



## Gastric modulation of startle eye blink

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### ABSTRACT

Previous assessment methods of gastric interoception either rely on self-reports, or imply invasive procedures. We investigated the reliability of startle methodology as a non-invasive alternative for the assessment of afferent gastric signals. Twenty-four participants were tested on three separate days, on which they were requested to ingest water (either 0, 300 or 600 ml), after 8 h of fasting. On each assessment day, eye blink responses (EMG) to 10 acoustic startle stimuli (105 dB) were assessed at 4 measurement points (before, 0, 7, 14 min. after ingestion). Increased normogastric responses (EGG), ratings of satiety and fullness, and higher heart rate variability (RMSSD) suggested effective non-invasive induction of gastric distention. Startle responses were lower directly after ingestion of 600 ml as compared to earlier and later measurements. These results suggest that startle methodology provides a reliable method to investigate afferent gastric signals. It could be useful to study possible dissociations between subjective reports and objective afferent gastric signals in eating or somatoform disorders.

### 1. Introduction

Interoception, i.e. the perception of visceral sensations, plays an important role in health, stress and disease (Schulz & Vögele, 2015). Altered interoception has been observed in patients with mental disorders that are associated with physical symptoms, such as somatoform disorders (Bogaerts et al., 2010; Pollatos et al., 2011; Weiss, Sack, Henningsen, & Pollatos, 2014) or eating disorders (Klabunde, Acheson, Boutelle, Matthews, & Kaye, 2013; Pollatos et al., 2008). Training of cardiac interoception can reduce symptom distress in patients with somatoform disorders (Schaefer, Egloff, Gerlach, & Witthoft, 2014). These findings underline the potential importance of interoception in the etiology of these disorders. The majority of these findings, however, is based on cardiac interoception. In contrast, specifically eating disorders and somatoform disorders, in which gastrointestinal symptoms are amongst the most prevalent complaints (Aiarzaguena, Grandes, Salazar, Gaminde, & Sanchez, 2008; Kroenke & Price, 1993; Rief, Hessel, & Braehler, 2001), also require the assessment of interoception related to the gastrointestinal system.

Two approaches have been used previously for the assessment of interoception related to the gastrointestinal system (Herbert, Muth, Pollatos, & Herbert, 2012; van Dyck et al., 2016; Whitehead & Drescher, 1980). In the first, participants are asked to assess the synchrony of exteroceptive signals and gastric contractions, which are monitored by a nasogastric catheter (Whitehead & Drescher, 1980). The second

approach involves a one-step (until fullness) (Herbert et al., 2012) or a two-step (until satiation and until fullness) water load test (WLT) (van Dyck et al., 2016), where the amount of water ingested is interpreted as an indicator for gastric function sensitivity.

The physiological basis for visceral sensations is provided by organ activation, the associated stimulation of interoceptors and subsequent afferent neural signal transmission. Interoception occurs when attention is directed towards physical sensations (Schulz, Lass-Hennemann, Sutterlin, Schächinger, & Vögele, 2013; Vaitl, 1996). On the one hand, accuracy of gastric interoception as assessed by catheter- and WLT-based tasks correlates substantially ( $r = 0.45\text{--}0.53$ ) with cardiac interoceptive accuracy as estimated by heartbeat perception tasks (Herbert et al., 2012; van Dyck et al., 2016; Whitehead & Drescher, 1980). On the other hand, previously used indicators of cardiac and gastric interoceptive accuracy have several shortcomings. For example, they cannot differentiate between the physiological basis of interoception and the psychological processes of attention focusing and evaluation, and they depend on self-reports and are, therefore, highly sensitive to psychological states, such as motivation, affect and attention (Montoya, Schandry, & Muller, 1993; Weitkunat & Schandry, 1990). Both aspects are of particular relevance for mental disorders, as the correspondence between physical sensations, their perception and interpretation may be altered in eating disorders (Jansen, Nederkoorn, & Mulken, 2005; Smeets, Jansen, & Roefs, 2011) or somatoform disorders (Gordon, Kraihuhin, Kelly, Mearns, & Howson, 1986; James, Gordon,

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Krauhin, & Meares, 1989). For example, in eating disorders physical sensations of hunger are perceived as pleasant instead of eliciting food intake (Stevenson, Mahmut, & Rooney, 2015), whereas in somatoform disorders ‘normal’ physical sensations are interpreted as harmful (Rief & Broadbent, 2007).

Startle methodology may offer an alternative to interoceptive tasks, which confound visceral sensations and their perception, as it provides a measure of visceral-afferent neural traffic, which is independent of its perception. Electromyographic (EMG) responses to acoustic startle stimuli, for example, have been demonstrated to be lower during the early cardiac cycle phase (R-wave +230 ms), when the arterial pulse wave is expected to stimulate arterial baroreceptors in large blood vessels, compared to the late cardiac cycle phase (R +530 ms) (Richter, Schulz, Port, Blumenthal, & Schachinger, 2009; Schulz, Lass-Hennemann, Nees et al., 2009; Schulz, Lass-Hennemann, Richter et al., 2009; Schulz, Reichert, Richter et al., 2009; Schulz, Plein, Richter, Blumenthal, & Schächinger, 2011). Since this cardiac modulation of startle (CMS) effect is largely diminished in individuals with degeneration of autonomic afferent nerves (Schulz, Lass-Hennemann, Nees et al., 2009), this approach provides an indirect method to reflect baro-afferent neural signals. Respiratory phases also have the potential to affect the startle responses in that startle magnitudes are higher during on-going expiration than during all other respiratory phases (Schulz, Schilling, Vögele, Larra, & Schächinger, 2016). This respiratory modulation of startle (RMS) effect may be due to afferent neural signals from slow-adapting pulmonary stretch receptors. Although both CMS and RMS likely reflect visceral-afferent signals from different origins, both represent important neural signals that are crucial for cardiac and respiratory interoception, respectively, while being independent of their actual perception.

With the current study we extended the method of natural (non-invasive) CMS and RMS to the gastrointestinal system. We aimed to elucidate the impact of gastric stimulation by a natural and non-invasive stimulus, such as the ingestion of water, on startle eye blink responses. Natural gastric distension induces vagal activation (Paintal, 1953; Schwartz, McHugh, & Moran, 1991; Schwartz, McHugh, & Moran, 1993), which is indicated by a subsequent decrease in heart rate (HR) (Wakisaka et al., 2012). Afferent vagal signals are relayed over the nucleus tractus solitarius (NTS), which project onto the hypothalamus, the amygdala and the thalamus (Stephan et al., 2003; Wang et al., 2008), and possibly evoke a (positive) reward response in case of distension (Geliebter, 2013). This limbic network plays an important role in emotion processing and the mediation of defensive reflexes, such as the startle response, resulting in a decrease of response amplitudes in positive affective states (Davis, 2006; Koch, 1999; Lang, Bradley, & Cuthbert, 1998). Parasympathetic activation should therefore, be associated with a decrease in startle response amplitudes. Furthermore, water ingestion induces a distension in the esophagus and the stomach that elicits regular gastric contractions with a frequency of about 3 cycles per minute in healthy individuals (Herbert et al., 2012; Koch & Stern, 2004; Stern, Koch, & Muth, 2007).

In the current study, participants ingested 0 ml, 300 ml and 600 ml of water on three separate days. Electromyographic eye blink responses to acoustic startle stimuli were assessed before, 0, 7 and 14 min after water ingestion. Based on previous observations on natural gastric distension, we expected (I.) that gastric distension is associated with an increase in gastric contractions (higher relative normogastric EGG (3 cpm) power), (II.) an increase in parasympathetic activation after water ingestion, indicated by lower HR and higher beat-to-beat heart rate variability (HRV), (III.) increases in self-reports of satiety and fullness in response to water ingestion of 300 and 600 ml, and (IV.) a decrease in startle response amplitudes after water ingestion of 300 and 600 ml.

## 2. Methods

### 2.1. Participants

Thirty healthy undergraduate students (18 females; mean age: 23.7 [3.9] years; mean BMI: 22.2 [2.6] kg/m<sup>2</sup>) participated in the study and received monetary compensation (€ 30, –). Physical health status, past and present mental disorders (e.g., eating disorders) were assessed by a customized interview. Somatoform symptoms within the past two years were assessed with the questionnaire “Screening for somatoform disorder” (SOMS-2) (Rief & Hiller, 2008), resulting in an average ICD-10 somatization index of 0. Exclusion criteria were hearing problems (impairments, tinnitus), regular use of contact lenses, any acute or chronic disease, any past or present mental disorder, use of medication, and a BMI < 19 or > 29. Participants provided written informed consent and were made aware of their right to discontinue participation in the study at any time. Data from six participants were excluded from further analyses due to strong habituation to acoustic startle stimulation resulting in less than 50% of valid startle responses (visible startle response 20–150 ms after stimulus onset, baseline without non-stereotyped artifacts). The final sample for startle analyses, therefore, consisted of 24 participants (14 females; mean age: 23.4 [3.9] years; BMI: 22.5 [2.7] kg/m<sup>2</sup>). The research design was approved by the Ethics Review Panel of the University of Luxembourg.

### 2.2. Procedure

Participants were invited to attend three laboratory sessions on separate days, which were between two and seven days apart. They were instructed to skip their last meal on all testing days (for morning sessions: breakfast; for afternoon sessions: lunch) and time of the laboratory session was scheduled accordingly in order to achieve a fasting period of at least 8 h. Furthermore, they were asked not to ingest fluids for the two hours preceding the experimental sessions. A cover story was used to reinforce participants’ compliance: they were told that on the day of the experimental sessions, a saliva sample would be collected, and that electrogastric activity would be evaluated to check their nutritional status, with loss of financial reimbursement if instructions were not followed. On all experimental days, saliva samples were collected and, as an additional way to control for compliance, participants were asked to recall their routine from waking until arriving at the laboratory (diary method: Stone, Kessler, & Haythornthwaite, 1991). In each laboratory session, participants were seated in front of a LCD computer display in a comfortable chair. Before electrode attachment, skin was gently cleaned and prepared with abrasive gel. Glasses were removed, and EMG, ECG, and EGG electrodes were attached. Headphones (Creative Labs EP-630) were placed, and participants were informed about the experimental procedures on the computer display. They were asked to relax, to neither speak nor move, avoid longer periods of eye closure, and listen carefully to all acoustic stimuli. The experimental setup began with a 5-min resting period, during which baseline EGG and ECG data was assessed (‘EGG/ECG baseline’). Thereafter, 16 acoustic startle stimuli were presented over headphones (‘startle baseline’), of which the first six stimuli served as habituation trials and were excluded from further analysis. The startle stimuli had a jittering inter-stimulus interval between 8 and 12 s. Next, participants were asked to drink all the water from a transparent glass within 5 min if there was any (‘water load test’: WLT; 0 ml, 300 ml or 600 ml). The maximum amount of 600 ml was chosen based on earlier studies demonstrating perceived fullness in healthy individuals after ingestion of approx. 600 ml of water (Jones, Hoffman, Shah, Patel, & Ebert, 2003; Jones, Roth, & Crowell, 2005; Koch, Hong, & Xu, 2000). Then, participants underwent a startle stimulation (10 stimuli; ‘startle post 1’), a five-min. resting period (‘EGG/ECG post 1’), another startle stimulation (10 stimuli; ‘startle post 2’), a second resting period (5-min; ‘EGG/ECG post

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