Learned Fear of Gastrointestinal Sensations in Healthy Adults

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BACKGROUND & AIMS: **Gastrointestinal symptom-specific fear and anxiety are important determinants of gastrointestinal symptom perception. We studied learning of fear toward 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 distention at a perceptible but nonpainful intensity (conditioned stimulus [CS], the innocuous sensation) and at a painful intensity (unconditioned stimulus [US]). Subjects were assigned randomly 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 distention—the painful US was no longer administered. Indexes of fear learning toward the innocuous CS distention included the skin conductance response, fear-potentiated startle (measured by the eye-blink 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 with the control group, $P = .04$), increased skin conductance response (compared with the control group, $P = .03$), and potentiated startle reflex (compared with the control group, $P = .001$) in response to the CS. The differences between the experimental and control groups in US expectancy and skin conductance, but not fear-potentiated startle, disappeared during the extinction phase.**

CONCLUSIONS: **Fear toward innocuous gastrointestinal sensations can be established through associative learning in healthy human beings. This may be an important mechanism in the development of fear of gastrointestinal symptoms, implicated in the pathophysiology of functional gastrointestinal disorders.**

Keywords: Functional Gastrointestinal Disorders; Visceral Pain; Interoceptive Conditioning; Gastrointestinal Symptom-Specific Fear.

See editorial on page 1559.

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, also occur often in the absence of any detectable physiological abnormalities, as is the case in functional gastrointestinal disorders (FGIDs).

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] because 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]

[a]Authors share co-first authorship.

Abbreviations used in this paper: CS, conditioned stimulus; EMG, electromyogram; FGID, functional gastrointestinal disorder; IBS, irritable bowel syndrome; ISI, interstimulus interval; ISI_{postCS}, interstimulus interval occurring after the conditioned stimulus; US, unconditioned stimulus.

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How gastrointestinal symptom-specific fear develops remains unclear, but it often is assumed that fear learning plays a key role. More specifically, originally benign visceral sensations may become associated with unpleasant or painful visceral sensations. For example, benign, nonpainful epigastric sensations may precede an episode of stomachache. As a consequence of this temporal contingency, the individual eventually experiences these benign sensations as unpleasant and may come to fear them, whereas previously 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 anatomic location as in the example described earlier, 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 (ie, 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.[10–12]

The purpose of the current study therefore was to study fear learning toward innocuous visceral sensations as a potential mechanism in the development of gastrointestinal symptom-specific fear. We set up a study with a painful esophageal stimulus as the US, and a detectable, nonpainful esophageal stimulus as the CS. We expected fear learning to the CS to occur when the CS immediately preceded the painful US (experimental group), but not when the CS and US were 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 form in line with the declaration of Helsinki before deciding whether or not to participate (see the Supplementary Methods section for more detail). Participants were assigned randomly to the experimental or the control group (see later). Both groups were matched for age and sex.

Esophageal Stimulation

Both the CS and the 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 determined individually for both the CS and the US using a variation of the ascending methods of limits, with the CS being perceptible but nonpainful stimulation of the esophagus, and the US being painful but still bearable stimulation at the same anatomic site (see the Supplementary Methods section for more detail). A pediatric catheter (used for gavage) with a diameter of 3 mm (TR-2008, Pennine Healthcare, Derby, United Kingdom) was inserted via the nose into the distal esophagus, 35 cm from the nostril. A deflated, custom-made, silicon medical balloon (diameter, 5 mm; length, 25 mm; Medasil, Leeds, United Kingdom) was attached firmly to the end of the catheter positioned in the esophagus (see the Supplementary Methods section for more detail).

Subjective Expectancy of the Unconditioned Stimulus 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 toward zero. The more certain they were to expect the US, the more they turned the dial from 50 upward to 100 (see the Supplementary Methods section for more detail).

Psychophysiological Measures

Eyeblink-startle electromyogram. 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 of 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 (see the Supplementary Methods section for more detail).

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 (see the Supplementary Methods section for more detail).

Study Design

The experiment consisted of the following 3 phases: (1) a baseline phase (4 trials), (2) a learning phase (16 trials), and (3) an extinction phase (16 trials).

During the baseline and extinction phases, both groups were treated identically and received 1 innocuous CS distention in every trial, and no painful US distentions. During the learning phase, both groups received 1 innocuous CS in every trial and in addition 1 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 with a 100% reinforcement scheme, because 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 2-s delay) by the US. The control group had an interval of 26 seconds between the CS and the US onset (Figure 1). In essence, in the experimental group the CS announced the imminence of the painful US, whereas in the control group it announced an imminent safe and pain-free period.

Every trial lasted 48 seconds, irrespective of phase. The innocuous CS distention always was administered 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 interstimulus interval [ISI_{postCS}]) of each trial (Figure 1) (see the Supplementary Methods section for more detail).

Response Definition and Statistical Analysis

Because US expectancy was measured continuously, data were reduced by selecting 5 time points of interest for each trial: the 7th second (before the CS, in the middle of the ISI from trial onset until onset of the CS), 20th second (during the CS), 24th second (end of the US for the experimental group/beginning of ISI_{postCS} for the control group), 33rd second (middle of ISI from CS offset till US onset in the control group), and the 45th second (end of trial; ie, right before the US onset for the control group).

Galvanic skin responses were calculated by subtracting the mean skin conductance level during baseline (2 s before the CS onset) from the maximum value in the window from 0 to 7 seconds after CS onset (see the Supplementary Methods section for more detail).

Eyeblink-startle electromyogram (EMG) responses were calculated by taking the difference between the peak value in the time window from 21 to 175 ms and the mean value from the time window from 0 to 20 ms after probe onset (see the Supplementary Methods section for more detail).

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 analysis of variance 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 of time was included (at the 7th, 20th, 24th, 33rd, and 45th second) with the 7th second (ie, trial onset, before the CS) as a reference. For startle EMG, a within-subject factor stimulus (CS, ISI_{postCS}) was included. Greenhouse–Geisser corrections were applied where appropriate. Uncorrected degrees of freedom and corrected *P* values are reported together with η_p^2 (partial eta squared, as a measure of effect size) and ε (as a measure of sphericity). All tests were 2-tailed. The α value was set at .05. All statistical analyses were performed using SPSS 20 (Brussels, Belgium).

To test the main hypothesis that fear learning to the CS would occur and extinguish again in the experimental

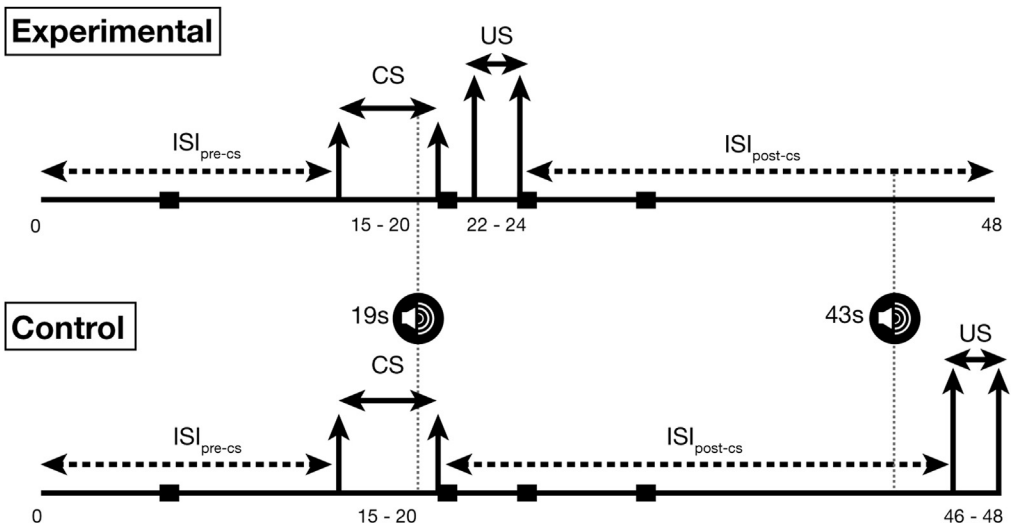

Figure 1. Trial structure during the learning phase. The CS was delivered from 15 to 20 seconds after trial onset, and preceded and followed by an ISI, respectively, labeled ISI_{preCS} and ISI_{postCS}. The US was delivered from 22 to 24 seconds after trial onset for the experimental group, and from 46 to 48 seconds for the control group. The *sound symbols* represent acoustic startle probes, which invariably were administered at 19 and 43 seconds after trial onset. The *black squares* on the timelines are the points in time that were included in the analysis of the subjective US anticipation.

group relative to the control group, for each measure we tested 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 (galvanic skin responses) to the CS compared with 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 (20 seconds after the start of a trial compared to 7 seconds after the start of the trial) to a greater extent than the control group in the late learning block. For the control group, the US was imminent toward 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 analysis of variance can be found in the Supplementary Methods section.

Results

Unconditioned Stimulus 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 with the control group (Figure 2). In addition, the increase in US expectancy from before the CS (second 7) toward the end of the trial (second 45) was greater for the control than for the experimental group ($F_{1,50} = 6.48$, $P = .014$, $\eta_p^2 = .12$) (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).

Galvanic Skin Response

Baseline phase. As expected, no group differences in skin conductance responses to the CS were observed during baseline ($F_{1,50} = .25$, $P = .62$) (Figure 3).

Learning phase. As expected, the CS elicited significantly stronger skin conductance responses in the experimental group compared with the control group during the late learning phase ($F_{1,50} = 5.72$, $P = .021$, $\eta_p^2 = .1$) (Figure 3).

Extinction phase. As expected, there were no group differences during the late extinction phase in skin

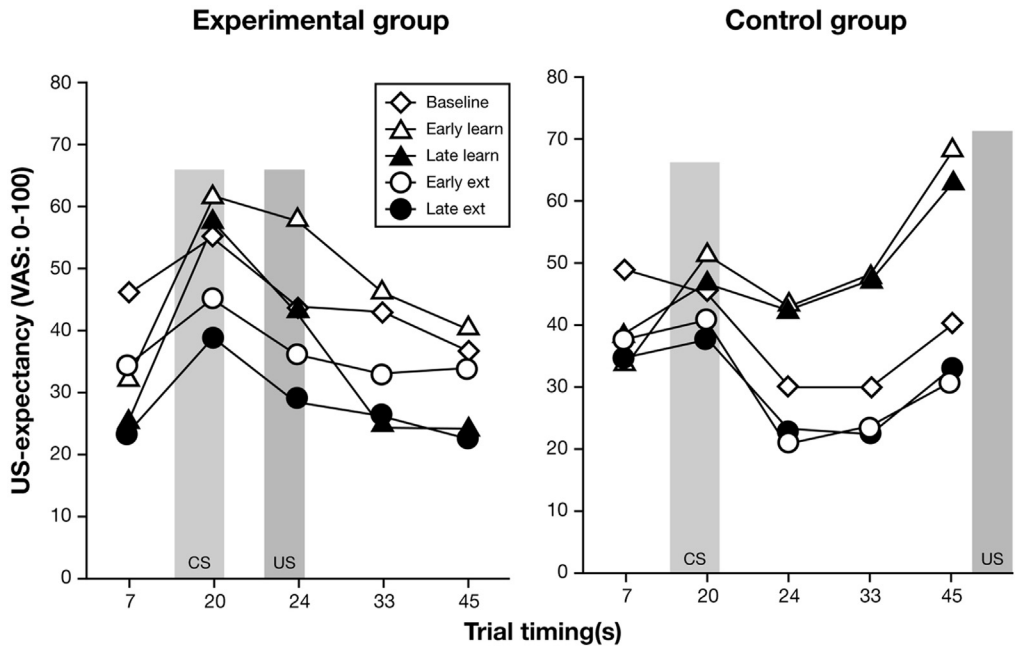

Figure 2. Mean US anticipation ratings at the 7th, 20th, 24th, 33rd, and 45th second for the experimental and control groups during the baseline phase, early learning phase (Early learn), late learning phase (Late learn), early extinction phase (Early ext), and late extinction phases (Late ext). On a visual analog scale (VAS) of 0–100, a rating of 50 reflects the point of uncertainty, a rating of 100 reflects 100% certainty that the US is imminent, and a rating of 0 reflects 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.

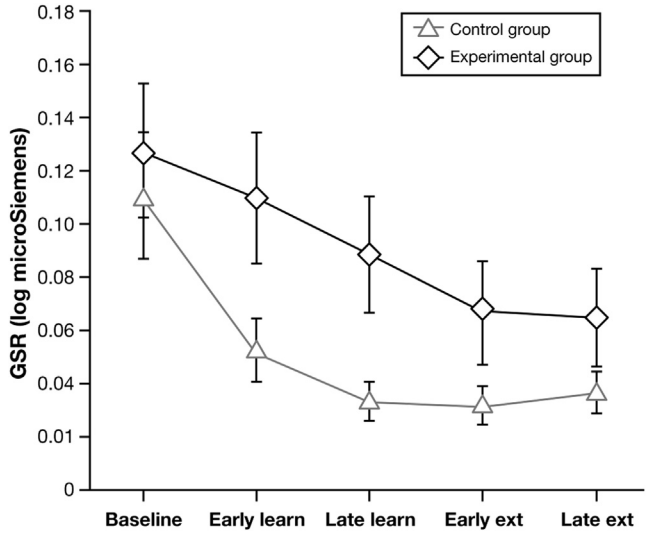

Figure 3. Mean log-transformed skin conductance responses of the experimental and control groups during the baseline phase, early and late learning phases, and early and late extinction phases. The error bars represent the standard error. GSR, galvanic skin response. Ext, extinction phase; learn, learning phase.

conductance responses to the CS ($F_{1,50} = 1.98$, $P = .17$) (Figure 3).

Startle Eyeblink Electromyogram

Baseline phase. During the baseline phase, no group differences were observed in startle amplitudes to the CS relative to startle amplitudes during the ISI_{postCS} ($F_{1,43} = 1.24$, $P = .27$) (Figure 4).

Learning phase. During the late learning block, a significant group difference in the startle magnitude during the CS relative to the ISI_{postCS} was found ($F_{1,43} = 13.37$, $P = .001$, $\eta_p^2 = .24$), with higher CS amplitudes compared with ISI amplitudes in the paired group and the opposite pattern in the unpaired group (Figure 4).

Extinction phase. Opposite to our hypothesis, there still was a significant group difference in startle amplitudes during the CS relative to the ISI_{postCS} during late extinction ($F_{1,43} = 5.8$, $P = .020$, $\eta_p^2 = .12$), with higher CS amplitudes compared with ISI amplitudes in the paired group and the opposite in the unpaired group (Figure 4).

Discussion

The current study sought to investigate whether fear toward innocuous gastrointestinal sensations can develop by means of associative learning between consecutive gastrointestinal events in healthy human beings. 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 toward innocuous gastrointestinal sensation can be established because 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 nonpainful visceral CS would be established by means of associative learning between interoceptive events, which

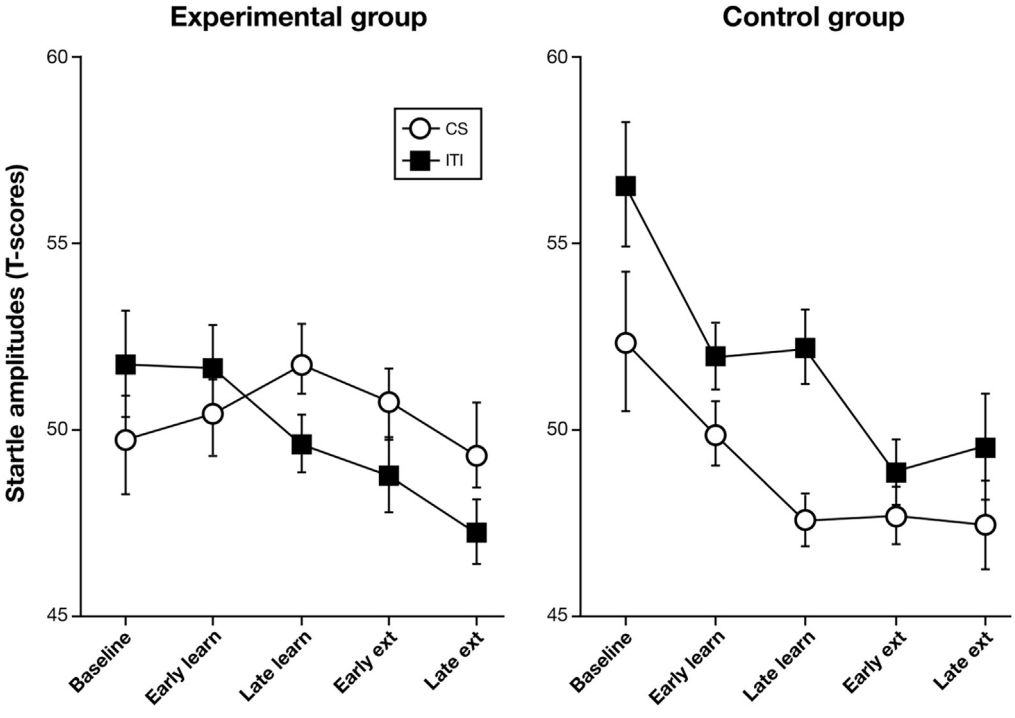

Figure 4. Mean startle amplitudes (T scores) for the (*A*) experimental group and the (*B*) control group. The error bars represent the standard error. See the Results section for statistical details. Ext, extinction phase; learn, learning phase.

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 with the control group provides evidence that the innocuous CS had gained emotional significance for participants from the experimental group. Finally, we also found fear learning effects in startle eyeblink EMG, which generally is accepted to be an index of covert, subcortical activation of fear circuits.[16] Together, these results convincingly show 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. Toward the end of extinction, both groups no longer differed in the skin conductance responses to or US expectancies during the CS. However, 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] Although 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 therefore may require a more in-depth and prolonged extinction training.

Clinical Implications

The present findings on how fear toward innocuous gastrointestinal sensations can come about through an associative learning process is relevant for any gastrointestinal disorder but for FGID in particular because many of those patients are characterized by excessive distress and fear toward certain types of gastrointestinal sensations.[4] Recently, we have found that associative learning leading to gastrointestinal sensations not only causes emotional distress, but also alters perceptual thresholds for those gastrointestinal sensations.[13] Thus, gastrointestinal symptom-specific fear and visceral hypersensitivity may be related closely, 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 (eg, spiders), but also applies to visceral sensations and therefore can be 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 being aware that the sensation will not be followed by or develop 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 physician and clearly accepts that a certain type of gastrointestinal sensations is not harmful and does not reflect disease activity, a patient still may show fear responses toward these innocuous gastrointestinal sensations, causing feelings of distress and potentially decreasing the threshold to perceive the sensations. Therefore, in-depth and prolonged exposure therapy may be required to extinguish learned fear responses to gastrointestinal sensations.

Conclusions

We can conclude from our study that innocuous gastrointestinal sensations can come to elicit fear once they have been associated with a painful sensation that shares perceptual similarities to the innocuous sensation and has an identical anatomic origin (ie, in this case, the gastrointestinal tract). The present study showed 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 classic 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 prolonged exposure therapy may be necessary for an in-depth extinction of gastrointestinal symptom-specific fear.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2016.04.035.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Methods

Subjects

The informed consent form outlined the experimental procedure including stimuli to be delivered, guaranteed anonymity, and stated that participation was voluntary (with a reimbursement of 50 Euros), and stated 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 the following: (1) psychiatric conditions; (2) abdominal or thoracic surgery (except an appendectomy or cholecystomy); (3) neurologic, endocrine, or digestive disorders; and/or (4) other medical disorders. Moreover, they also had to indicate whether at the time of the experiment they (1) were pregnant, (2) had pain symptoms, (3) used medication affecting the function of the digestive tract and/or the nervous system, (4) had a recent accident from which they were not fully recovered, and/or (5) had a serious hearing impairment. Any patient who affirmed any or several of these was deemed unfit for participation and was thanked kindly 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 owing to peristaltic contractions of the esophagus, which 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 an intensity of 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, equal to 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 because the first may have been more prone to novelty effects.

Subjective Expectancy of Unconditioned Stimulus Onset

The position of the dial on the scale was registered digitally at 10 Hz and transmitted via a data acquisition card to a computer throughout the entire experiment. The recorded digital values provided 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 could be used to assess whether participants learned to make correct predictions of US onset.

Psychophysiological Measures

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

Eyeblink Startle Electromyogram

The startle was elicited and measured as based on the guidelines of Blumenthal et al.[3] A 50-ms burst of white noise with a volume of 102 dB was used as an acoustic startle probe. The raw EMG signal was amplified by a LabLinc (Hollistone, MA) v75-04 Coulbourn Isolated Bioamplifier with bandpass filter; the recording bandwidth was between 13 Hz 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 20 ms. The EMG signal was digitized at 1 kHz, starting 500 ms before the onset of the acoustic probe until 1000 ms after the probe onset.

Galvanic Skin Response

After cleaning the hypothenar side of the nondominant hand with alcohol, 2 standard silver chloride electrodes (diameter, 1 cm) filled with water-soluble KY*gel (Johnson & Johnson, New Brunswick, NJ) were attached here, spaced approximately 2.5-cm 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 0.5 V over the electrodes; the analog signal was digitized at 10 Hz.

Study Design

After the determination of the individualized thresholds for CS and US, electrodes for measuring startle and galvanic skin response were attached. Subjects were informed verbally what these electrodes would be used for, including information about the occurrence of acoustic startle probes throughout the experiment. After 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 laboratory with the participant 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 the 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 also was placed outside the field of vision of the participant. On this monitor, a countdown occurred 5 seconds before 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 until deflation.

The startle magnitude in response to the startle probe tends to be exaggerated on initial presentation, and becomes more stable after repeated stimulation. Participants first were exposed to 12 startle probes, all administered with a fixed interval of 10 seconds immediately before 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 eyeblink electromyogram. EMG signals were inspected visually offline to detect artifacts (eg, excessive noise from muscular activity before 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 nonresponse 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 Analysis of Variance

Unconditioned stimulus expectancy. There was a main effect of block ($F_{4,200} = 9.45$, $P < .001$, $\eta_p^2 = .16$, $\varepsilon = .86$) and 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 toward 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 with 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 Eyeblink Electromyogram

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 3-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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