Learned Fear of Gastrointestinal Sensations in Healthy Adults

Article *in* Clinical Gastroenterology and Hepatology · May 2016

DOI: 10.1016/j.cgh.2016.04.035

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Grant Support:

EC was supported by the grant for "the Psychology of Pain and Disability Research Program" funded by the Fund for Scientific Research – Flanders awarded to JWSV. LVO is assistant research professor of the KU Leuven Special Research Fund. JZ was supported by a KU Leuven grant (PF/10/005) to the Centre for Excellence of Generalization Research in Ill Health and Psychopathology awarded to IVD and JWSV.

Abbreviations:

CS = Conditioned Stimulus

EMG = Electromyogram

FGID = Functional Gastrointestinal Disorder

IBS = Irritable Bowel Syndrome

ISI = Inter-Stimulus Interval

$ISI_{post\text{-}CS}$ = Inter-Stimulus Interval occurring after the CS

$ISI_{pre\text{-}CS}$ = Inter-Stimulus Interval occurring prior to onset of the CS.

US = Unconditioned Stimulus

Disclosures:

The authors have nothing to disclose that may be relevant to the manuscript.

Author Contributions:

Study concept and design: EC, JV, LVO, IVD; acquisition of data: EC, ES, NW; data preparation: VA, EC, IVD; statistical analysis: JZ; drafting of the manuscript: EC, JZ, IVD; revision of the manuscript and acceptation of the final draft: EC, JZ, ES, VA, NW, JV, LVO, IVD; material support: NW.

Abstract

Background & Aims: Gastrointestinal symptom-specific fear and anxiety are important determinants of gastrointestinal symptom perception. This study aimed to study fear learning towards innocuous gastrointestinal sensations as a putative mechanism in the development of gastrointestinal symptom-specific fear and anxiety.

Methods: Fifty-two healthy subjects (26 women) received 2 types of esophageal balloon distentions at a perceptible but non-painful intensity (conditioned stimulus [CS], the innocuous sensation) and at a painful intensity (unconditioned stimulus [US]). Subjects were randomly assigned to 1 of 2 groups. . During the learning phase, the innocuous CS preceded the painful US in the experimental group (n=26). In the control group (n=26), on the contrary, the US never followed the CS directly. During a subsequent extinction phase, both groups received only CS distentions—the painful US was no longer administered. Indexes of fear learning towards the innocuous CS distention included the skin conductance response, fear-potentiated startle (measured by the eyeblink electromyogram), and self-reported expectancy of the US.

Results: During the learning phase, only the experimental group learned to fear the innocuous gastrointestinal CS, based on the increase in US expectancy (compared to the control group, P=.04), increased skin conductance response (compared to the control group, P=.03), and potentiated startle reflex (compared to the control group, P=.001) in response to the CS. The differences between experimental and control groups in US expectancy and skin conductance, but not fear-potentiated startle, disappeared during the extinction phase.

Conclusions: Fear towards innocuous gastrointestinal sensations can be established through associative learning in healthy humans. This may be an important mechanism in the development of fear of gastrointestinal symptoms, implicated in the pathophysiology of functional gastrointestinal disorders.

KEY WORDS: functional gastro-intestinal disorders; visceral pain; interoceptive conditioning; gastrointestinal symptom-specific fear

Introduction

Visceral pain is one of the primary causes for seeking medical attention and the most common form of pain resulting from disease[1]. Gastrointestinal symptoms, including visceral pain, often occur without the presence of any detectable physiological abnormalities, as is the case in functional gastrointestinal disorders (FGID).

Stress-related affective and cognitive psychobiological processes play an important role in the pathophysiology of FGID through the brain-gut axis, the bidirectional neurohumoral communication system between the central nervous system and the gastrointestinal tract[2,3]. Gastrointestinal symptom-specific fear, the apprehension of specific visceral sensations, is one of the most important cognitive-affective processes in this context[4,5] as it is associated with symptom severity and quality of life in FGID, specifically in irritable bowel syndrome (IBS)[6]. Furthermore, decreases in gastrointestinal symptom-specific fear mediates the effect of exposure-based cognitive behavioral therapy on IBS symptoms[7,8].

How gastrointestinal symptom-specific fear develops remains unclear, but it is often assumed that fear learning plays a key role in its development. More specifically, originally benign visceral sensations may become associated with unpleasant or painful visceral sensations. For example, benign, non-painful epigastric sensations may precede an episode of stomach ache. As a consequence of this temporal contingency, the individual eventually experiences these benign sensations as unpleasant and may come to fear them, whereas before the same sensations were experienced as relatively neutral. This natural learning process is a case of Pavlovian aversive conditioning in which a relatively neutral stimulus becomes a conditioned stimulus (CS) predicting the inherently unpleasant unconditioned stimulus (US). When both the benign CS and the painful US are experienced at the same anatomical location as in the example above, this is referred to as homoreflexive conditioning. When either the CS or US, or both, are perceived as informative about the internal state of the body, i.e. interoceptive, this is referred to as interoceptive conditioning[9]. Homoreflexive interoceptive fear conditioning is an interesting candidate mechanism in the development and maintenance of gastrointestinal symptom-specific fear, but to the best of our knowledge, this has not been directly studied[10–12].

The purpose of the current study was therefore to study fear learning towards innocuous visceral sensations as a potential mechanism in the development of gastrointestinal symptom-specific fear. In order to address this void in the current knowledge, we set up a study with a painful esophageal stimulus as US, and a detectable, non-painful esophageal stimulus as CS.

We expected fear learning to the CS to occur when the CS immediately precedes the painful US (experimental group), but not when the CS and US are separated by a relatively long time interval (control group).

Materials and Methods

Subjects

Fifty-two healthy participants (26 women) were recruited via advertisements on social media. Interested individuals received an informed consent in line with the declaration of Helsinki prior to deciding whether or not to participate (more details in *Supplementary Material*). Participants were randomly assigned to the experimental or the control group (see later). Both groups were matched for age and gender.

Esophageal Stimulation

Both the CS and US consisted of mechanical stimulation of the distal, autonomously innervated part of the esophagus[13]. The CS and the US lasted 5 and 2 seconds, respectively. The intensity of stimulation was individually determined for both CS and US using a variation of the ascending methods of limits, with the CS being perceptible but non-painful stimulation of the esophagus, and the US being painful but still bearable stimulation at the same anatomical site (more details in *Supplementary Material*). A pediatric catheter (used for gavage) with a diameter of 3mm (TR-2008, Pennine©) was inserted via the nose into the distal esophagus, 35 cm from the nostril. A deflated custom made, silicon medical balloon (diameter: 5mm, length: 25mm, Medasil©) was firmly attached to the end of the catheter positioned in the esophagus (more details in *Supplementary Material*).

Subjective expectancy of US onset

Throughout the study, participants posed their dominant hand on a custom-built dial[14,15], continuously rating the extent to which they expected the US in the following seconds. The scale of the dial ranged from 0 to 100. A score in the middle (50) meant the participant totally did not know whether or not to expect the US. The more certain they were that the US would not come, the more participants turned the dial below 50 and towards zero. The more certain they were to expect the US, the more they turned the dial from 50 upwards to 100. More details are provided in *Supplementary Material*.

Psychophysiological measures

Eyeblink startle EMG

The startle eyeblink reflex is a brief increase in activation of the muscle surrounding the eye, which can be elicited using a sudden burst of sound. The magnitude of the elicited muscle activation can be used as a measure of activation subcortical fear circuits [16]. In fear conditioning studies, increased startle magnitudes during the CS relative to magnitudes during the absence of the CS are thought to reflect motor preparation (an aspect of fear) in response to the CS (more details in *Supplementary Material*).

Galvanic Skin Response

The skin conductance response is a measure of changes in electrodermal activity. These changes occur in response to activation of sweat glands. Sweat gland activity increases as a function of increase in emotional (and/or sympathetic) arousal, with more exciting stimuli increasing skin conductance responses[17]. This measure was included based on the reasoning that an increase in skin conductance response would occur when the CS gains emotional significance, because participants have learned it will be followed shortly by the painful US (more details in *Supplementary Material*).

Study Design

The experiment consisted of three phases: (a) a baseline phase (4 trials), (b) a learning phase (16 trials), and (c) an extinction phase (16 trials).

During the baseline and extinction phases, both groups were treated identically and received one innocuous CS distention in every trial, and no painful US distentions. During the learning phase, both groups received one innocuous CS in every trial and in addition one painful US in 75% of the trials (the 3rd, 8th, 11th, and 15th trial of the learning phase had no US). Such partial (75%) reinforcement of the CS with the US during the learning phase is known to strengthen conditioning[18]. In addition, it may better reflect clinical reality compared to a 100% reinforcement scheme, as also in patients, not every innocuous abdominal sensation is always followed by a painful sensation. For the experimental group, the CS was followed almost immediately (with a 2s delay) by the US. The control group had an interval of 26 seconds between the CS and the US onset (see *Figure 1*). In essence, in the experimental group the CS announces the imminence of the painful US, whereas in the control group it announces an imminent 'safe' and pain-free period.

Every trial lasted 48 seconds, irrespective of phase. The innocuous CS distention was administered always from the 15th up to the 20th second after trial onset. Acoustic startle probes occurred at the 19th second (during the CS) and the 43rd second (during the post-CS inter stimulus interval, ISI_{postCS}) of each trial (see *Figure 1*). More details are provided in *Supplementary Material.*

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Response definition and statistical analysis

As US-expectancy was measured continuously, data were reduced by selecting 5 time points of interest for each trial: the 7th second (prior to the CS, in the middle of $ISI_{pre\text{-}CS}$[a]), 20th second (during the CS), 24th second (end of US for the experimental group / beginning of $ISI_{post\text{-}CS}$ for the control group), 33rd second (middle of $ISI_{post\text{-}CS}$[b]) and the 45th second (end of trial; i.e., right before US onset for the control group).

Galvanic skin responses were calculated by subtracting the mean skin conductance level during "baseline" (2s before the CS onset) from the maximum value in the window 0-7 s following CS onset (more details in *Supplementary Material*).

Eyeblink startle (EMG) responses were calculated by taking the difference between the peak value in the 21 - 175 ms time window and the mean value from the 0 -20 ms time window following probe onset (more details in *Supplementary Material*).

The learning and extinction phases were subdivided into an early and late block comprising 8 trials each. Hypotheses were tested with planned comparisons in repeated measure ANOVAs with Block (baseline, early learning, late learning, early extinction, and late extinction) as a within-subject factor and Group (experimental, control) as a between-subject factor. For US-expectancy, an additional within-subject factor Time was included (7th, 20th, 24th, 33rd, 45th second) with the 7th second (i.e., trial onset, prior to the CS) as reference. For startle EMG, a within-subject factor Stimulus (CS, $ISI_{post\text{-}CS}$) was included. Greenhouse-Geisser corrections were applied where appropriate. Uncorrected degrees of freedom and corrected *p*'s are reported together with η_p^2 (as a measure of effect size) and ε (as a measure of sphericity). All contrasts were tested two-tailed. Alpha was set at .05. All statistical analyses were performed using SPSS 20.

[a] $ISI_{pre\text{-}cs}$ = Inter Stimulus Interval from trial onset till onset of the CS.
[b] $ISI_{post\text{-}cs}$ = Inter Stimulus Interval from CS offset till US onset in the control group.

To test the main hypothesis that fear learning to the CS would occur and extinguish again in the experimental relative to the control group, we tested for each measure specific planned contrasts. We expected no group differences to occur during the baseline and the last extinction block. During the late learning block, we expected the experimental group to have higher skin conductance responses (GSRs) to the CS compared to the control group in the learning phase, because the CS announced US-imminence only in the experimental group. In a similar vein, we expected that participants from the experimental group would increase their US-expectancy during the CS (second 20 relative to second 7) to a greater extent than the control group in the late learning block. For the control group, the US was imminent towards the end of the ISI_{postCS} during the learning phase; therefore, we expected that only participants from the control group would have higher US-expectancies at second 45 relative to second 7 during the late learning block. For startle EMG, we expected that startle potentiation during the CS (startle eyeblink response magnitude during the CS relative to during the ISI) would increase from baseline to late acquisition in the experimental group only. The result section reports on the planned contrasts. Findings on the omnibus tests in the repeated measure ANOVAs can be found in *Supplementary Materials*.

Results

US-expectancy

Baseline phase

As expected, groups did not differ in their increase in US-expectancy during the CS (second 20 relative to second 7) or near the end of a trial (second 45 relative to second 7), $F(1,50) = .31$, $p = .58$ and $F(1,50) = .05$, $p = .83$, respectively.

Learning phase

As expected, both groups differed in their change in US expectancy during the CS during the late learning phase, $F(1,50) = 4.54$, $p = .038$, $\eta_p^2 = .08$: the experimental group had a greater increase in US-expectancy during the CS (second 20 relative to second 7) compared the control group (see Figure 2). In addition, the increase in US-expectancy from prior to the CS (second 7) towards the end of the trial (second 45) was greater for the control than for the experimental group, $F(1,50) = 6.48$, $p = .014$, $\eta_p^2 = .12$ (see Figure 2).

Extinction phase

During the late extinction block, group differences in US-expectancy during the CS (second 20 relative to second 7) and near the end of a trial (second 45 relative to second 7) were no longer significant, $F(1,50) = .54$, $p = .47$ and $F(1,50) = .02$, $p = .87$, respectively.

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Galvanic Skin Response

Baseline phase

As expected, no group differences in skin conductance responses to the CS were observed during baseline $F(1, 50) = .25$, $p = .62$ (*see Figure 3*).

Learning phase

As expected, the CS elicited significantly stronger skin conductance responses in the experimental group compared to the control group during the late learning phase $F(1,50) = 5.72$, $p = .021$, $\eta_p^2 = .1$ (*see Figure 3*).

Extinction phase

As expected, there were no group differences during the late extinction phase, in skin conductance responses to the CS $F(1,50) = 1.98$, $p = .17$ (*see Figure 3*).

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Startle eye blink EMG

Baseline phase

During the baseline phase, no group differences were observed in startle amplitudes to the CS relative to startle amplitudes during the $ISI_{post\text{-}CS}$ $F(1, 43) = 1.24$, $p = .27$ (*see Figure 4A & 4B*).

Learning phase

During the late learning block, a significant group difference in the startle magnitude during the CS relative to the $ISI_{post\text{-}CS}$ was found, $F(1,43) = 13.37$, $p = .001$, $\eta_p^2 = .24$, with higher CS amplitudes compared to ISI amplitudes in the paired group and the opposite pattern in the unpaired group (*see Figure 4A & 4B*).

Extinction phase

Opposite to our hypothesis, there still was a significant group difference in startle amplitudes during the CS relative to the $ISI_{post\text{-}CS}$ during late extinction $F(1,43) = 5.8$, $p = .020$, $\eta_p^2 = .12$, with higher CS amplitudes compared to ISI amplitudes in the paired group and the opposite in the unpaired group. (*see Figure 4A & 4B*).

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Discussion

The current study sought to investigate for the first time whether fear towards innocuous gastrointestinal sensations can develop by means of associative learning between consecutive gastrointestinal events in healthy humans. To this end, a novel homoreflexive interoceptive conditioning paradigm was developed with experimentally induced visceral sensations at the level of the distal esophagus, as CS and US. The general aim of the current study was to assess whether associative fear learning towards innocuous gastrointestinal sensation can be established, as such learning processes are considered central in the generation and maintenance of gastrointestinal symptom-specific fear, a key factor in the pathophysiology of FGID.

Principal findings

We hypothesized that fear learning to the innocuous non-painful visceral CS would be established by means of associative learning between interoceptive events, which should be reflected in increases in subjective anticipation of the US during the CS, increased skin conductance responses to the CS and fear potentiated startle responses in the presence of the CS relative to the absence of the CS. We also hypothesized that these learned fear responses would disappear again in the extinction phase, when the innocuous CS was no longer followed by the US.

Associative fear learning. Participants assigned to the experimental group learned to fear the innocuous gastrointestinal sensation (CS) during the learning phase, because for them it signaled the imminence of a painful gastrointestinal sensation (US). Such fear learning to the CS was absent in the control group for whom the innocuous sensation (CS) signaled a relatively safe period without pain. Importantly, fear learning to the innocuous sensation (CS) was established at different levels of learning and for all outcomes. The effects on US-

expectancy indicate that in the late block of the learning phase, participants from the experimental group had to some extent acquired explicit knowledge of the CS-US contingency. In addition, the relatively increased skin conductance responses to the CS in the experimental compared to the control group provides evidence that the innocuous CS had gained emotional significance for participants from the experimental group. Finally, we found also fear learning effects in startle eyeblink EMG, which is generally accepted to be an index of covert, subcortical activation of fear circuits[16]. Together, these results convincingly demonstrate that fear for innocuous gastrointestinal sensations can arise from temporal contingencies between gastrointestinal events, giving rise to associative learning. This in turn can be linked to earlier hypotheses on the generation of gastrointestinal symptom-specific fear, which attribute an important role to associative learning processes by which initially relatively 'neutral' bodily sensations start provoking fear through activation of fear circuits in the brain[10–12].

Extinction of learned fear. Our hypothesis that fear would extinguish in the experimental group when the innocuous CS was no longer followed by the painful US was only partially confirmed. Towards the end of extinction, both groups did no longer differ in the skin conductance responses to or US-expectancies during the CS. Yet, the experimental group still responded with a fear-potentiated startle to the CS, relative to the control group. This is very much in line with earlier findings using respiratory sensations as CS and US in an interoceptive associative learning paradigm[14]. Whereas fear-conditioned changes in skin conductance responses and in US expectancy primarily reflect explicit knowledge of the CS–US contingency, startle potentiation is thought to more directly reflect subcortical, amygdala-dependent *emotional* learning that can dissociate from the former measures[19,20]. Our findings suggest that extinction of unconscious, emotional learning to visceral sensations is particularly slow and rather difficult to establish, and may therefore require a more in-depth and prolonged extinction training.

Clinical Implications

The present findings on how fear towards innocuous gastrointestinal sensations can come about through an associative learning process is relevant for any gastrointestinal disorder but for FGID in particular, as many of those patients are characterized by excessive distress and fear towards certain types of gastrointestinal sensations[4]. Recently, we have found that associative learning leading to gastrointestinal sensations does not only cause

emotional distress, but also alters perceptual thresholds for those gastrointestinal sensations[13]. Thus, gastrointestinal symptom-specific fear and visceral hypersensitivity may be closely related, likely because associative learning between gastrointestinal events is a key common mechanism underlying both phenomena[11].

Our findings further support the value of exposure-based cognitive-behavioral treatment as an important treatment option for FGID, particularly in patients with high levels of gastrointestinal symptom-specific fear. Previous research found exposure-based treatment to be effective in symptomatic improvement of IBS, with its effects being mediated by reduction in gastrointestinal symptom-specific fear [8,10]. In line with this, the present study confirms that extinction learning as a process is not limited to external feared objects (e.g., spiders), but also applies to visceral sensations and can therefore considered the major active ingredient of successful interoceptive exposure therapies.

Interestingly, our findings from the extinction phase suggest that innocuous visceral sensations can activate subcortical emotional responses, despite one is aware that the sensation will not be followed by or develops into a painful sensation. Such dissociation between fear indices during extinction may have clinical relevance. For example, even though a patient may have understood from the treating MD and clearly accepts that a certain type of gastrointestinal sensations is not harmful and does not reflect disease activity, a patient may still show fear responses towards these innocuous gastrointestinal sensations, causing feelings of distress and potentially lowering the threshold to perceive the sensations. Therefore, in-depth and prolonged exposure therapy may be required to extinguish learned fear responses to gastrointestinal sensations.

Conclusion

We can conclude from our study that innocuous gastrointestinal sensations can come to elicit fear once they have been associated to a painful sensation that shares perceptual similarities to the innocuous sensation and has an identical anatomical origin (i.e. in this case, the gastrointestinal tract). The present study demonstrated that it is possible to form an association between an originally benign visceral sensation and an unpleasant visceral sensation merely through the basic process of associative learning. Thus, the present study established that classical conditioning is a viable mechanism to create gastrointestinal symptom-specific fear, which may in turn trigger the development of FGID and maintain or exacerbate symptoms. Furthermore, our findings suggest that a prolonged exposure therapy may be necessary for an in-depth extinction of gastrointestinal symptom-specific fear.

Figure legends

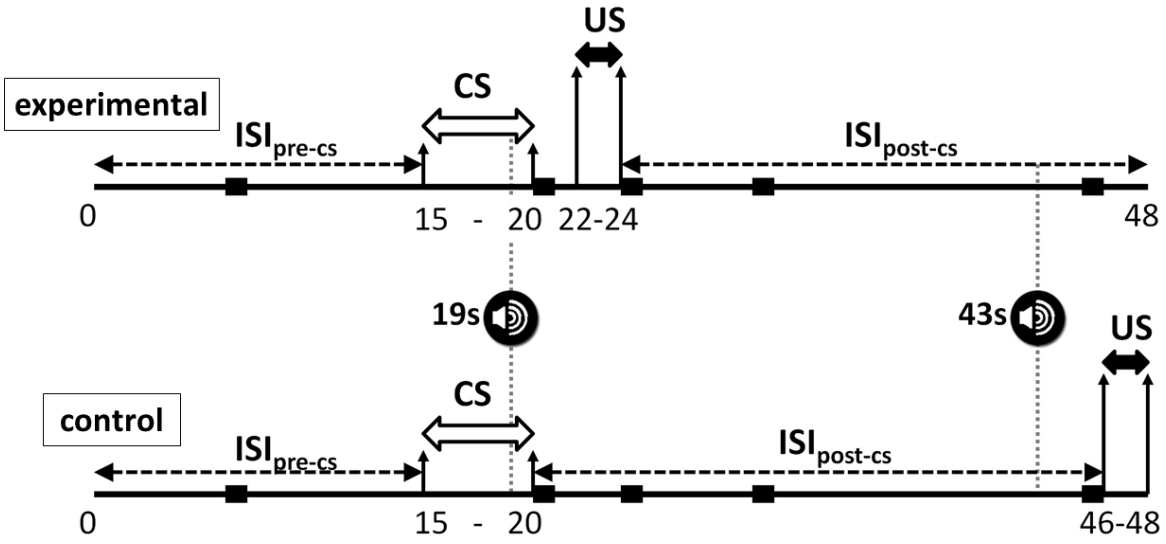

Figure 1. Schematic illustration of trial structure during the learning phase.
The conditioned stimulus (CS) was delivered from 15 to 20 seconds after trial onset, and preceded and followed by an inter-stimulus interval (ISI), respectively labeled $ISI_{pre\text{-}cs}$ and $ISI_{post\text{-}cs}$. An unconditioned stimulus (US) was delivered from 22 to 24 seconds after trial onset for the experimental group, and from 46 to 48 seconds for the control group. The sound symbols represent acoustic startle probes, which were invariably administered at 19s and 43s after trial onset. The black squares on the timelines are the points in time which were included in the analysis of the subjective US-anticipation.

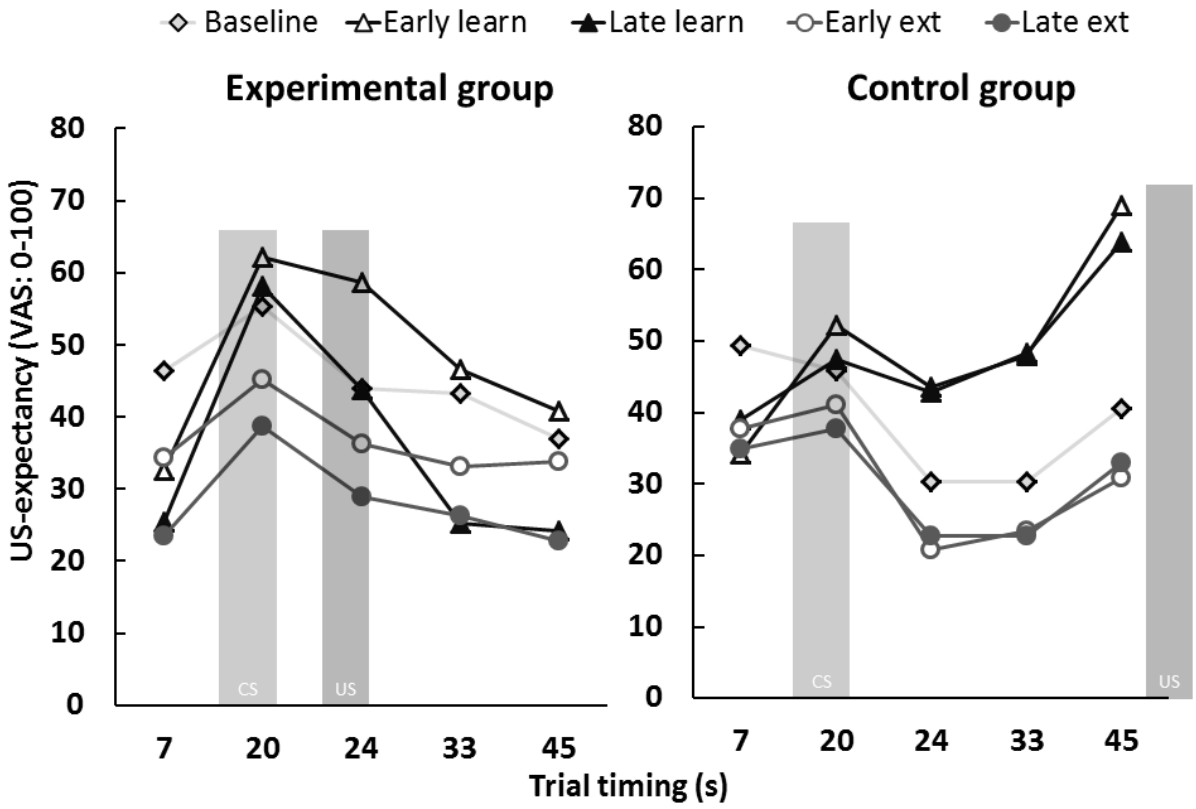

Figure 2. Mean US-anticipation ratings at the 7th, 20th, 24th, 33rd and 45th second for the experimental and control group during the baseline phase, early learning phase (Early learn), late learning phase (Late learn), early extinction phase (Early ext) and late extinction phase (Late ext).

On a 0-100 scale, a rating of '50' reflects the point of uncertainty, '100' reflects 100% certainty that the US is imminent, while '0' 100% certainty that the US is not-imminent The light grey bars represent the presentation of the CS, the darker grey bars represent the presentation of the US.

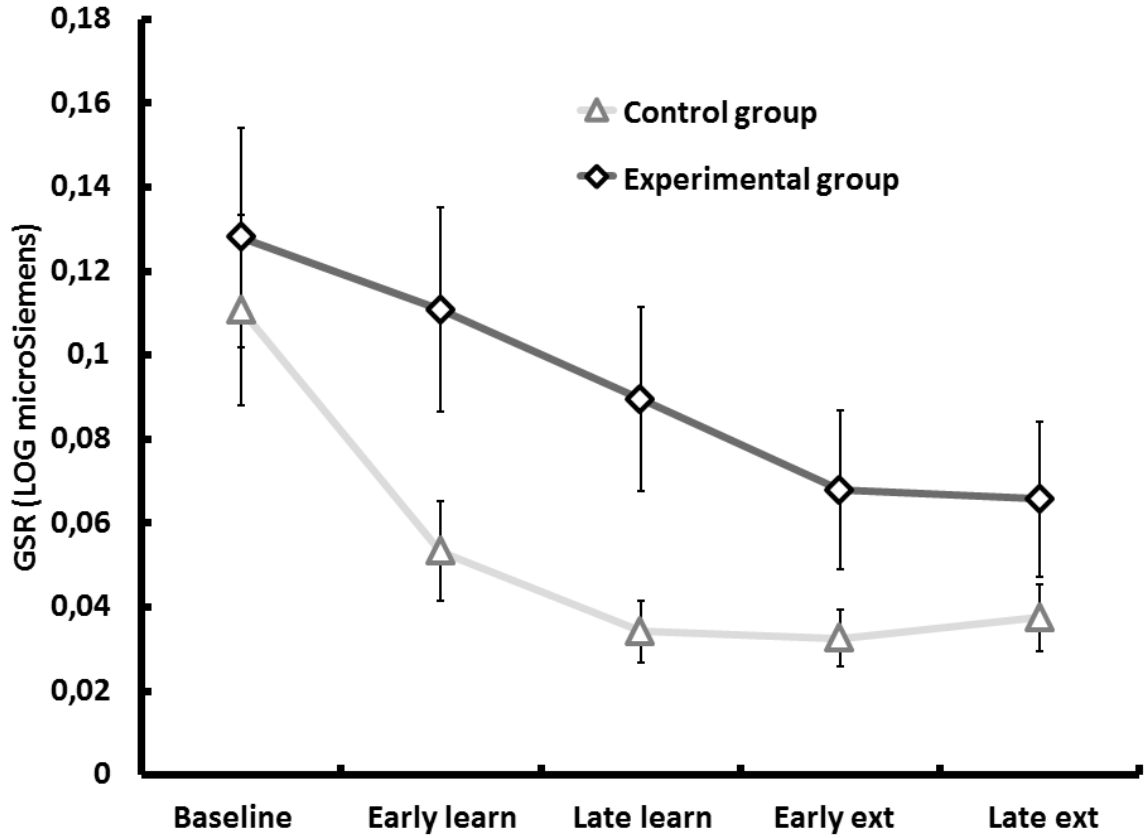

Figure 3. Mean log transformed skin Conductance responses of the experimental and ccontrol group during the baseline phase, early and late learning phase, early and late extinction phase.

The error bars represent the standard error.

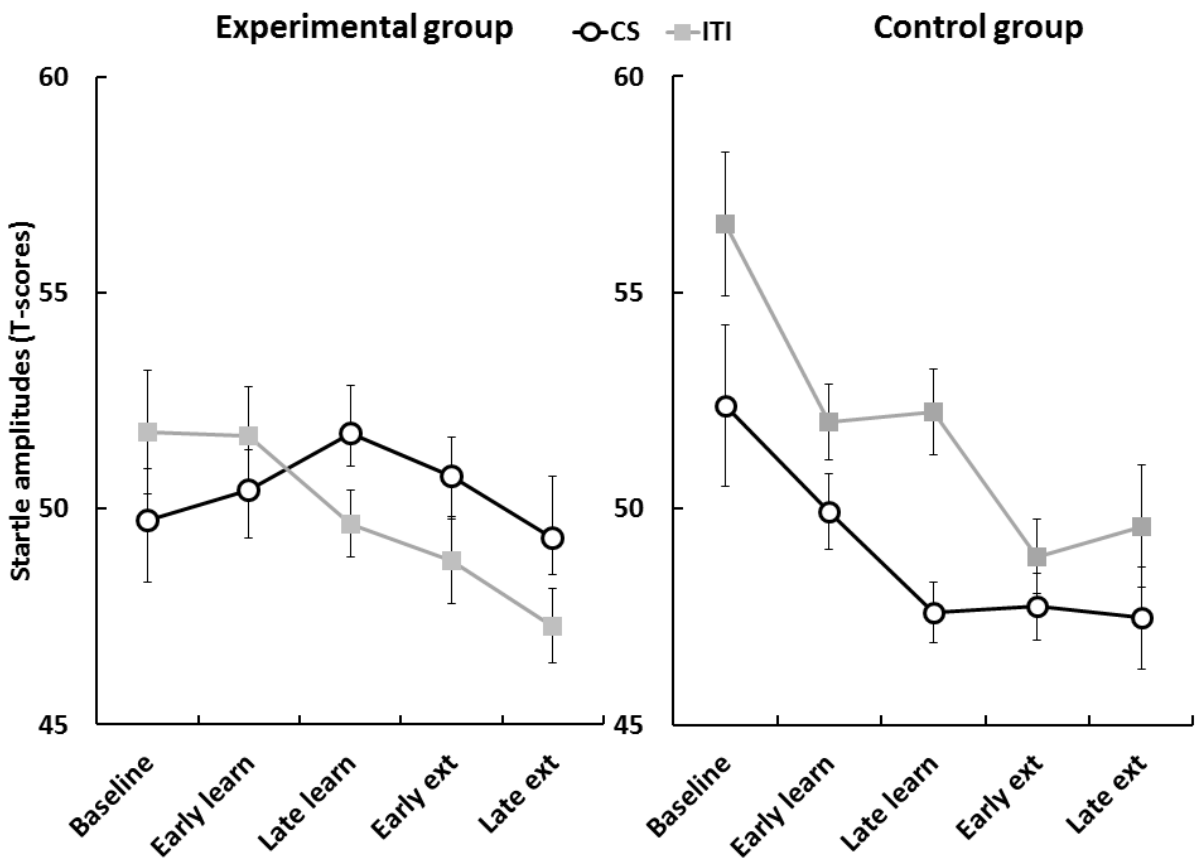

Figure 4. Mean startle amplitudes (T-scores) for the (A) experimental group and the (B) control group .

The error bars represent the standard error. See Results section for statistical details. CS = conditioned stimulus; ISI = Inter-stimulus interval (after CS); learn = learning phase; ext = extinction phase.

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Supplementary Material

Methods

Subjects

The informed consent outlined the experimental procedure including stimuli to be delivered, guaranteed anonymity, and stated that participation was voluntary (with a reimbursement of 50 Euros), and mentioned that participation could be halted at any moment if the participant so desired, without loss of the promised reimbursement.

If still interested, participants were required to indicate whether or not they had a history or presence of: (a) psychiatric conditions; (b) abdominal or thoracic surgery (except appendectomy and cholecystomy); (c) neurological, endocrine, or digestive disorders, and/or (d) other medical disorders. Moreover they also had to indicate whether at the time of the experiment they (e) were pregnant, (f) had pain symptoms, (g) used medication affecting the function of the digestive tract and/or the nervous system, (h) had a recent accident of which they weren't fully recovered yet, and/or (i) had a serious hearing impairment. Anyone affirming any or several of these, was deemed unfit for participation and was kindly thanked for their interest in participating.

Approval for conducting the experiment was obtained from the Medical Ethical Committee of the University of Leuven (reference number ML8570).

Esophageal Stimulation

To prevent the catheter from moving due to peristaltic contractions of the esophagus that occur in response to balloon inflation, tape was used to gently attach the extraneous part of the catheter to the cheeks. The remainder of the catheter was draped over the ear and attached to an air filled syringe that was used for inflating the esophageal balloon.

For this threshold determination, the volume of the balloon increased with 1 ml relative to each previous inflation. Between each 1 ml inflation, the balloon was deflated. Immediately after each inflation, subjects indicated whether they felt something, and rated what they felt on a scale from 0 to 10, with zero being no sensation at all, 1 indicating possibly a sensation (not being entirely certain), 2 indicating a sensation definitely being present but not yet painful, 8 being a clearly painful but still tolerable sensation, and 10 being the maximally tolerable intensity of pain. Participants were warned that intensity 10 would never be used, and that it was always possible to reduce the volume if the subjective intensity was too high. During threshold determination, up to and including intensity 3, the balloon was

inflated for 5 seconds, equaling the duration of the CS to be used in the experiment. Beyond this point on the scale, the balloon was inflated for 2 seconds only, which was the duration of the US to be used in the experiment. The entire threshold determination procedure was repeated a second time to make sure the thresholds were accurate. In case the second threshold determination yielded different results than the first, the thresholds obtained during the second determination were used as the first may be more prone to novelty effects.

Subjective expectancy of US onset

The position of the dial in the scale was digitally registered at 10Hz and transmitted via a data acquisition card to a computer throughout the entire experiment. The recorded digital values give an indication of the subjective estimation of each participant on how likely they felt they were to receive the US in the following seconds. As such, this dial can be used to assess whether participants learned to make correct predictions of US onset.

Psychophysiological Measures

All signals described below were recorded using Affect 4.0 software[1] and transmitted via a 16-Bit PCI-6221 data acquisition card (National Instruments, Austin, Texas) to a computer, and treated offline with Psychophysiological Analysis software (PSPHA)[2].

Eyeblink startle EMG

The startle was elicited and measured as based on the guidelines of Blumenthal et al.[3]. A 50ms burst of white noise with a volume of 102dB was used as an acoustic startle probe. The raw EMG signal was amplified by a LabLinc v75-04 Coulbourn Isolated Bioamplifier with Bandpass filter; the recording bandwidth was between 13Hz and 1 kHz. This signal was transmitted to a LabLinc v76-24 Coulbourn 4 Channel Integrator, which rectified and smoothed the signal online with a time constant of 20ms. The EMG signal was digitized at 1kHz starting 500ms prior to onset of the acoustic probe, until 1000ms after probe onset.

Galvanic Skin Response

After cleaning the hypothenar side of the non-dominant hand with alcohol, two standard Ag/AgCl electrodes (diameter 1cm) filled with water-soluble KY*gel were attached here, spaced approximately 2.5cm apart. The galvanic skin response measured via these electrodes was transmitted to the LabLinc v71-23 Coulbourn Isolated Skin Conductance Coupler, which maintained a constant voltage of 0.5V over the electrodes; the analog signal was digitized at 10Hz.

Study design

Following the determination of the individualized thresholds for CS and US, electrodes for measuring startle and GSR were attached. Subjects were verbally informed what these electrodes would be used for, including information about the occurrence of acoustic startle probes throughout the experiment. Following electrode attachment, the intended use of the dial was explained to participants, and after they indicated they had no more questions, earphones were mounted on their head.

Throughout the entire experiment, an experimenter remained in the lab with the participant in order to be able to administer the CS and US when required. Inflation and deflation of the esophageal balloon occurred outside the field of vision of the participant for both CS and US, by means of a manually operated air filled syringe. The experimenter administering the CS and US was cued to do so via a monitor, which was also placed outside the field of vision of the participant. On this monitor, a countdown occurred 5 seconds prior to inflation while indicating whether a CS or US had to be administered, and a second countdown occurred starting from onset of inflation, showing the remaining time till deflation.

As the startle magnitude in response to the startle probe tends to be exaggerated upon initial presentation, and becomes more stable after repeated stimulation. Participants were first exposed to twelve startle probes, all administered with a fixed interval of ten seconds immediately prior to the onset of the actual experiment. After habituating to the probes, the participant started using the US expectancy dial and continued doing so until the end of the experiment. The dial was fixed in place within arm's length in front of the participant.

Response definition and statistical analysis

Startle eye blink EMG

EMG signals were visually inspected off-line to detect artifacts (e.g., excessive noise from muscular activity prior to the startle probe). Artifacts were rejected from analysis and defined as missing. The average percentage of rejected responses per participant was 8% (SD 6%). If responses to the probe were not visible, responses were classified as a non-response and set at zero. Five participants were excluded from the startle analysis because they either had more than 33% rejected responses, or had no visible response to the probe more than 66% of the time. All startle responses were T-transformed within persons to correct for interindividual variability that was unrelated to the experimental conditions of interest[3].

Galvanic Skin Response

After skin conductance responses were averaged across trials, skin conductance data were LOG_{10}(1 + skin conductance response)-transformed before being analyzed.

Results: Omnibus Test of Repeated Measure ANOVAs

US-expectancy

There was a main effect of Block $F(4,200) = 9.45$, $p < .001$, $\eta_p^2 = .16$, $\varepsilon = .86$, a main effect of Time $F(4,200) = 8.32$, $p < .001$, $\eta_p^2 = .14$, $\varepsilon = .72$ but no main effect of Group $F(1,50) = .1$, $p = .8$) and a trend towards significance for the Block × Group interaction $F(4,200) = 2.06$, $p = .099$, $\eta_p^2 = .04$, $\varepsilon = .86$. Furthermore, there was a significant Time × Group interaction $F(4,200) = 8.87$, $p < .001$, $\eta_p^2 = .15$, $\varepsilon = .72$, and a significant Block × Time Interaction $F(16,800) = 3.11$, $p = .001$, $\eta_p^2 = .06$, $\varepsilon = .56$. The Block × Time × Group interaction reached significance $F(16,800) = 2.41$, $p = .011$, $\eta_p^2 = .05$, $\varepsilon = .56$.

Galvanic Skin Response

There was a main effect of Block $F(4, 200) = 16.48$, $p < .001$, $\eta_p^2 = .25$, $\varepsilon = .495$ as skin responses habituated across blocks. Furthermore, there was a trend for stronger skin responses in the experimental group across blocks compared to the control group (Main effect of group: $F(1,50) = 3.05$, $p = .087$, $\eta_p^2 = .06$. The interaction between Block and Group did not reach significance $F(4,200) = 1.49$, $p = .23$.

Startle eye blink EMG

There was a main effect of Block $F(4,172) = 5.03$, $p = .005$, $\eta_p^2 = .11$, $\varepsilon = .63$, but no Block × Group interaction $F(4,172) = 1.87$, $p = .15$, $\varepsilon = .63$. There was a significant Stimulus × Block $F(4,172) = 3.22$, $p = .014$, $\eta_p^2 = .07$ and a Stimulus × Group interaction $F(1,43) = 7.93$, $p = .007$, $\eta_p^2 = .16$. The main effect of Stimulus $F(1,43) = 3.58$, $p = .065$, $\eta_p^2 = .08$ as well as the three-way interaction between Block × Stimulus × Group failed to reach significance $F(4,172) = 2.05$, $p = .089$, $\eta_p^2 = .05$.

References

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