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ORIGINAL ARTICLE

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The role of serotonin in the control of esophageal sensitivity assessed by multimodal stimulation in health

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Abstract

Background: Esophageal hypersensitivity is considered an important pathophysiological mechanism in refractory gastroesophageal reflux disease (GERD) patients. Serotonin (5-HT) plays an important role in the regulation of GI (gastrointestinal) secretion, motility and sensitivity. Previous studies found that altered 5-HT availability has no clear effects on esophageal/GI sensations. Our aim was therefore to investigate the role of 5-HT in esophageal sensitivity in healthy volunteers (HV).

Methods: Esophageal sensitivity to thermal, mechanical, electrical, and chemical stimuli was assessed in 3 different placebo-controlled studies. In the first study, the effect of citalopram (40 mg; 5-HT reuptake inhibitor; intravenous) was investigated (n = 14). In the second study, the effect of buspirone (20 mg; 5HT1A agonist; oral) was investigated (n = 10). In the third study, acute tryptophan depletion (ATD) was used to decrease 5-HT levels to investigate the effect of reduced 5-HT availability on esophageal sensitivity (n = 15).

Key Results: No difference was observed in esophageal sensitivity after the administration of citalopram or buspirone (all $p > 0.06$). In contrast, pain perception threshold to chemical stimulation was increased after ATD ($p = 0.017$, Cohen's d+ = 0.67). No effect was found on the first perception or pain tolerance threshold. ATD had no influence on esophageal sensitivity to thermal, mechanical, and electrical stimulation compared with placebo.

Conclusions and Inferences: ATD, which induces 5-HT depletion, significantly decreased pain perception threshold during chemical stimulation, without affecting sensitivity to mechanical, thermal, or electrical stimulation. These findings confirm the involvement of 5-HT in the control of esophageal acid sensitivity, but identifying the receptors involved requires more ligands and studies.

KEYWORDS
buspirone, citalopram, electric stimulation, esophagus, healthy volunteers, hot temperature, pain, physical stimulation, physiopathology, serotonin

Charlotte Broers and Annelies Geeraerts shared first author.

1 | INTRODUCTION

Gastroesophageal reflux disease (GERD) is characterized by typical symptoms such as heartburn and regurgitation, caused by the retrograde movement of gastric content into the esophagus.[1] Acid-suppressive therapy, such as proton pomp inhibitors (PPIs), is the first-line treatment for GERD.[2–5] Despite treatment with a double dose of PPIs (for at least 12 weeks), 10% to 40% of the patients fail to achieve complete symptom relief and are referred to as having refractory GERD (rGERD) symptoms. Esophageal hypersensitivity has been proposed as a major contributing factor in the pathophysiology of refractory reflux symptoms.[18]

The gastrointestinal (GI) tract is highly integrated and communicates in a bidirectional way with the brain through extensive sensory innervation, and this is referred to as the brain-gut axis.[6,7] It has been shown that esophageal mechanosensitivity can be enhanced by acid, present in gastric contents during the occurrence of gastroesophageal reflux. Furthermore, previously mechanically insensitive afferents can develop mechanosensitivity during inflammation and a wide range of signaling molecules such as serotonin (5-HT), prostaglandins, adenosine, histamine, proteases, and many others are involved in this sensitization process.[8–10]

Extensive research has revealed that 5-HT plays a pivotal role in the regulation of GI function and has also long been associated with emotion regulation and psychological disorders such as depression, anxiety, and phobia.[11,12] 5-HT, present both in the GI tract and in the central nervous system (CNS), can be considered as one of the key mediators of the brain-gut axis, although its exact role in esophageal sensitivity and hypersensitivity is still incompletely understood. The pharmacology of 5-HT is highly complex, due to the existence of many different receptors and subtypes, which may mediate opposite effects.[12] Current knowledge on the modulating role of 5-HT in GI function and the brain-gut axis has mainly been obtained from a few studies, using selective 5-HT reuptake inhibitors (SSRIs) such as citalopram and a limited number of 5-HT receptor ligand studies. Our group previously demonstrated that acute administration of citalopram, widely used in the treatment of depression, significantly lowered chemical and mechanical esophageal sensitivity in hypersensitive healthy volunteers (HV).[13] Furthermore, buspirone, a partial 5-HT_{1A}—receptor agonist, mainly used as an anxiolytic for generalized anxiety disorder,[14] is able to modify esophageal motility by enhancing the esophageal peristaltic amplitude in health.[15] A number of studies have reported enhanced esophageal contractility in response to administration of 5-HT4 receptor agonists.[16] However, there is a lack of information concerning the effect of 5-HT ligands on esophageal sensitivity in humans and the effect of citalopram in non-selected subjects.

On the other hand, there is a lack of studies with suitable and selective 5-HT receptor antagonists for use in human research which could be a plausible explanation for these discrepancies. One possible method to overcome this problem is the application of the acute tryptophan depletion (ATD) technique. ATD is a validated technique to acutely lower central and peripheral 5-HT concentration by temporarily reducing the availability of the essential amino acid tryptophan (TRP), which is the precursor of 5-HT, and thereby decreasing the synthesis of 5-HT.[17] TRP depletion is accomplished by administration of an amino acid mixture lacking TRP. This technique is widely used in psychiatric research to investigate the role of central 5-HT in affective disorders. Further research also demonstrated that ATD affects GI physiology by delaying gastric emptying and enhancing visceral pain perception during rectal balloon distension.[18,19] Furthermore, ATD has been shown to alter gastric postprandial motor function and distension-induced nausea. These findings establish involvement of 5-HT in the control of gastric accommodation and sensitivity.[20]

Key points

- No difference was observed in esophageal sensitivity after administration of citalopram or buspirone.
- Pain perception threshold to chemical stimulation was increased after ATD, but no effect was found on the first perception or pain tolerance threshold.
- ATD had no influence on esophageal sensitivity to thermal, mechanical, and electrical stimulation compared with placebo.

Therefore, three proof-of-concept studies were developed to investigate the following hypotheses: (1) and (2) increasing 5-HT availability or activation of the 5-HT1A receptor by acute administration of citalopram and buspirone, respectively, in normosensitive HV lowers chemical and mechanical esophageal sensitivity; and (3) lowering central and peripheral 5-HT levels increases chemical esophageal sensitivity.

The aim of these preliminary proof-of-concept studies is to further unravel the role of 5-HT in esophageal sensation in HV and thereby gain more insight into the involvement of the 5-HT system in symptom perception in rGERD patients by performing three studies: (1) and (2) to explore the effect of a single-dose administration of an unexplored ligand (buspirone) and the SSRI (citalopram) on esophageal sensitivity in normosensitive HV; and (3) to explore the effect of ATD (blocking the 5-HT system) on esophageal sensitivity in HV.

2 | MATERIALS AND METHODS

2.1 | Subjects

We performed these sensitivity studies in normosensitive HV, and written informed consent was obtained from participants before inclusion in the study. Inclusion criteria included age between 18 and 60 years. Exclusion criteria included a history of psychiatric disease or a positive first-degree psychiatric family history, pregnancy, or lactation, concomitant administration of any centrally activating medication (antidepressive medication,

hypnotics, sedatives, anxiolytics, etc.) or medication affecting esophageal motility, significant comorbidities (neuromuscular, psychiatric, cardiovascular, pulmonary, endocrine, autoimmune, renal, and hepatic), prior history of esophageal, gastric surgery or endoscopic antireflux procedure, and history of gastrointestinal disease. During the last 2 weeks before the study, the volunteers needed to be free from medication, except for oral contraceptives. The study protocols have been registered to ClinicalTrials.gov (NCT04355455, NCT04352686, NCT03017768), and the study was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Leuven (approval numbers S53359, S53603, S57087).

2.2 | Study design

After an overnight fast, HV came to the endoscopy unit of the university hospital and the study was performed according to the outline shown in Figure 1. During the study protocol, emotional status of the study subjects was assessed using the State-Trait Anxiety

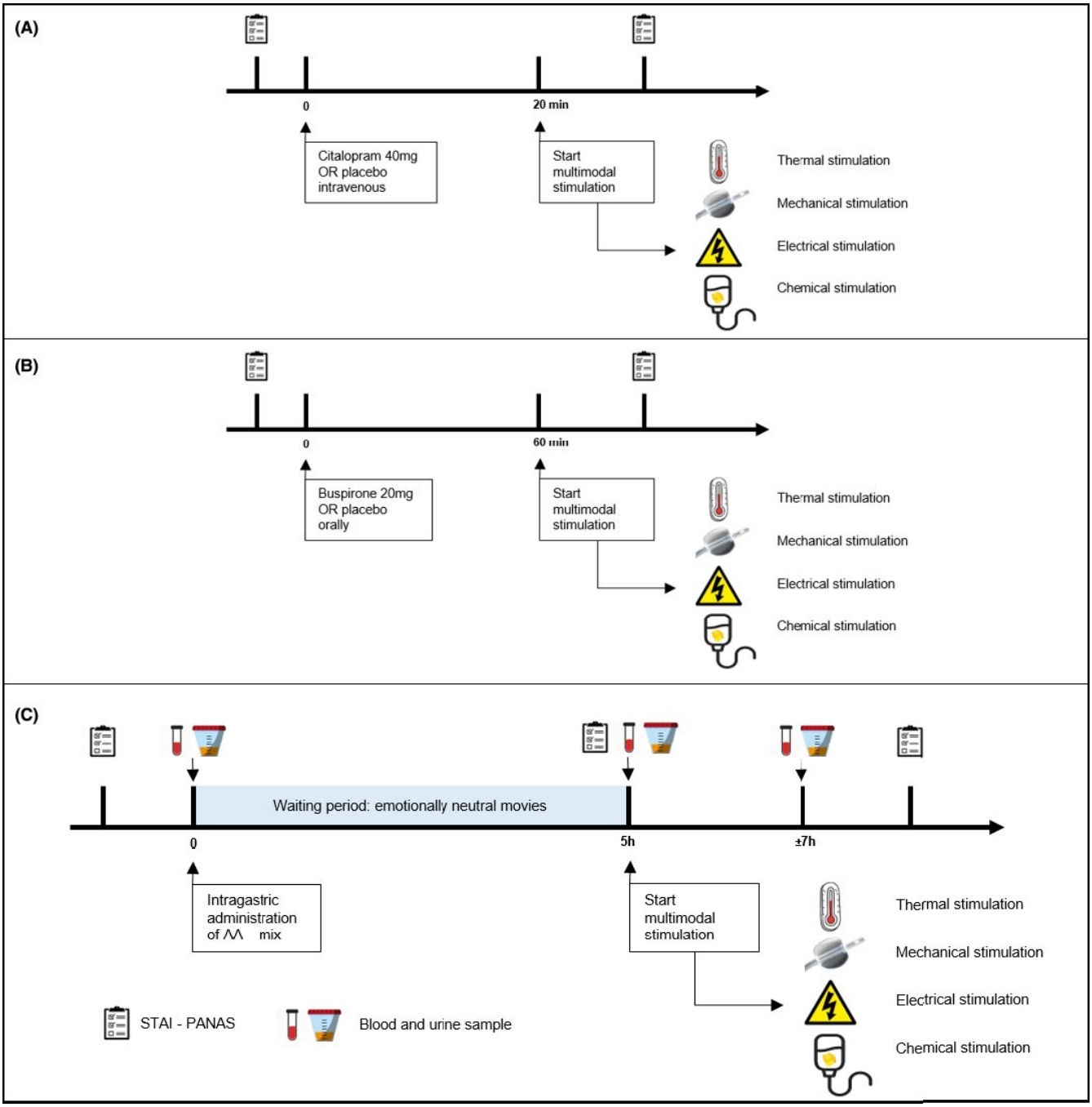

FIGURE 1 Outline of the three study protocols. (A) Citalopram study, (B) buspirone study, and (C) acute tryptophan depletion study. AA-mix, amino acid mixture; PANAS, Positive and Negative Affect Schedule; STAI, State-Trait Anxiety Inventory

Inventory (STAI) and the Positive and Negative Affect Schedule (PANAS) questionnaires at the beginning and at the end of the study protocol. After administration of the active product or placebo, a multimodal stimulation probe (as described in the technical note by Broers et al.[21]) was placed with the balloon positioned 10 cm proximal to the lower esophageal sphincter (LES). Next, esophageal sensitivity was evaluated by the multimodal esophageal stimulation probe (Ditens, Aalborg, Denmark), which allows stimulation of the esophagus in one single protocol and in the same order (thermal, mechanical, electrical, and chemical stimulation) as previously evaluated and reported.[22,23] During the four different esophageal stimulations, subjects were instructed to report perception of the stimuli using the visual analogue scale (VAS). Next, these numerical scores were interpreted as follows: VAS 1 = first perception threshold, VAS 5 = pain perception threshold (PPT), and VAS 7 = pain tolerance threshold (PTT).[21,23]

Thermal stimulation was performed by recirculating a saline solution (0.9% NaCl) through the balloon mounted on the probe, and infusion water was heated by a water bath with a maximal temperature of 62°C. The volume in the balloon was kept constant at 5 ml to avoid mechanical stimulation of the esophagus. Thermal stimulation was terminated when the subject reached VAS = 7 (PTT). Mechanical stimulation of the esophagus was executed by distension of the balloon. A saline solution (0.9% NaCl) of 37°C was used, to avoid thermal stimulation of the esophagus. The stimulation was terminated when the subject reached VAS = 7 (PTT). Two electrodes, mounted on the probe proximal to the inflatable balloon, were used to administer short electrical pulses. Stimulation was terminated when the subject reached VAS = 5 (PPT). Finally, after pulling back the probe 3 cm, chemical stimulation was performed in the distal esophagus by infusing an acidic solution (0.1 N HCl). The stimulation lasted for a maximum period of 30 min or was terminated when subjects reached VAS = 7 (PTT).[21] Sensitivity tests were performed in a semi-recumbent position.

2.2.1 | Citalopram study

On two separate occasions, with at least 1-week interval, participants were randomly assigned to receive 40 mg citalopram intravenously (IV) or saline 20 min prior to the sensitivity tests (as peripheral and central effects after acute IV administration of citalopram require up to 30 min to reach significance). Previous research showed alteration of GI sensorimotor function in HV after administration of 20 mg of citalopram IV.[13,24,25]

2.2.2 | Buspirone study

On two separate occasions, with at least 1-week interval, participants were randomly assigned to receive 20 mg buspirone or placebo via oral administration 60 min prior to the sensitivity tests. Previous research showed significant stimulatory effects of buspirone on esophageal peristalsis and LES function after a single dose of buspirone 20 mg orally in HV.[15]

2.2.3 | Acute tryptophan depletion study

On two separate occasions, with at least 1-week interval, participants were randomly assigned to receiving an amino acid mixture containing TRP (control) or TRP-deficient amino acid mixture, which was administered directly intragastrically via a nasogastric catheter (RT12/100, Polyurethane Enteral Feeding Tube; Eurosteriel Medical, Dronten, NL) to avoid nausea due to the unpleasant taste and smell of the mixture. Since maximal TRP depletion is obtained approximately 5 h after intake of the amino acid mixture, the mixture was administered 5 h prior to the actual start of the multimodal esophageal stimulation test.[17,20]

Since the amino acid mixture has an influence on the levels of brain 5-HT synthesis of the study participants, the Mini International Neuropsychiatric Interview (Dutch, version 5.0.0, DSM-IV) was used to evaluate the psychosocial condition of the volunteers during a prior screening visit. Based on the outcome of the neuropsychiatric interview, candidates were considered eligible for participation in the study if no psychiatric condition was present. Additionally, blood samples were collected at baseline (T = 0), T = 5 h, and T = 7 h to measure plasma TRP levels and plasma ratio TRP/$\sum$ large neutral amino acids (LNAA; sum of tyrosine, leucine, phenylalanine, isoleucine, valine). Furthermore, urine samples were collected to measure levels of urinary 5-hydroxyindoleacetic acid (5-HIAA) which is the most important metabolite of 5-HT. The analysis of these biochemical parameters was performed by the Laboratory Medicine Unit of the University Hospital Leuven (Leuven, Belgium). During the time between administration of the amino acid mixture and the actual start of the multimodal esophageal stimulation test, study participants were asked to watch standardized movies with a neutral emotional content.

2.3 | Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.02 (GraphPad Software, Inc.). Thermal, mechanical, electrical, and chemical sensitivity was measured at pain perception threshold (PPT) and pain tolerance threshold (PTT). The 1st perception threshold was additionally measured during the electrical and chemical sensitivity test. Next, these thresholds were used to assess esophageal sensitivity. Esophageal sensitivity for the four different stimulation modalities was compared between test conditions (citalopram, buspirone, and ATD) and control conditions using two-tailed paired *t* test or the nonparametric paired Wilcoxon signed rank test. Deviations from the Gaussian distribution were tested using the Shapiro-Wilk normality test. Two-way ANOVA with a post hoc *t* test per time point with the Bonferroni correction for multiple testing was used to evaluate the change in parameters of interest over time

in male and female volunteers in the ATD study. Sample sizes were based on previously performed studies with the same technique in our group and the group from Denmark.[21–23,26,27] A p-value <0.05 was considered statistically significant. Data are presented as median [25th–75th percentiles], unless stated otherwise.

3 | RESULTS

3.1 | Citalopram study

Fourteen unique HV were included in the citalopram study (7 male/7 female, mean age 31 years [21–50 years]) to compare the effects of placebo and citalopram on esophageal sensitivity. No significant differences were observed in the PPT and PTT thresholds between the citalopram administration and the placebo condition for the thermal and electrical stimulation (Table 1). For mechanical and chemical stimulation, a large proportion of the HV failed to reach PTT at the endpoint of the study, which precludes the interpretation of the results (Appendix Table S1). Furthermore, no significant differences were observed in STAI or PANAS scores before and after the stimulation tests and no side effects were observed.

3.2 | Buspirone study

Ten unique HV were included in the buspirone protocol (4 male/6 female, mean age 34 years [21–50 years]) to investigate the effect of buspirone on esophageal sensitivity. No significant differences were observed in the PPT and PTT thresholds after the administration of buspirone compared with placebo for the thermal and electrical stimulation (Table 2). For mechanical and chemical stimulation, the same phenomenon was observed that a large proportion of the HV failed to reach PTT at the endpoint of the study, which precludes the interpretation of the results (Appendix Table S2). Finally, no significant differences were observed in STAI or PANAS scores before and after the stimulation tests and no side effects were observed.

3.3 | Acute tryptophan depletion study

Fifteen unique HV were included in this protocol (7 male/8 female, mean age 24 years [21–33 years]) to investigate the effect of blocking the 5-HT system on esophageal sensitivity.

The biochemical parameters showed the following results: Baseline values (time point 0) were comparable under both conditions (Table 3). ATD significantly reduced plasma levels of TRP 5 and 7 h after administration of the amino acid mixture ($p < 0.0001$). The ratio of TRP and the sum of LNAAs (TRP/∑LNAA) was also significantly lower with the ATD protocol. In urine samples, the levels of 5-HIAA, the major metabolite of 5-HT, were significantly decreased 5 and 7 h after ATD compared with the control condition (Table 3).

ATD did decrease PPT during chemical stimulation ($p = 0.017$) with a pronounced effect size (Cohen's d+ = 0.67) (Figure 2B). No effect on the other two sensitivity thresholds (1st perception: $p = 0.21$, PTT: $p = 0.36$) was found (Figure 2A,C). When comparing ATD to the control condition, we found no influence on esophageal sensitivity to thermal stimulation. The thresholds for pain perception and pain tolerance were not altered after administration of the TRP-deficient amino acid mixture (PPT $p = 0.19$, PTT $p = 0.08$) (Appendix Figure S2A,B). Similar results were found for mechanical (PPT: $p = 0.71$, PTT: $p = 0.05$) and electrical stimulation (1st perception: $p = 0.50$, PPT: $p = 0.39$): ATD did not alter the sensitivity thresholds compared with the control mixture (Appendix Figure S2C–F).

When we looked further into the differences in PPT between placebo and ATD, women appeared to be more sensitive to acid infusion compared with men in both conditions ($p = 0.002$). However, this difference was not more pronounced in the ATD condition: Women did not respond significantly stronger to ATD than men, and there was no interaction effect of gender and treatment ($p = 0.96$) (Figure 3).

As mentioned above, there was no effect of ATD on esophageal sensitivity to thermal, mechanical and electrical stimulation. However, we performed a two-way ANOVA analysis to investigate the effect of gender and as was the case with the chemical stimulation, gender differences were also present for thermal and

TABLE 1 Values for thermal, mechanical, electrical, and chemical stimulation after citalopram administration compared with placebo

		Citalopram	Placebo	p-value
Thermal stimulation (in °C)	PPT	46.20	47.00	0.40
	PTT	49.10	49.60	0.56
Mechanical stimulation (in ml)	PPT	30.83	29.11	0.98
	PTT	42.00	32.86	0.19
Electrical stimulation (in mA)	1st perception	10.32	6.69	0.06
	PPT	14.78	11.81	0.22
Chemical stimulation (in ml)	1st perception	19.29	21.08	0.72
	PPT	30.40	33.25	0.93
	PTT	34.25	38.00	0.73

Note: Results are presented as mean. Between-group differences (citalopram vs. placebo).
Abbreviations: PPT, pain perception threshold; PTT, pain tolerance threshold.

TABLE 2 Values for thermal, mechanical, electrical, and chemical stimulation after buspirone administration compared with placebo

		Buspirone	Placebo	*p*-value
Thermal stimulation (in °C)	PPT	47.40	47.50	0.94
	PTT	49.40	49.60	0.87
Mechanical stimulation (in ml)	PPT	23.76	30.31	0.37
	PTT	30.73	30.24	1.00
Electrical stimulation (in mA)	1st perception	9.00	9.00	1.00
	PPT	17.00	15.00	0.44
Chemical stimulation (in ml)	1st perception	14.33	23.25	0.17
	PPT	27.00	29.33	0.63
	PTT	32.67	41.60	0.08

Note: Results are presented as mean. Between-group differences (buspirone vs. placebo).
Abbreviations: PPT, pain perception threshold; PTT, pain tolerance threshold.

TABLE 3 Biochemical parameters at time point 0, time point 5, and time point 7 during the ATD condition and control condition

	T0	T5	T7
TRP (µmol/L)			
Control	65.0 [49.3–69.5]	141.9 [102.6–168.3]***,°°°	73.6 [59.4–105.0]***,°
ATD	62.5 [51.8–74.8]	7.4 [5.0–18.2]°°	10.5 [6.8–15.6]°°°
TRP/∑LNAA (×100)			
Control	12.2 [11.2–16.0]	10.2 [9.0–11.1]***,°°	8.5 [7.3–11.4]***,°°°
ATD	12.7 [10.4–15.2]	0.5 [0.3–1.2]°°°	0.9 [0.6–2.3]°°°
5-HIAA (mg/L)			
Control	3.9 [1.9–5.7]	2.4 [1.4–4.1]**	1.4 [0.9–1.0]**,°°
ATD	3.5 [1.9–4.5]	1.0 [0.5–2.0]°	0.7 [0.0–1.3]°°

Note: Results are presented as median [25th–75th percentile], n = 15. **$p < 0.01$, ***$p < 0.0001$ for between-group differences (ATD vs. control) and °$p < 0.05$, °°$p < 0.01$, °°°$p < 0.0001$ within-group differences (compared with baseline). *p*-values corrected for multiple testing. TRP (µmol/L) and TRP/∑LNAA (x100) assessed in blood samples and 5-HIAA (mg/L) in urine.
Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; ATD, acute tryptophan depletion; LNAA, large neutral amino acids; TRP, tryptophan.

mechanical sensitivity. For thermal stimulation, thresholds for PPT and PTT ($p = 0.0058$ and $p = 0.0001$, respectively) were lower in women than in men. Thresholds for mechanical stimulation were significantly lower in women than in men (PPT: $p = 0.008$, PTT: $p = 0.03$). No gender differences were seen for electrical stimulation (1st perception: $p = 0.24$, PPT: $p = 0.53$).

No differences in positive and negative affect scores were present at time points 0, 5, and 7 in ATD or control condition (Figure 4A,B). In addition, STAI scores remained stable throughout the study period in the ATD and in the control condition (Figure 4C). Seven out of 8 female volunteers experienced nausea during the ATD condition. In comparison, in the condition with the placebo amino acid mixture, 4 out of 8 female HV reported nausea. The occurrence of side effects in women was not different between the ATD and placebo condition ($p = 0.28$). Two out of 7 male volunteers reported nausea in the ATD condition, 1 out of 7 male HV reported nausea in the placebo condition. No difference in the occurrence of nausea was present between the 2 conditions in male HV ($p > 0.9999$), although women reported significantly more nausea than men ($p = 0.04$, Fisher's exact test).

4 | DISCUSSION

5-HT is a major neuromodulator and neurotransmitter in the control of GI sensorimotor function, is also a key mediator in the pathophysiology of mood and anxiety disorders, and is involved in afferent signaling from the GI tract to the brain.[28,29] Hence, 5-HT has the potential to influence visceral sensitivity by modulating processing, sensation, and perception of visceral afferent information both in the periphery and at the level of the CNS.[30-32] The effect of 5-HT receptor agonists and antagonists on esophageal sensitivity is poorly elucidated. Furthermore, studies on the influence of 5-HT on GI function are mainly performed using 5-HT agonists such as 5-HT_4 agonists, while the effect of 5-HT antagonism is studied less extensively.

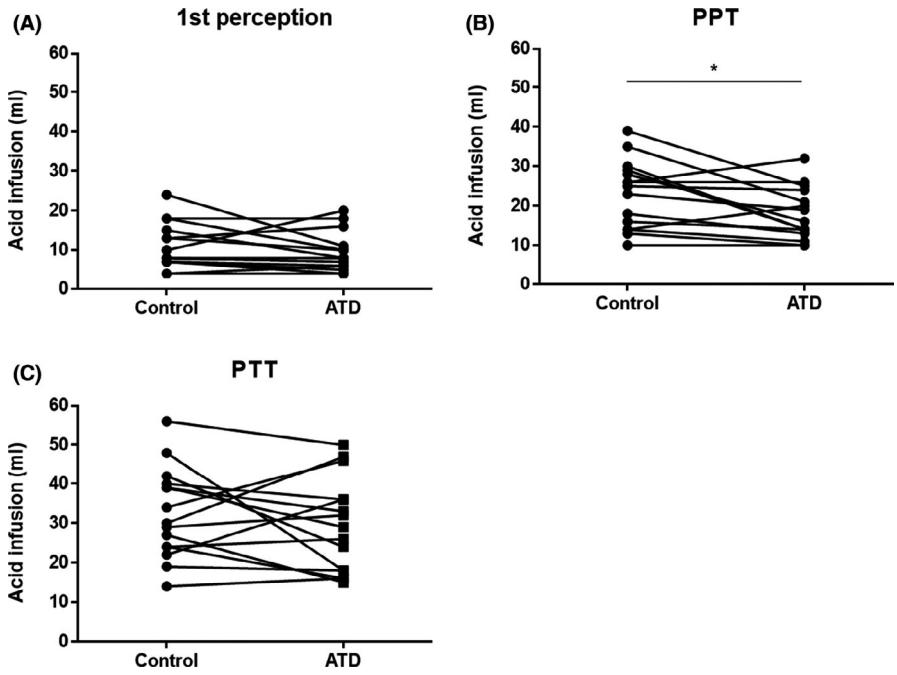

FIGURE 2 Results of esophageal chemical stimulation after ATD or in the control condition. (A, C) No differences were seen for the 1st perception threshold and PTT. (B) A significant decrease in PPT was seen after ATD compared with control. *$p < 0.05$, corrected for multiple testing. ATD, acute tryptophan depletion; PPT, pain perception threshold; PTT, pain tolerance threshold

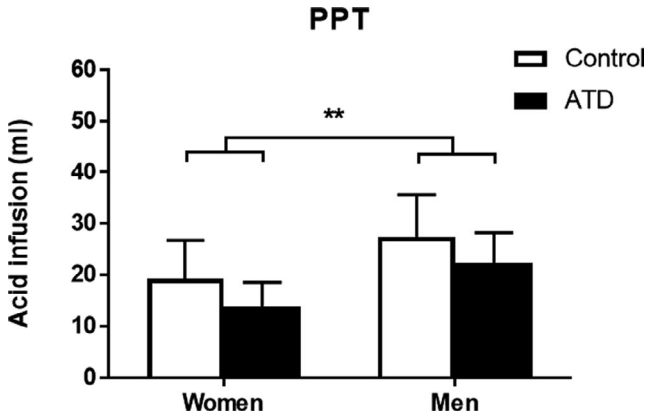

FIGURE 3 Comparison of chemical stimulation between women and men. Two-way ANOVA revealed a significant difference between the volume of acid infusion at which women reached PPT compared with the PPT threshold in men. There was no interaction effect of ATD and gender. **$p < 0.01$, corrected for multiple testing. ATD, acute tryptophan depletion; PPT, pain perception threshold

4.1 | Citalopram

In contrast with previous findings in hypersensitive subjects,[13] this study showed no significant effect of citalopram on esophageal sensitivity assessed by multimodal stimulation. As HV in the current study were not preselected on hypersensitivity, the findings of this study show that citalopram has no major influence on esophageal sensitivity in healthy, normosensitive subjects. The findings support the notion that 5-HT is playing a role under hypersensitivity conditions, but identifying the nature of this role requires additional confirmatory studies.

4.2 | Buspirone

Furthermore, in the second study, the influence of 5-HT_{1A}, buspirone, on esophageal sensitivity was evaluated. Previous research observed that buspirone significantly enhances esophageal peristaltic amplitude in HV.[15] Additionally, buspirone has been shown to have anxiolytic and antidepressant properties.[14,33] Psychological comorbidity, especially anxiety, is very frequent in GERD patients and is thought to contribute to symptom generation and to failure of response to PPI treatment.[7,34,35] This study observed no alteration of esophageal sensitivity assessed by multimodal stimulation after acute administration of buspirone. However, in the present study, anxiety levels were low and not correlated to sensory thresholds. Hence, there is still a rationale to study the impact of buspirone in patients with GERD, especially in those with comorbid anxiety or depression.

4.3 | ATD

In the third and last study, our aim was to evaluate the effect of low levels of 5-HT, both peripheral and central, achieved by ATD, on esophageal sensitivity to multimodal stimulation in HV as the effect of 5-HT antagonism is studied less extensively. ATD is an established technique using the ingestion of an amino acid load that lacks

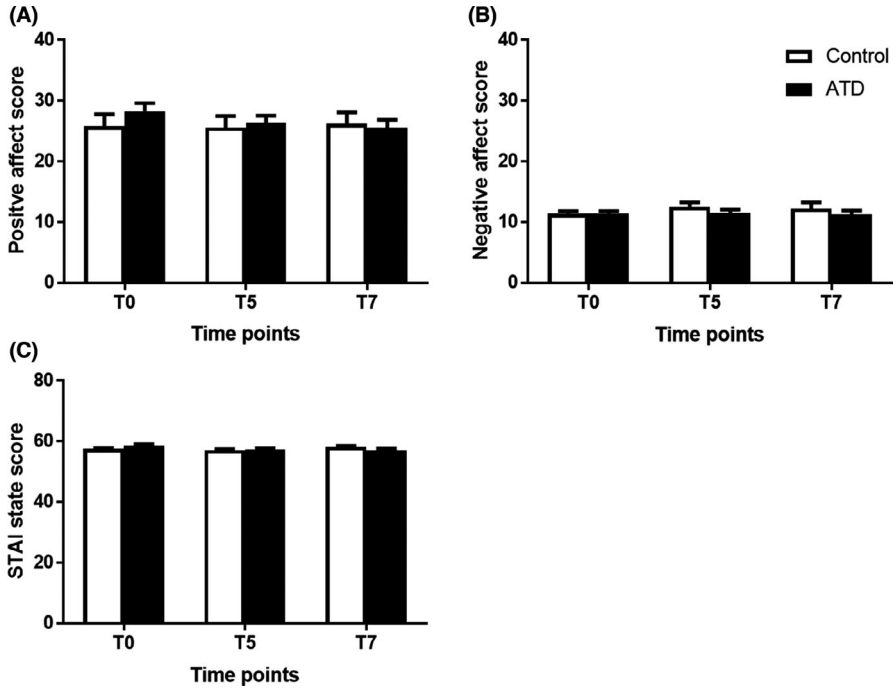

FIGURE 4 Results of questionnaires before and after multimodal stimulation in the control and ATD condition. (A) Positive affect, (B) negative affect, and (C) STAI questionnaire scores. No significant differences in questionnaire scores were observed. ATD, acute tryptophan depletion; PANAS, Positive and Negative Affect Schedule; STAI, State-Trait Anxiety Inventory

tryptophan, the precursor of 5-HT, to deplete the levels of this essential amino acid. It has been established that reducing the plasma levels of TRP causes a consequent reduction in 5-HT synthesis.[17,36] The major finding of this study was an increased esophageal sensitivity to esophageal acid infusion when TRP was depleted. Finally, no effect of ATD on anxiety scores and positive and negative affect scores was present.

The biochemical analysis of blood and urine samples at 3 different time points in our study protocol confirmed that plasma levels of TRP decreased in all subjects as a result of ATD. The ratio of plasma TRP/∑LNAAs, which is considered to be an accurate predictor of brain TRP levels,[37] was significantly lower after ATD compared with the ingestion of the control mixture. The concentration of 5-HIAA, the most important metabolite of 5-HT synthesis, was also significantly lower after ATD compared with the control condition. Based on the results of the biochemical analysis, we concluded that ATD was effective in inducing decreased 5-HT levels in our study participants.

In contrast with the two previous experiments, which used 5-HT agonists or increased the local availability of 5-HT, we investigated the influence of blocking (in fact depleting) the 5-HT system by ATD in the third study. We found a significantly lower PPT during esophageal chemical stimulation in HV. The differences in study outcome can be explained by the differential effects of 5-HT agonists or blocking the 5-HT neurotransmitter system. Furthermore, in contrast to receptor agonists, ATD alters the general availability of peripheral and central 5-HT and does not act in a receptor specific fashion. The fact that ATD lowers sensitivity thresholds to acid infusion can indicate that normal levels of 5-HT are involved in suppressing esophageal acid sensitivity under physiological conditions. Alterations in 5-HT regulation are associated with comorbidities such as anxiety and depression.[38] Therefore, our findings may have implications for the understanding and treatment of patients with rGERD or functional heartburn since in this population psychosocial comorbidities such as anxiety, are known to be more frequent and they are assumed to display hypersensitivity to (acid) reflux.[38–40]

Furthermore, we observed a differential effect of esophageal multimodal stimulation in women and men. When we compared the sensitivity thresholds for women and men in the control condition and after ATD, we observed that for temperature, mechanical and chemical stimulation, women were more sensitive for all thresholds compared with men. This confirms and extends findings of a study by Krarup et al. in which the authors found that women had lower pain thresholds to mechanical stimulation of the esophagus. Furthermore, they observed a smaller number of women which tolerated the maximum acid challenge during chemical stimulation. In contrast to our results, there were no differences between men and women for thermal stimulation. Similar to our findings, also no differences were present between men and women for electrical stimulation.[41] Nguygen et al. reported a lower pain threshold to balloon distension in women compared with men.[42] In a study by Reddy et al. the opposite results were reported: Men appeared to be more sensitive to esophageal balloon distension than women. However, the authors of the latter study conclude that not balloon volume or pressure are valid to score sensory responses but rather strain is associated with stimulation of mechanosensitive receptors. Based on measurements of strain, no differences were found between mechanical sensitivity in men and women.[43] In that study, it was also

demonstrated that women have larger referred pain areas than men, indicating a differential mechanism of central pain processing.[43] The authors postulate that men are more able to inhibit visceral pain at the central level and conclude that this may contribute to the observation of a female predominance in functional GI disorders since an aberrant central processing of pain signals is one of the hypotheses explaining functional disorders.[40,43–45]

ATD did not have a measurable effect on mood in our study participants. This finding is in agreement with other studies in which anxiety ratings have been recorded following ATD. When HV were subjected to ATD, very little effects on anxiety scores were reported.[36,46,47] Although we did not find an alteration in mood or anxiety scores in HV, the effects of ATD on mood and anxiety are dependent on the characteristics of the study population: Mood alterations after ATD have been described in patients with a history of depression, and changes in anxiety scores were reported in patients with social anxiety disorders.[36]

The exact mechanism by which ATD influences esophageal sensitivity is not fully clear: ATD decreases the synthesis of 5-HT both at central and peripheral levels. Apart from measurements of 5-HIAA in urine samples, we did not assess the peripheral level of 5-HT, so we are not able to distinguish whether the sensitivity changes to acid infusion are mediated through alterations of peripheral or central 5-HT availability. Since ATD is an experimental technique developed in psychiatric research to investigate the role of central 5-HT in affective disorders, it is not possible to rule out a centrally mediated working mechanism of ATD.[17]

4.4 | Limitations

A potential limitation of the citalopram was the acute setting with only one single dose of the drug. In the current study, citalopram was not used to evaluate its long-term effects similar to its use in the treatment for depression. However, we have previously demonstrated clear effects of a single dose of citalopram.[25] We cannot exclude that results may be different after a more prolonged treatment, which can be explored in a future study, although a chronic treatment approach is less feasible in the context of a study in healthy volunteers. Another limitation is the lower number of HV in the buspirone study and the lower number of HV that were able to reach the PTT. Therefore, we need to take into account that statistically insignificant results can be due to a type II error. Next, a potential limitation of this study was the fact that the majority of our female participants had side effects after the administration of the amino acid mixture. Women experienced mild nausea during both the control condition and the ATD condition, although this was more pronounced during ATD condition. These feelings of nausea were mainly present in female volunteers and therefore could be a potential explanation for the higher sensitivity to esophageal stimulation in women compared with men in this study. Furthermore, all studies were conducted in healthy subjects and not in a model of esophageal hypersensitivity. To counterbalance this limitation, these proof-of-concept studies tried to explore both the effect of increased and decreased availability of 5-HT in normosensitive HV.

In conclusion, in the first and second studies, we observed no significant difference in esophageal sensitivity assessed by multimodal esophageal stimulation after acute administration of buspirone or citalopram at standard doses in a group of healthy volunteers. In the third study, we evaluated the effect of 5-HT antagonists on esophageal sensitivity and observed that ATD was able to alter sensitivity to acid perfusion, with a lower pain perception threshold compared with the condition where a control mixture was used. Furthermore, ATD did not affect the 1st perception threshold or PTT to chemical stimulation and other stimulation modalities were unaffected by ATD. It remains to be further investigated whether ATD alters local GI 5-HT concentrations. More research is needed to clarify the exact role of 5-HT in esophageal sensitivity and acid sensitivity in particular. Therefore, future studies can investigate the involvement of 5-HT in acid sensitivity by using “neuromodulators” as a treatment strategy in patients with reflux hypersensitivity and functional heartburn.

AUTHOR CONTRIBUTIONS

CB, VB, BVH, TV, JT, and AP were responsible for the study concept and design. CB, VB, BVH, and AP were involved in the acquisition of data. Data analysis was performed by CB, VB, and AP. Interpretation of data was performed by CB, VB, BVH, TV, JT, and AP. CB, AG, and VB, TV, JT, and AP were responsible for the draft of the manuscript. Biochemical parameter analysis was performed by NP and PV. All authors were involved in the critical revision of the manuscript for important intellectual content, and all authors reviewed and approved the final version of the manuscript.

DISCLOSURE

Jan Tack has given Scientific advice to AlfaWassermann, Allergan, Christian Hansen, Danone, Grünenthal, Ironwood, Janssen, Kiowa Kirin, Menarini, Mylan, Neutec, Novartis, Noventure, Nutricia, Shionogi, Shire, Takeda, Theravance, Tramedico, Truvion, Tsumura, Zealand and Zeria pharmaceuticals, has received research support from Shire, Sofar and Tsumura, and has served on the Speaker bureau for Abbott, Allergan, AstraZeneca, Janssen, Kyowa Kirin, Menarini, Mylan, Novartis, Shire, Takeda, Truvion and Zeria.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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