportunity to better understand the role of gut-related symptoms in human pathological conditions and might yield fundamental insights into how gut feelings are communicated to the human brain.

Introduction

The human brain must decipher a multitude of complex signals originating from within the body in order to maintain homeostasis and optimally sustain life. Interoception, the process by which the nervous system senses, interprets, and integrates signals originating from within the body across conscious and nonconscious levels (1), is a vital component of this homeostatic machinery maintaining internal stability in the face of changing environments. However, interoception remains poorly understood despite both growing scientific interest (2-5) and the recognition that certain psychiatric (6) and neurological (7, 8) disorders may manifest through abnormal neural processing of interoceptive signals.

Most interoception research on the gut-brain connection has focused on the cellular and molecular mechanisms of afferent interoceptive signal transmission in nonhuman animals (9). For example, in the alimentary tract, rapid cell-specific peripheral sensors of osmotic balance (10, 11), glucose (12, 13), and mechanical stretch (14, 15) have been identified that enable organisms to quickly regulate feeding/drinking behaviors before the onset of relevant blood-level changes. Neurons in the insular cortex have been identified to play a key role in the integration of these viscerosensory signals and the adaptive estimation of upcoming needs (16) (17) – providing a focal point within the central nervous system for understanding interoceptive predictive processing. However, the brain also exerts powerful influences on the gut and multiple descending pathways linking the stomach and brain have been identified. For example, the insular and medial prefrontal cortices send parasympathetic projections to the stomach through the vagus nerve, whereas the primary motor and somatosensory cortices send sympathetic projections to the stomach through spinal efferents (18).

In humans, the lack of appropriate techniques for easily accessing the body's interior has hindered the study of interoception. Some of these limitations have begun to be addressed through the development of pharmacological or mechanosensory perturbations of cardiac and respiratory sensation (19-21), yet there remains a dearth of minimally invasive probes for assessing other organ systems such as the gut. Most prior studies evaluating the conscious perception of gastrointestinal sensations have used invasive approaches involving the insertion of inflatable balloons or electrical probes into the esophagus (22, 23), stomach (24), colon (25), or rectum (26). While such approaches have shown the ability to engage putative interoceptive cortical circuitry (i.e. insular and somatosensory cortices)(27) and have revealed insights into the gut-brain axis in gastrointestinal disorders (28-30), the invasiveness of these approaches has made it challenging to conduct such research in human participants.

In the current study, we developed a minimally invasive probe targeting perceptions of the gastrointestinal system via ingestion of a vibrating capsule. Using a design inspired by signal detection theory, we combined the mechanosensory stimulation of stomach signals with perceptual measurement of stomach sensations and continuous recording of stomach electrogastrogram (EGG) and electroencephalogram (EEG) signals. We identify (1) reliable signatures of gastrointestinal perception at the individual subject level, and (2) differential effects in the brain based on the degree of stimulation, as measured by evoked response potentials (ERP) using EEG. We suggest that this minimally invasive

approach could serve as a useful method for understanding gut-brain interactions across a variety of human health conditions.

Results

40 healthy human participants (19 female, average age = 22.90 ± 4.56 years, range between 19 and 39 years, average BMI = 24.18 ± 3.03, range between 18.24 and 32.28) completed the study and met quality assurance criteria for inclusion in the analysis. Figure S1 shows the study Consort diagram.

Vibratory Stomach Stimulation Modulates Gut Sensation.

We examined whether the noninvasive delivery of vibratory stimulation to the stomach would yield reliable signatures of gastrointestinal perception at the individual subject level. Following ingestion of the capsule, subjects were instructed to press a button anytime they consciously perceived a feeling in their gut (Figure 1).

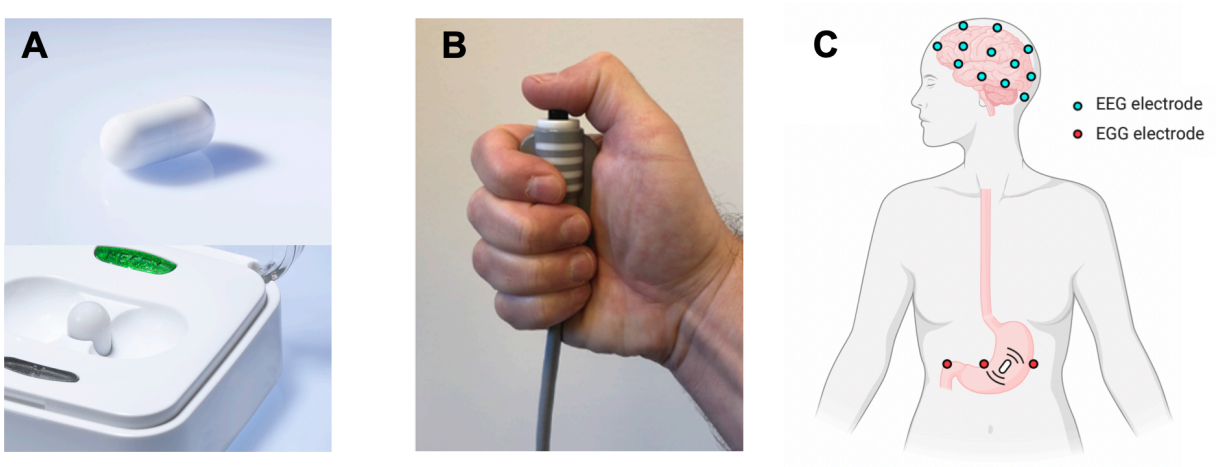

Figure 1: A) Vibrating capsule and activation base. **B)** The push button which participants were asked to press each time they detected a capsule-induced stomach sensation. **C)** Scalp electroencephalogram (EEG) and stomach electrogastrogram (EGG) lead placement.

The analysis of button-press responses showed that participants could successfully detect vibration stimuli under both normal and enhanced stimulation, *Normalized A prime* (mean ± standard deviation [STD]) = 2.49 ± 0.40 and 2.84 ± 0.25, respectively. Perceptual accuracy increased significantly under enhanced versus normal stimulation (p = 4.11e-08, $t(39)$ = 6.79, Cohen's d = 1.1) (Figure 2A). Response latencies (i.e., the time from capsule stimulation to button press) during normal and enhanced blocks were as follows: average response latency (mean ± STD) = 1.06 ± 0.33 seconds (normal stimulation) and 0.74 ± 0.23 seconds (enhanced stimulation); STD of response latency (mean ± STD) = 0.46 ± 0.14 (normal stimulation) and 0.28 ± 0.13 seconds (enhanced stimulation). The response latency decreased significantly under enhanced versus normal stimulation for both the average response latency (p = 7.61e-07, and $t(38)$ = 5.91, Cohen's d = 1.0) and for the STD of response latency (p = 3.20e-08, and $t(38)$ = 6.92, Cohen's d = 1.1) (Figure 2B and C). The binomial test showed that all 40 participants performed significantly above

chance ($p < 0.05$) during enhanced stimulation, whereas 5 subjects performed below chance ($p \geq 0.05$) during normal stimulation.

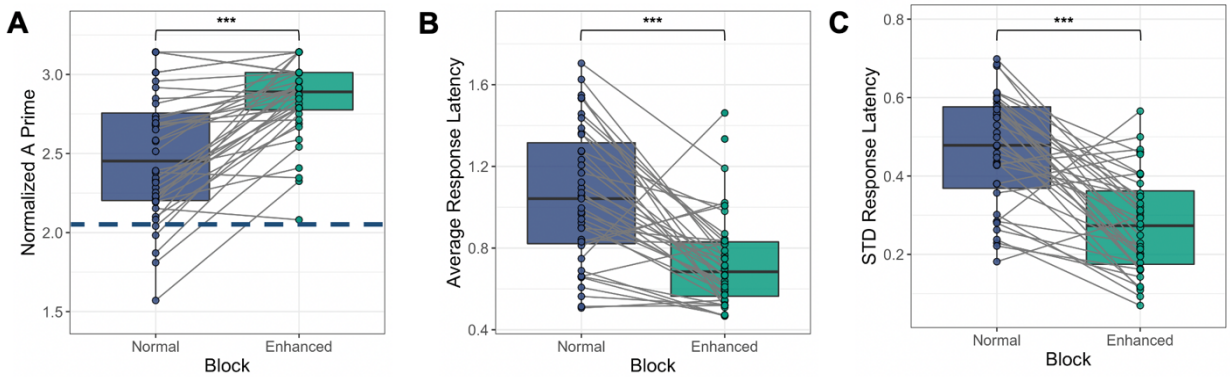

Figure 2: Perceptual accuracy measures during the normal and enhanced stimulation blocks based on button-presses signifying gut feelings during vibratory stimulation of the stomach. Gray lines present changes in individual subject performance from the normal to enhanced block. **A)** Normalized A prime (dashed line shows chance performance based on binomial expansion); **B)** Average response latency (in seconds); and **C)** Standard deviation (STD) of the response latency (in seconds). The participants' performance increased significantly with enhanced stimulation across all three measures, indicating the paradigm effectively induced changes in gastric interoceptive awareness. ***$P < 0.001$.

Sex Effects Do Not Account for Observed Changes in Gut Sensation

Repeated measures analysis of variances (ANOVAs) of the normalized A prime, average response latency, and the STD of response latency as dependent variables (which they applied separately for each variable), with sex and block as fixed factors showed no significant differences between the males and females for any of these dependent factors, despite significant differences between normal and enhanced stimulation for all dependent variables (Table S1).

Parieto-occipital ERP Indicators of Gut Sensation

Slow positive deflections in the ERP signal emerged around 300 milliseconds after stimulation onset, with a peak around 600 ms, and lasting up to 3000 ms in duration. These were maximally located over the midline centro-parietal electrode sites and within the time window of 300-600ms, a signature consistent with the arousal-modulated late positive potential (LPP) observed in studies of motivated attention (31). The degrees of freedom were adjusted using Greenhouse-Geisser corrections for the effects that violated sphericity assumptions, including channel (32). A significant main effect of condition, $F(1) = 23.79$, $p < 0.001$, $\eta 2 = 0.48$, revealed that the enhanced stimulation condition (Mean (M) = 3.52 *microvolts* (μV)) resulted in a larger LPP amplitude than the normal stimulation condition ($M = 2.18\ \mu V$). There also was a main effect of Channel, $F(2.03) = 12.93$, $p < 0.001$, $\eta 2 = 0.33$; however, the two-way interaction between Channel and Condition, $F(1.72) = 2.84$, $p = 0.077$, was non-significant. Figure 3A displays the ERP waveform for the six aforementioned channels, and Figure 3B shows the scalp topography maps for each block. Thus, the enhanced condition resulted in a larger LPP amplitude than the normal condition and this

enhancement was not significantly modulated by measurement site (Tables S2 and S3). Over the whole experiment, the capsule vibration resulted in an increased amplitude of the ERP signal for the parieto-occipital electrodes between 300 and 600 ms and 600 to 1000 ms windows (Figure S2). The repeated measures ANOVA applied to the averaged LPPs among midline centro-parietal electrodes between 300 and 600 ms showed no significant sex differences between males and females, with sex and block as fixed factors, $F(1) = 1.110$, $p = 0.297$; but a significant block difference, $F(2.03) = 5.422$, $p = 0.024$.

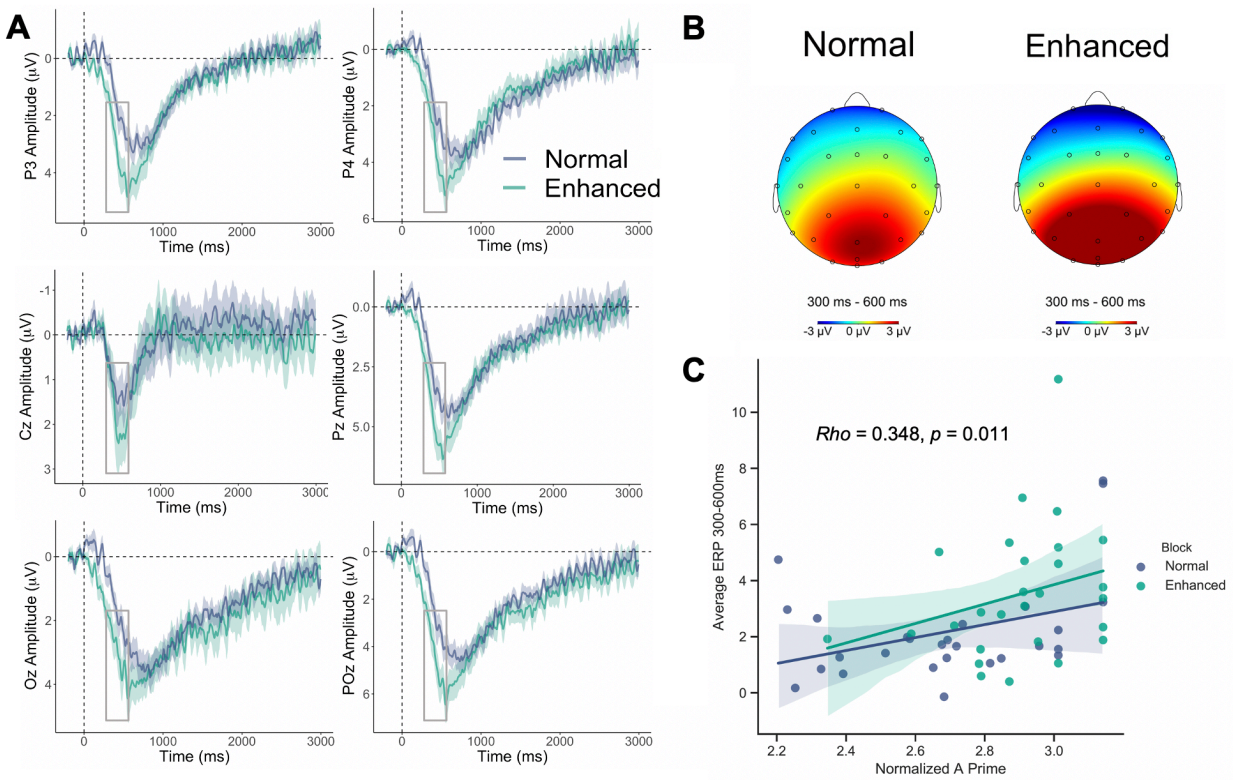

Figure 3: Parieto-occipital event related potential (ERP) indicators of gut sensation and association with perceptual accuracy measures during normal and enhanced stimulation. **A)** The ERP waveforms for channels P3, P4, Cz, Pz, Oz, and POz during the normal (blue) and enhanced (green) blocks. A dose-dependent increase was observed across all six electrodes during the 300 to 600 ms window (outlined in gray). Shaded areas represent the standard error of the mean for the ERP signal at each time point. Time-zero represents the onset of vibration stimulus. The presented waveforms were calculated from the average mastoid-referenced EEG. **B)** Late positive potential (LPP) scalp topography between 300 and 600 ms window after the vibration onset relative to the 200 ms pre-stimulus baseline for the normal and enhanced blocks. **C)** The positive association between LPP signal strength (average ERP signal among P3, P4, Cz, Pz, Oz, and Poz channels) and perceptual accuracy (normalized A prime) was significant across both blocks. Shaded areas correspond to the 95% confidence interval for the regressions.

Neural Gut Sensation Indicators Relate to Perceptual Accuracy

There was an association between the average LPP amplitude and perceptual accuracy (as measured by normalized A prime) after controlling for condition (i.e., normal/enhanced), $Rho = 0.348$, $p = 0.011$ (Figure 3C). In comparison, there was a negative association between LPP and STD of response latency, $Rho = -0.292$, $p = 0.034$ but no association between LPP and the average response latency, $Rho = -0.250$,

p = 0.071. However, only the correlation between LPP and normalized A prime remained significant after Bonferroni correction (p = 0.033).

Vibratory Stomach Stimulation Does Not Modulate EGG signals

The repeated measures ANOVA for the EGG total power showed no significant differences between the baseline, normal, and enhanced blocks ($F(2)$ = 2.50, p = 0.089 , $\eta 2$ = 0.062) (Figure 4A). To evaluate potential frequency-specific changes in the EGG signal, we conducted a separate repeated measures ANOVA for the three main frequency ranges (bradygastria [0.5 to 2.5 cycles per minute, cpm], normogastria [2.5 to 3.5 cpm], and tachygastria [3.75 to 9.75 cpm]) as a function of the three blocks (baseline, normal, enhanced). There was a significant main effect of frequency band, $F(1.25)$ = 5.75, p = 0.015, $\eta 2$ = 0.13; however, no significant main effect of block, $F(1.99)$ = 3.0, p = 0.056, $\eta 2$ = 0.07) was observed, and the two-way interaction between frequency bands and block, $F(2.17)$ = 1.49, p = 0.230, $\eta 2$ = 0.038, was non-significant. Further, EGG power at baseline was significantly correlated with EGG total power in both the normal and enhanced blocks: *Rho* = 0.495, p = 0.001; and *Rho* = 0.718, p < 0.001, respectively. Figure 4B displays the EGG power in different frequency bands for each block.

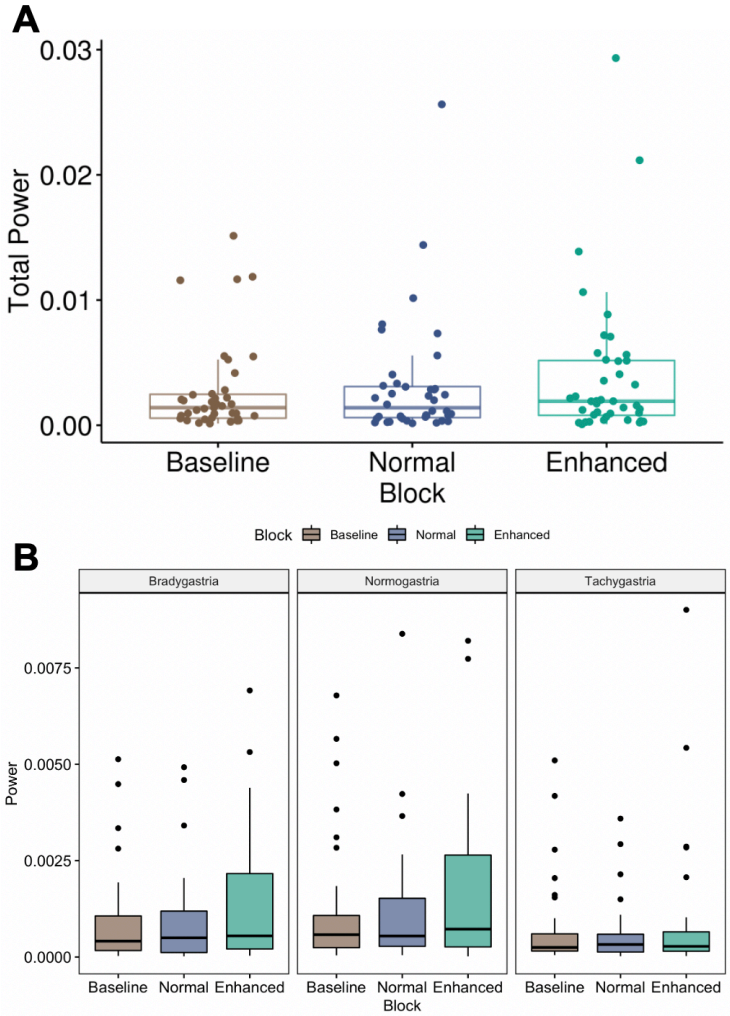

Figure 4: Electrogastrogram (EGG) power measured during the baseline, normal stimulation, and enhanced stimulation blocks. **A)** Total EGG power across all physiologically relevant spectrums. **B)** EGG power within the bradygastria (0.5 –2.5 cycles per minute, cpm), normogastria (2.5 to 3.5 cpm), and tachygastria (3.75 to 9.75 cpm) frequency spectrums. There was no evidence of significant stimulation-induced EGG changes across any power spectrum.

Vibratory Stomach Stimulation Induces Cross-Channel Respiratory Interference

The repeated measures analysis of pre- and post- stimulation interoceptive intensity ratings revealed significant differences between rating types, $F(2.367) = 45.214$, $p < 0.001$, $\eta 2 = 0.537$; the different time points, $F(1) = 35.254$, $p < 0.001$, $\eta 2 = 0.475$, and a significant interaction between time point and rating types, $F(2.51) = 29.909$, $p < 0.001$, $\eta 2 = 0.434$. The post-hoc comparison showed significant increases in both stomach/digestive and respiratory sensation intensity after Bonferroni correction ($p < 0.001$, Cohen's $d = 1.56$ for stomach/digestive sensation intensity and $p = 0.032$, Cohen's $d = 0.44$ for respiratory sensation intensity). The estimated marginal means of pre- and post- stimulation for all four measures are presented in Table S4. The magnitude of the stomach sensation intensity rating changes was larger than that for respiratory sensation. There were no significant differences for heartbeat or muscle tension ratings (corrected *p-values* are 0.181 and 1, respectively). Figure 5 illustrates the intensity ratings across the

experiment. There were no significant differences between the males and females observed for any of the interoceptive intensity ratings (Table S5).

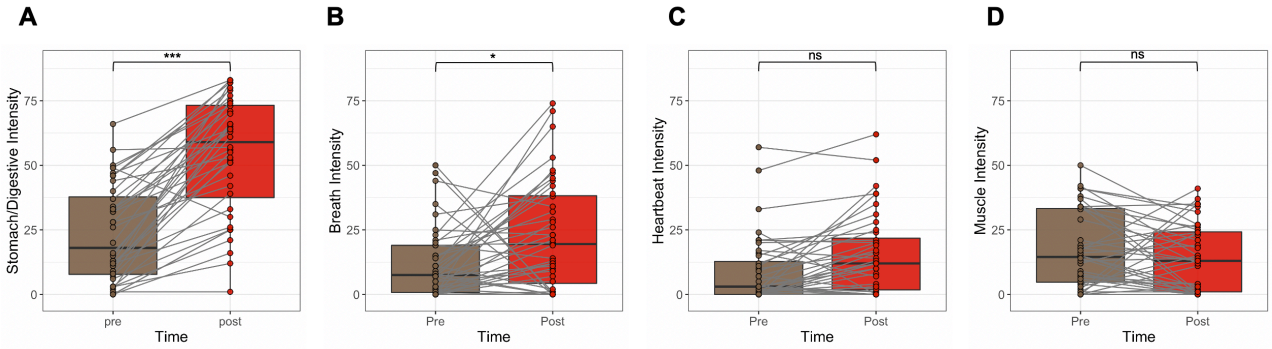

Figure 5: Self-reported intensity ratings of different interoceptive sensations experienced before (Pre) and during stimulation (Post; these ratings were provided retrospectively, and encompassed sensations experienced during both blocks). **A)** Stomach/Digestive, **B)** Breath, **C)** Heartbeat, and **D)** Muscle tension ratings. Stimulation-induced intensity ratings increased for both stomach and breath sensations. Gray lines show changes in ratings for each individual. *$P < 0.05$, ***$P < 0.001$; ns, not significant.

Vibratory Stomach Stimulation was Safe and Tolerable

There were no adverse events in any participant during the study. One female participant temporarily stopped button-press performance during the first capsule stimulation block due to scalp irritation from the EEG cap. After cap removal this individual completed the remainder of the session without incident, but due to missing data they were not included in the EEG analysis (see Figure S1: Consort diagram). Interviews of participants after each stimulation session revealed no clinical evidence of increased abdominal pain (0 out of 43 total subjects tested[1]), nausea (1 out of 43 subjects[2]) or dizziness (1 out of 43 subjects[2]). At a quantitative level, there was no evidence of acute state anxiety changes (pre STAI state score: 28.15 ± 6.82, post STAI state score: 28.35 ± 7.18, $t(39) = 0.2368$, $p = 0.814$). No sex differences in reported anxiety were observed by applying ANOVA, by having STAI state score as the dependent variable and sex and block as the fixed factors, Sex: $F(1) = 0.404$, $p = 0.527$; Block: $F(1) = 0.020$, $p = 0.887$; and interaction between sex and block: $F(1) = 0.029$, $p = 0.864$. In addition, there were no significant correlations between the total EGG power and pre- and post-stimulus STAI state anxiety STAI scores, and there were no significant correlations between changes in total EGG power and changes in STAI state anxiety scores for the normal or enhanced conditions. These findings provide further evidence that the vibration stimulation did not significantly modulate anxiety or gastric myoelectric signals in this sample of healthy individuals.

[1] Clinical interview data was missing for 1 subject. Note that a total of 44 subjects received capsule stimulation.
[2] Mild and transiently increased nausea and dizziness was reported by one subject related to a concern that the capsule might "get stuck". These symptoms were resolved prior to the end of the stimulation visit.

Discussion

In the current study we demonstrated that a minimally invasive form of mechanosensory gastrointestinal stimulation reliably changes the perception of gut feelings. Vibrating stimulation in the stomach induced evoked responses in the brain in midline parieto-occipital electrodes ~300-600ms following vibration of the capsule, consistent with a late positive potential. These 'gastric evoked potentials' (GEPs) responded to stimulation and were significantly correlated with perceptual accuracy. Furthermore, our observations of dose-dependent increases in both perceptual accuracy measures and GEP amplitudes between the normal and enhanced forms of stimulation indicate the reliability and effectiveness of this minimally invasive assay of gastrointestinal interoception. Although mechanosensory stomach stimulation was not associated with modulation of intrinsic gastric myoelectric rhythms, it was accompanied by retrospective reports of increased respiratory sensation. Overall, these results highlight the presence of a distinct form of enterically-focused sensory monitoring within the human brain.

Prior functional neuroimaging literature during mechanosensory balloon distension of the esophagus and colorectum showed activation in the primary/secondary somatosensory (SI/SII) and insular cortices (27-30). In the current study there was no ERP pattern consistent with activation in these areas, and instead we observed midline parieto-occipital evoked potentials during mechanosensory stomach stimulation. However, these ERP findings concur with a recent study documenting the presence of a 'gastric network' in the brain (33). This gastric network included bilateral SI/SII nodes, but notably, a greater number of nodes were located in the posterior cingulate sulcus, superior parieto-occipital sulcus, dorsal precuneus, retrosplenial cortex, as well as dorsal and ventral occipital cortex. In the study by Rebollo et al., it was predominantly the posterior midline brain regions that coactivated with the EGG signal under resting conditions, and different nodes within this network showed patterns of early and delayed changes in functional connectivity in relation to the EGG signal. Although 32-channel EEG measurements do not provide the spatial resolution necessary to optimally pinpoint the cerebral source of the observed parieto-occipital LPPs, the posterior midline brain regions observed by Rebollo et al. are located within a plausible set of brain regions for generating this result. Despite our focus on interoceptive awareness, the ERP results failed to show patterns suggestive of an insular or somatosensory genesis. Instead, our findings highlight the possibility that posterior midline brain regions could play a role in interoceptive awareness for stomach sensations. This is consistent with another study based on magnetoencephalography showing an association between EGG signals and midline posterior parietal and occipital regions (34). Using a causal interaction analysis, Richter et al. found greater evidence for information transfer from the gut to brain than the opposing direction (brain to gut). Taken together, our data support the hypothesis that gastric and intestinal interoception may be processed in posteromedial brain regions.

A recent landmark study found that deep posteromedial cortex stimulation was associated with an 'out of body' experience (i.e., dissociation) in an epileptic individual with intracranial recording electrodes implanted for seizure localization (35). In particular, the patient exhibited abnormal oscillatory activity in deep posteromedial cortex prior to the onset of seizures associated with dissociation, and electrical

stimulation of this region triggered the same oscillations and dissociation symptoms that mimicked the pre-seizure aura (a similar pattern of findings was firmly established in the mouse using optogenetic stimulation of layer 5 retrosplenial neurons and induction of dissociation-like episodes using ketamine). The posterior cingulate cortex, which is adjacent to retrosplenial cortex, is another region which has shown selective recruitment during stimulation of the gastric fundus to elicit fullness sensations as well as pain (36). This same region has been implicated in the hierarchical mapping of autonomic nervous system input via demonstrations of abnormal activity in this region in the setting of pure autonomic failure (37). Theoretical proposals have also pinpointed this region as a key substrate for the re-mapping of first-order body representations subserving emotion and conscious awareness in response to ongoing behavioral and environmental contexts (38). Collectively, these studies further emphasize the role of posterior midline structures in gastrointestinal interoception.

Neither group nor individual level analyses provided evidence that the vibrating capsule stimulation changed the frequency of stomach activity as indexed by the EGG signal. This result is important because it suggests that the afferent/ascending mechanosensory stimulation delivered by the capsule did not evoke a detectable efferent/descending visceromotor regulatory response, at least at the systems level of measurement employed here. Additional studies would be needed to establish the same finding in clinical populations, particularly for those in which gastric dysrhythmias commonly encountered (39).

Minimally invasive measurement of gut sensation is an important advance given the difficulty of gaining access to the gastrointestinal system and opens the possibility for future clinical disorders investigations. There are a number of poorly understood conditions in which abnormal gut sensation are part of symptom-based care settings, including eating disorders (e.g., anorexia and bulimia nervosa)(40-42), somatic symptom disorders (43, 44), functional neurological disorders (8, 45, 46), and functional bowel disorders (e.g., irritable bowel syndrome, functional dyspepsia, or functional bloating (47-49)). Clinicians are often faced with a patient reporting prominent gut symptoms without a clear medical explanation, despite the understanding that these disorders involve abnormal nervous system representation of internal sensory information (6-8). The current approach enables examinations of the relationship between gastrointestinal symptoms, illness severity, GEPs, and accuracy for detecting stomach stimulation in these clinical populations. Based on the sensitivity of this measure, particularly the ability to derive robust perceptual estimates at the individual level, such studies could potentially contribute to a better accounting of the pathophysiological manifestation of symptoms (e.g., how do internally arousing stimuli contribute to formation of the 'symptom scaffold', a process whereby bodily sensations are systematically interpreted as threatening? (45, 50)). Another promising future direction could be to combine this paradigm with more sophisticated computational modelling approaches, which is an area of interoception research that has been substantially limited by a lack of compatible tools (5, 6).

Studies using invasive approaches have previously identified an evoked potential during esophageal, duodenal, and anorectal stimulation (23, 51). This 'visceral evoked potential' was characterized by a triphasic response pattern (P1, N1, P2) usually resolving within 300 ms. It differs from the LPP we observed,

which consisted of a monophasic peak with a delayed onset starting around 200 ms after stimulation, peaking at 600 ms, and lasting up to 3000 ms centered over the Pz and POz electrode sites. There are several potential explanations for this discrepancy. First, all the studies in question used painful or aversive forms of stimulation (e.g., mechanical distension or electrical shock) to induce a sensory response, whereas capsule stimulation evoked nonpainful and nonaversive sensations via vibration. Second, the location of gut stimulation sites was not identical. The gut is a long hollow organ, and the associated afferent neuronal relays differ substantially (for example, splanchnic pathways would be engaged by anorectal stimulation vs. vagal pathways for stomach vs. a combination of spinal and vagal pathways depending on the location in the esophagus). Third, the aforementioned studies used a single recording channel via an electrode placed at the vertex (Cz), as opposed to the 32 channels recorded across the head in the current study. We are unable to speculate on whether these studies would have seen a stronger signal localized to more posterior parietal or occipital leads if they had included a larger array of recording electrodes. Finally, the invasive studies involved stimulation under passive instruction conditions whereas the current study paired a continuous top-down (i.e., goal-directed) focus of interoceptive attention with a motor response (the button press), a cognitive task which presumably recruits a broader set of cortical regions. This distinction is important when considering the neural processes potentially contributing to the different phases of observed neural responses. For example, motor reaction times to auditory, visual, and somatosensory stimulation are typically on the order of 120 to 150 ms in duration (52), whereas the average reaction times in the current study were about 1 second for the normal condition and 0.75 seconds for the enhanced condition. Thus, while neural processing related to the motor response following perception of stomach stimulation likely contributed to some of the delay in the observed LPP signal, it is insufficient to completely explain it. The prolonged time course of the LPP is generally thought to reflect reciprocal interactions between frontal and parieto-occipital regions and it is sensitive to autonomic and self-reported indices of arousal (31) and emotion regulation strategies (53). It is unclear whether such explanations account for the LPP timecourse in the current study.

This study has several limitations. We cannot completely rule out the contribution of afferent respiratory stimulation to the observed posteromedial LPP signals given the increased respiratory sensation reports (for example, due to potential transmission of vibratory stimulation to the lungs through the respiratory diaphragm, which sits on top of the stomach). We would expect this degree of interference to be minimal given the disproportionate magnitude of the effect on stomach sensation. Another possibility is that attending to stomach sensations also promotes attention to respiration. One important limitation of scalp EEG recordings is that they cannot easily detect localized signals originating from deep within the cortex. Thus, we cannot conclusively exclude the possibility that the capsule stimulation also evoked changes in deep subcortical structures previously implicated in gut sensation such as the insular cortex, thalamus, and brainstem. Studies involving magnetoencephalography (MEG) or positron emission tomography (PET) scanning could overcome this limitation (functional magnetic resonance imaging (fMRI) would not be an option due to capsule ferromagnetic components), and as previously mentioned one MEG study detailed evidence of parieto-occipital activity linked to stomach input under resting conditions (34). Several

unanswered questions remain. For example, what are the molecular and cellular entities transducing the delivered stimulation? And what are the associated peripheral (presumably neural) pathways conveying the mechanosensory vibration signals to the brain? Answering these questions would likely require the application of invasive (i.e., nonhuman) studies capable of evaluating mechanotransducers at molecular levels. One candidate would be PIEZO channels (14, 15) which are heavily expressed in the stomach (54, 55) – though many other mechanical (e.g., transient receptor potential (TRP) channels) and voltage-gated ion channels are possibilities (either individually or in combination). At the cellular level, several types of mechanosensitive neurons within the enteric nervous system are known to play a role in transmitting tensile and mechanical forces including extrinsic vagal afferent nerve endings in the upper gut and Dogiel type II neurons (spinal afferent nerve endings, which predominantly innervate the lower gut would be considered less likely)(56). Stomach stretching also elicits mechanosensory signaling that is relayed via GLP1R neurons (a vagal afferent subtype) to autonomic brainstem nuclei (nucleus tractus solitarius and area postrema)(57), providing a plausible pathway by which vibratory stimulation might reach the brain. Speculation as to the subsequent trafficking of signals in the brain is beyond the scope of the current study, though others have pointed to the role of hierarchical homeostatic reflexes in the transmission (18) and regulation (58) of information across the brain-gut loop. These feedback loops are certainly amenable to perturbation in a variety of contexts using the gut-brain probe demonstrated here.

In conclusion, a minimally invasive mechanosensory probe of stomach sensation elicits dose-dependent increases in perceptual accuracy and late positive potentials in parieto-occipital EEG leads. These changes are significantly associated with one another and are unrelated to gastric myoelectric indices of stomach rhythm. This finding of a gastric evoked potential provides an opportunity to better understand the role of gastrointestinal symptoms in a variety of human pathological conditions and might ultimately provide insights into how gut feelings are processed by the human brain.

Materials and Methods

Participants

Healthy adult male and female volunteers between the ages of 18 to 40 years were recruited from the general Tulsa community through electronic and print advertisements. Eligibility was verified via completion of structured medical and psychiatric screening evaluations including the MINI (Mini-International Neuropsychiatric Interview) (59). Exclusion criteria included current pregnancy or positive for drugs of abuse as defined by a urine screen during screening and during the stimulation visit, current diagnosis of a psychiatric disorder per the MINI (59), history or current diagnosis of a significant gastrointestinal disorder, gastrointestinal surgery, or other medical disorder involving respiratory, cardiovascular, renal, hepatic, biliary or endocrine disease, as well as chronic use of psychotropic medications or non-steroidal anti-inflammatory drugs. The study was conducted at the Laureate Institute for Brain Research and the research protocol was approved by the Western Institutional Review Board (IRB). All participants provided written informed consent and received financial compensation for participation.

Vibrating Capsule

The vibrating capsule was developed by Vibrant Ltd and is under investigation as a non-pharmacologic therapeutic option for chronic constipation via delivery of stimulation in the colon. It consists of an orally administered non-biodegradable capsule that is wirelessly activated using an activation base unit (Figure 1A). The Vibrant capsule is a non-significant risk device (NSR). The safety of this approach has been established in healthy human volunteers (60) and in patients with chronic constipation (61, 62).

Masking Procedure

To constrain expectancies participants were told that two different modes of the Vibrant capsule were being evaluated, and that they would be randomly assigned to one of three arms of the study: capsule mode A, capsule mode B (during which the capsule would vibrate), or a placebo capsule that did not vibrate. Participants were further informed that neither they nor the experimenter would know whether any stimulations will occur. However, in actuality, every participant received a capsule that delivered vibratory stimulations, making this a single-blinded protocol. To ensure that the contents of the stomach were empty at the time of capsule ingestion participants were instructed to begin fasting (defined as no food or drink for 3 hours prior to the study visit).

Mechanosensory Stimulation

Delivery of mechanosensory stimulations to the stomach started shortly following ingestion of the Vibrant capsule. Capsule activation occurred by placing the capsule in the base unit. Shortly after activation participants ingested the capsule with approximately 240 milliliters of water while seated in a chair. They were subsequently asked to attend to their stomach sensations while resting their eyes on a fixation cross displayed on a monitor approximately 60 centimeters away. They were instructed to use their dominant hand to press and hold a button each time they felt a sensation that they ascribed to the capsule, and to release the button as soon as this sensation had ended (Figure 1B). Stimulations began approximately 3 minutes after capsule activation in the base unit. Participants remained seated throughout the experiment

in order to reduce movement artifact in the EEG and EGG signals. They were told to rest their non-dominant hand in their lap as well as to avoid palpating their abdomen. They were visually observed throughout the experiment by a research assistant seated behind them to verify alertness and compliance with these instructions.

In the experiment, each participant received two blocks of vibratory stimulation (normal enhanced) in counterbalanced order. The normal condition entailed the delivery of a standard level of mechanosensory stimulation (as developed by Vibrant) matching the level of stimulation delivered during chronic constipation trials targeting the colon. The enhanced condition entailed delivery of an increased level of mechanosensory stimulation, which was expected to facilitate gastrointestinal perception. Each block included a total of 60 stimulations (each 3 seconds in duration) which were delivered in a pseudorandom order across a 13-minute period. After a 4-minute pause, a second round of 60 stimulations were delivered in pseudorandom order during a 13-minute period. Thus, participants rated the presence of gastrointestinal sensations throughout a 33-minute period following capsule ingestion. This timing ensured the capsule remained in the stomach during stimulations, since the normal gastric emptying time is estimated to be approximately 30 minutes (63-65)). Due to a technical error 3 vibrations were missing from the enhanced vibration block in the normal/enhanced sequence.

Vibration Detection

To precisely verify the vibration timing a digital stethoscope (Thinklabs Inc.) was gently secured against the anterior surface of the lower right quadrant of the abdomen using a Tegaderm patch (15 x 20 centimeters). The associated signal was continuously recorded during the entire experiment and fed into the physiological recording software at a sampling rate of 1000 Hz. To identify vibrations, we developed custom analysis scripts in Matlab (Mathworks, Inc.) and used a two-step procedure to detect the onset and offset of each delivered vibration. In the first step, the script detected the vibration timings automatically using the "*findchangepts*" function in Matlab. In the second step, the timing graph for each vibration was visually inspected and adjusted if needed. Subjects for whom the amplitude of vibrations could not be confidently identified using this procedure were excluded from analysis (2 subjects; 1 subject had a faulty capsule that did not vibrate and was also excluded, see Supplemental Figure 1: Consort diagram).

Physiological Recordings

Electrogastrogram (EGG) signals were recorded continuously at a sampling rate of 1000 Hz using a Biopac MP150 Acquisition Unit (Goleta, California). Cutaneous EGG signals were captured via two active abdominal electrodes positioned below the left costal margin and between the xyphoid process and umbilicus. The reference electrode was positioned in the right upper quadrant in line with the others (66) (Figure 1C). EEG signals were recorded continuously using a 32-channel EEG system from Brain Products GmbH, Munich, Germany. The EEG cap consisted of 32 channels, including references, arranged according to the international 10-20 system (Figure 1C). One of these channels recorded the electrocardiogram (ECG) signal via electrode placement on the back, leaving 31 EEG signals available for

analysis. The EEG signal was acquired at a sampling rate of 5000 Hz and a measurement resolution of 0.1 microvolts (μV).

EEG Data Processing

The pre/post processing of EEG data was completed using BrainVision Analyzer 2 software (Brain Products GmbH, Munich, Germany). EEG data was downsampled to 250 Hz. Next, a fourth order Butterworth (i.e., 24 dB/octave roll off) band-rejection filter (1 Hz bandwidth) was used to remove alternating current (AC) power line noise (60 Hz). Then, a bandpass filter between 0.1 and 80 Hz (eighth order Butterworth Filter, 48 dB/octave roll off) was utilized to remove signals unrelated to brain activity. Afterward, the infomax independent component analysis (ICA) was applied for independent component decomposition (67) over the entire data length after excluding intervals with excessive motion-artifact. ICA was run on the data from 31 EEG channels yielding 31 independent components (ICs). The time course signal, topographic map, power spectrum density, and energy of these ICs was utilized to detect and remove artifactual ICs (i.e. ocular, muscle and single channel artifacts) (68). In addition to these preprocessing steps, the data was segmented from the 200 milliseconds (ms) prior to the 3000 ms post onset of each vibration. Then the data were baseline corrected to the average of the 200 ms interval preceding the vibration onset. EEG data was referenced to the average of the mastoid channels (TP9 and TP10). Finally, automated procedures were used to detect bad intervals and flatlining in the data. Bad intervals were defined as any change in amplitude between data points that exceeded 50μv or absolute fluctuations exceeding 200μV in any 200 ms interval of the segments (i.e., -200 to 3000 ms); flat lining was defined as any change of less than 0.5μV in a 200 ms period. Trials were excluded if they included any of these artifacts. To ensure adequate power to detect ERP signals within each block of stimulation, only subjects with at least a 50% correct button-press response rate in both the normal and enhanced blocks were included (27 participants) for the further analyses involved ERP. Examination of the ERP waveform revealed a positive deflection with a magnitude, time course, and scalp distribution consistent with the late positive potential (LPP), an ERP component associated with motivated attention and modulated by arousal (e.g., (31, 53)) typically during picture viewing paradigms. In accordance with the LPP literature we chose to measure this deflection as the mean amplitude of activation from 300 to 600 ms post vibration onset baselined to the average of the EEG signal 200 ms prior to onset.

EGG Data Processing

The single channel EGG recording from each subject was divided into pre-stimulation baseline (30 minutes), normal stimulation (17 minutes), and enhanced stimulation (17 minutes) windows based on the counterbalanced protocol. For each window, the spectral power was computed to identify the location with the largest activity in the normogastria range (2.5-3.5 cycle per minute (cpm)). The spectral power analysis retained peaks of frequency in each condition for each subject. Fast Fourier Transform (FFT) from the FieldTrip toolbox (69) was used to estimate the spectral power with a Hanning taper to reduce spectral leakage and control frequency smoothing. To further characterize the gastric rhythm, we adopted a finite impulse response (FIR) filter to filter the EGG signal into low frequency ranges. FIR copes very well with very low frequency filtering (as shown in (70)). Then, we applied a Hilbert transform to compute the

instantaneous phase and amplitude envelope of the gastric rhythm. To further account for bad segments in the data, we adopted the artifact detection method described in (70). This method relies on the regularity of the computed cycle durations (the standard deviation (STD) of cycle duration from the condition). More specifically, a segment was considered an artifact if the cycle length was greater than the mean ± STD of the cycle length distribution, or if the cycle showed a nonmonotonic change in phase. Following the decision tree approach, any cycle with either of these conditions was considered as an artifact and excluded from the signal. The power spectral analysis was calculated again after excluding bad segments from the EGG signal, including subsequent filtering. We report the absolute power for four gastric ranges: normogastria [2.5-3.5] cpm, tachygastria [3.75 –9.75] cpm, bradygastria [0.5 –2.5] cpm, and total power [0.5 –11] cpm (in line with (71)).

Subjective Ratings Measures

Participants completed several self-report surveys including the Spielberger State Trait Anxiety Inventory (STAI (72)) and Visual Analog Scales indexing subjective and metacognitive experiences related to capsule stimulation including the perceived intensity of stomach/digestive, breath, heartbeat, and muscle sensations. The rating scale for the intensity ratings ranged from 0 ("Not at all/None") to 100 ("Extremely/The most I have ever felt"). The trait scales were completed during the screening visit, with the remainder of the state scales completed before and after the capsule stimulation. These scales were intended to provide additional information on safety and tolerability, and to facilitate analysis of perceptual experiences related to capsule stimulation. The assessment procedure at each event time point is reported in table S6.

Perceptual Discrimination Measures

We calculated measures of interoceptive accuracy for each block and participant, adopting a non-parametric signal detection analog of d′ used for conditions with low trial numbers (73, 74) as follows:

$$A' = \frac{1}{2} + \frac{(HR - FP)(1 + HR - FP)}{4HR(1 - FP)}$$

where HR represents the Hit Rate and FP is the False positive rate. We further normalized the A′ (A prime) scores using the following equation: $2\sin^{-1}(\sqrt{A'})$, resulting in values between 0 and π. In addition, we calculated the average and the STD of the response latency for each block and each participant. Response latency was defined as the difference between the vibration onset and the participant's button press response indicating a perceived sensation. Each participant's performance was further evaluated using a binomial test to check if they performed above chance during the normal and enhanced blocks, defined as ≥ 70 correct trials (out of a possible total of 120, counting the 60 normal vibration and 60 nonvibration intervals as trials) and ≥ 67 correct trials (out of a possible total of 114, counting the 57 enhanced vibration and 57 nonvibration intervals as trials) corresponding to an individual performance threshold statistically above chance ($p < 0.05$) according to the binomial distribution (as in (74)). *Note:* One participant did not generate any button press responses during the normal block; therefore, there was no average and STD response latency available for this individual, and no line connecting their performance from the normal to enhanced blocks for those measures (e.g., in Figure 2B).

Statistical Analysis

Two-way analyses of variance (ANOVA) with block (normal, enhanced) and sex (male, female) as independent variables, and separated subject-level performance measures (normalized A', response latency, STD response latency) and LPP as the dependent variable were conducted to investigate the effect of sex and block in each of those perception measures. 2-way repeated measure ANOVAs were also utilized to examine the differences between EEG channels (i.e., Pz, P3, P4, Cz, POz, Oz) and blocks (normal, enhanced), EGG power in different frequency bands (normogastria, tachygastria, bradygastria) and different blocks (baseline, normal, and enhanced), as well as comparing the subjective measure of 4 different intensities (i.e., stomach/digestive, breath, heartbeat, and muscle) and time (pre-, post-stimulus). For the repeated measures ANOVA, if the assumption of sphericity was violated according to Mauchly's test for any variables, degrees of freedom were adjusted using Greenhouse-Geisser estimates of sphericity. We applied 1-way repeated measure of ANOVA for comparing the total EGG power in 3 different blocks (baseline, normal, enhanced). The nonparametric Spearman's rank correlation was used to evaluate the association between neurophysiological measurements and accuracy measurements, due to the non-normal distributions of such measurements. The statistical analyses were performed using SPSS version 26 (IBM SPSS Inc., Chicago, Ill).

Data and code availability: Access to the data collected using the Vibrant capsule is covered by nondisclosure and material transfer agreements between LIBR and Vibrant Ltd. The raw and/or processed data may be made available upon request to researchers pending the establishment of similar agreements between all parties.

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Abbreviations: Cycles per minute (CPM), Electroencephalogram (EEG), Electrogastrogram (EGG), Evoked response potential (ERP), False positive (FP), Hit rate (HR), Late positive potential (LPP), Mean (M), Microvolts (μV), Spielberger State Trait Anxiety Inventory (STAI), Standard deviation (STD).

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Figure Captions

Figure 1: A) Vibrating capsule and activation base. **B)** The push button which participants were asked to press each time they detected a capsule-induced stomach sensation. **C)** Scalp electroencephalogram (EEG) and stomach electrogastrogram (EGG) lead placement.

Figure 2: Perceptual accuracy measures during the normal and enhanced stimulation blocks based on button-presses signifying gut feelings during vibratory stimulation of the stomach. Gray lines present changes in individual subject performance from the normal to enhanced block. **A)** Normalized A prime (dashed line shows chance performance based on binomial expansion); **B)** Average response latency (in seconds); and **C)** Standard deviation (STD) of the response latency (in seconds). The participants' performance increased significantly with enhanced stimulation across all three measures, indicating the paradigm effectively induced changes in gastric interoceptive awareness. ***$P < 0.001$.

Figure 3: Parieto-occipital event related potential (ERP) indicators of gut sensation and association with perceptual accuracy measures during normal and enhanced stimulation. **A)** The ERP waveforms for channels P3, P4, Cz, Pz, Oz, and POz during the normal (blue) and enhanced (green) blocks. A dose-dependent increase was observed across all six electrodes during the 300 to 600 ms window (outlined in gray). Shaded areas represent the standard error of the mean for the ERP signal at each time point. Time-zero represents the onset of vibration stimulus. The presented waveforms were calculated from the average mastoid-referenced EEG. **B)** Late positive potential (LPP) scalp topography between 300 and 600 ms window after the vibration onset relative to the 200 ms pre-stimulus baseline for the normal and enhanced blocks. **C)** The positive association between LPP signal strength (average ERP signal among P3, P4, Cz, Pz, Oz, and Poz channels) and perceptual accuracy (normalized A prime) was significant across both blocks. The area shaded, corresponding 95% confidence interval for the regressions.

Figure 4: Electrogastrogram (EGG) power measured during the baseline, normal stimulation, and enhanced stimulation blocks. **A)** Total EGG power across all physiologically relevant spectrums. **B)** EGG power within the bradygastria (0.5 –2.5 cycles per minute, cpm), normogastria (2.5 to 3.5 cpm), and tachygastria (3.75 to 9.75 cpm) frequency spectrums. There was no evidence of significant stimulation-induced EGG changes across any power spectrum.

Figure 5: Self-reported intensity ratings of different interoceptive sensations experienced before (Pre) and during stimulation (Post; these ratings were provided retrospectively, and encompassed sensations experienced during both blocks). **A)** Stomach/Digestive, **B)** Breath, **C)** Heartbeat, and **D)** Muscle tension ratings. Stimulation-induced intensity ratings increased for both stomach and breath sensations. Gray lines show changes in ratings from the pre- to post- stimulation for each individual. *$P < 0.05$, ***$P < 0.001$; ns, not significant.