Interoceptive awareness and unaware fear conditioning: Are subliminal conditioning effects influenced by the manipulation of visceral self-perception?

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Abstract

Research has shown repeatedly that attention influences implicit learning effects. In a similar vein, interoceptive awareness might be involved in unaware fear conditioning: The fact that the CS is repeatedly presented in the context of aversive bodily experiences might facilitate the development of conditioned responding. We investigated the role of interoceptive attention in a subliminal conditioning paradigm. Conditioning was embedded in a spatial cueing task with subliminally presented cues that were followed by a masking stimulus. Response times to the targets that were either validly or invalidly predicted by the cues served as index of conditioning. Interoceptive attention was manipulated between-subjects. Half the participants completed a heartbeat detection task before conditioning. This task tunes attention to one's own bodily signals. We found that conditioned responding was facilitated in this latter group of participants. These results are in line with the hypothesis that a rise interoceptive attention enhances unaware conditioned responding.

Keywords: fear conditioning; contingency awareness; unconscious learning; interoceptive awareness; attention; spatial cueing

1.1 Introduction

Fear conditioning refers to the repeated pairing of a neutral stimulus with a threatening stimulus (unconditioned stimulus; US) as a result of which the neutral stimulus (conditioned stimulus; CS) starts evoking an anticipatory (fear) response to the US (conditioned response; CR). Fear conditioning research is a key source of information about the pathogenesis of anxiety, as it provides us with a good model of what might happen in real life. An important question in fear conditioning literature is whether conditioned responding can be installed in the absence of awareness of the CS-US contingency (Lovibond & Shanks, 2002; Purkis & Lipp, 2001). A search of the literature shows that the evidence for unaware conditioning is inconsistent. In the course of the last decade, some interesting methods have been used to study conditioning with restricted contingency awareness, for instance through manipulating the discriminability of the CS (Knight, Nguyen, & Bandettini, 2003; Schultz & Helmstetter, 2010), or through the study of conditioning in (sleeping) infants (Fifer et al., 2010) or in patients with brain lesions (Bechara et al., 1995). In addition, a large number of experiments have found indications for unaware conditioning using more conventional methods such as subliminal conditioning (Ohman & Soares, 1998; Raes, Koster, Van Damme, Fias, & De Raedt, 2010) or conditioning with a distracter task (Smith, Clark, Manns, & Squire, 2005; Tabbert et al., 2010; Tabbert, Stark, Kirsch, & Vaitl, 2006; Weike, Schupp, & Hamm, 2007). Still, several other studies have failed to find conditioning effects in the absence of awareness (Dawson, Rissling, Schell, & Wilcox, 2007; Purkis & Lipp, 2001).

One explanation for inconsistent findings in the field of unaware conditioning, is that the possibility to detect unaware conditioned responding depends on an interplay of several methodological requirements. A first requirement relates to the use of a sensitive outcome measure. Apparently, some measures fail to pick up unaware conditioning effects that are successfully indexed with other measures (Klucken et al., 2009). A second requirement concerns the conditioning method. For example, several authors have discussed the potential impact of using fear-relevant CSs in subliminal conditioning (Ohman & Soares, 1998) and of the use of trace versus delay conditioning in autonomous (Knight, Nguyen, & Bandettini, 2006) or eyeblink conditioning (Clark & Squire, 1998). A third requirement might pertain to attentional processes. In the vast literature on implicit learning, an increasing number of studies indicates that the learning of predictive relations, in which responding to a first stimulus is influenced through its relationship with a second stimulus, depends on top-down attention rather than on awareness (Junge, Scholl, & Chun, 2007; Van Boxtel, Tsuchiya, & Koch, 2010). For instance, in two experiments of Eitam, Schul and Hassin (2009), an implicit learning task was preceded by a unrelated task in which an achievement goal was primed. Interestingly, mere participation in the goal-inducing task influenced participants' performance on the implicit learning task. Similarly, Custers and Aarts (2010) showed that unconscious learning of predictive relations in a subliminal priming task depended on the task-set that was implicitly imposed in a preceding task. Importantly, in both studies, participants were unaware of the goal of the first task and of the relationship between both tasks. These results indicate that participants' attentional set can be tuned or prepared to process a specific type of stimuli or relations between stimuli.

A similar mechanism might apply to unaware fear conditioning. If participants are encouraged to attend to a class of stimuli or signals relevant to conditioning in a first task, this might facilitate the development of unaware conditioned responding in a subsequent conditioning task. The crucial question then is which stimuli should be attended to in order to increase unaware conditioned responding. In contrast with implicit learning, unaware fear conditioning includes emotionally relevant stimuli (USs), some of which (e.g., electric stimuli, loud noise) have a direct impact on our body. After repeated CS-US pairings, the CS gets associated not only with an aversive external stimulus (US), but also with an internal state of arousal (referred to as the unconditioned response, UR) (Bliss-Moreau & Barrett, 2009). The CS-UR side of the conditioning procedure has received relatively little attention. Still, this association might be particularly relevant in the context of unaware conditioning, in which affective learning is assumed to be the principle underlying mechanism as expectancy-based propositional processes are excluded.

Therefore, one hypothesis is that unaware conditioning effects can be increased through enhancing attention to the CS-UR relationship. This can be achieved by manipulating the self-perception of visceral signals (interoception; e.g., Craig, 2002). Our brain preferentially processes information that impacts on our affective state (Vuilleumier & Driver, 2007). Because an increase in interoception includes increased attention to aversive internal states, such as those induced by the US (i.e., the UR), it is likely that it will also enhance the processing of the stimuli that are associated with this negative internal events (i.e., the CS). Previous studies have shown that individuals who are sensitive to their own bodily signals show advantages in the processing and displaying of emotions in general (Pollatos, Traut-Mattausch, Schroeder, & Schandry, 2007; Wiens, Mezzacappa, & Katkin, 2000). More specifically, however, it has been demonstrated that a higher sensitivity to experiencing affect, which is associated with interoception (Barrett, Quigley, Bliss-Moreau, & Aronson, 2004), enhances conditioning effects under conditions of unrestricted awareness (Bliss-Moreau, Barrett, & Wright, 2008). It is our suggestion, however, that this mechanism might be even more crucial in the context of unaware fear conditioning. Therefore, we would expect that encouraging participants to attend to their own bodily signals will enhance conditioned responding.

1.1.1 The present research

In the present study, we examined whether encouraging participants to process their own visceral signals leads to enhanced conditioning effects in the context of unaware fear conditioning. To measure conditioning effects in the absence of contingency awareness, we used a differential conditioning paradigm that was embedded in a spatial cueing task (Posner, 1980). In this task, participants respond to peripheral targets that are preceded by peripheral cues, which predict target location correctly on most of the trials (valid trials). On the remaining trials, the cues appear on the other side of the screen (invalid trials). Response times on invalid trials are generally slower than response times on valid trials. This is referred to as the cue validity effect.

In our emotional modification of this task, two cues are used, one of which, the CS+, is repeatedly paired with a threatening US. The other cue, the CS-, is never paired with the US. This version of the spatial cueing task has been used repeatedly to investigate attentional processing of threat (Koster, Crombez, Van Damme, Verschuere, & De Houwer, 2004; Koster, Crombez, Verschuere, Vanvolsem, & De Houwer, 2007). Most previous studies using this version of the spatial cueing task found a larger cue validity effect for the CS+ than for the CS- during acquisition (i.e., the phase in which the CS+ is paired with the US), indicating enhanced attentional processing by the CS+ (Koster et al., 2004; Koster, Crombez, Van Damme, Verschuere, & De Houwer, 2005; Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006). In the current experiment, the CSs were presented subliminally and masked to prevent visual discrimination between the cues. A former study (Raes et al., 2010) indicated that this method is suitable to prevent contingency awareness and that differential conditioning effects can be attained with this task. It can be assumed that a subliminal conditioning effect is more difficult to establish than a regular (supraliminal) conditioning effect, because the former is solely based on trial-by-trial learning and not on expectancy learning. For that reason, subliminal conditioned responding might develop more slowly than regular conditioned responding. Therefore, we decided to perform a time course analysis of the acquisition phase, with separate analyses for the first and second half of acquisition (104 trials each).

The spatial cueing task was followed by an assessment of awareness, which served to exclude the possibility that participants had been contingency aware. To this aim, contingency awareness was explicitly questioned. We also used a forced-choice task that assessed perceptual awareness of the CSs.

To manipulate interoceptive attention, we used a heartbeat detection task (HDT; Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004; Domschke, Stevens, Pfleiderer, & Gerlach, 2010; Katkin, Wiens, & Ohman, 2001). In this task, participants judge whether a tone is presented either together with or delayed from their own heartbeat. Although most participants perform at chance level in this task (Domschke et al., 2010), engaging in heartbeat perception encourages them to attend to their own visceral signals. Neurobiological data show that HDT participation is associated with enhanced activity in the insula (Critchley et al., 2004), a region that is crucial in interoceptive awareness (Craig, 2002; Khalsa, Rudrauf, Feinstein, & Tranel, 2009). In our study, half of the participants performed the HDT before the conditioning task, whereas the others completed the tasks in the reverse order. We compared the two groups with regard to their performance on the HDT to exclude between-group differences in trait interoceptive awareness.

Our main hypothesis was that participants who had participated in the HDT before conditioning would show more pronounced unaware conditioning effects than the other participants. In line with previous studies that used a spatial cueing task in a conditioning experiment (Koster et al., 2004; Van Damme et al., 2006), successful conditioning was defined as a larger cue validity effect for the CS+ than for the CS-. Therefore, we specifically expected an enhanced CS+/CS- cue validity discrimination in participants that had been encouraged to engage in visceral self-perception relative to the control participants.

1.2 Method

1.2.1 Participants

Thirty-five undergraduate students took part in this study in exchange for course credits or financial compensation. One participant was excluded from further analysis because his accuracy level on the spatial cueing task (49 erroneous responses) deviated more than 3 SD from the group mean of 10.06 (*SD* = 9.51). Mean age of the remaining sample was 20.76 (*SD* = 2.94). The sample was predominantly female (79.4%). All participants gave their written informed consent.

1.2.2 Material

1.2.2.1 Spatial cueing task.

1.2.2.1.1 Apparatus and Stimuli.

The spatial cueing task was programmed in e-prime (Psychology Software Tools, Inc., 2001). The experiment was run on a Fujitsu Siemens Amilo Pro V3505 laptop coupled with a Philips 107P4 CRT 17-inch screen with a resolution of 1024 x 768 pixels. Screen refresh rate was 60 Hz. Before the experiment was performed, timing accuracy was checked by technical staff. An optocoupler circuit was connected to the CRT screen by glass fibre. This circuit was connected to a digital oscilloscope on which the number of refresh rates of each stimulus presentation could be assessed.

Three 261 x 261 pixels pictures of male faces from the Karolinska Directed Emotional Faces (KDEF; Lundqvist, Flykt, & Ohman, 1998) were used as CSs and masking face. Two angry faces were chosen as CSs. A neutral face served as masking stimulus. The allocation of the faces to the function of CS+ and CS- was counterbalanced across subjects. An additional male angry face (also from the KDEF) was used during the perceptual awareness task. The US consisted of a 170 ms 98 dB(A) white noise. This stimulus was presented binaurally via a Sony MDR-XD100 head phone. A white square of 1 x 1 cm served as target stimulus.

Responses to the targets were collected with a Cedrus RB-730 response box (Cedrus Corporation, San Pedro, CA).

***1.2.2.1.2 Trial description*.**

Figure 1 gives an overview of trial course within the spatial cueing task. Each trial started with a black screen presentation. After a variable interval (200 ms – 1200 ms), a white fixation cross was presented for 500 ms in the middle of the screen, accompanied by two white rectangles, right and left of fixation. The peripheral presentations subtended 9 degrees of the visual field.

"(Figure 1 about here)"

Subsequently, one of the CSs (50% CS+ trials, 50% CS- trials) replaced the left or the right rectangle during one refresh rate (16.7 ms). The CS+ and the CS- were presented equally often on either side of the screen. The CSs were then replaced by the neutral masking face, which was displayed at the same location for 183 ms (see Figure 1). This neutral face was followed by a shortly presented black screen (16.7 ms). Then the target appeared for a duration of 300 ms. Allocation of target position was dependent on allocation of CS position: in 75% of the trials, CS and target appeared on the same side of the screen (valid trials), while in 25% of the trials, CS and target were presented on opposite sides of the screen (invalid trials). Participants responded to the target with both index fingers, pressing the left key of the response box in response to left targets and the right key in response to right targets. On reinforced trials (see below) the US was presented 600 ms after target onset. Participants' responses that exceeded 600 ms were excluded as outliers (0.75% of all trials). Allocation of trial type (CS+/CS-; right/left cue position; valid/invalid; reinforced/non-reinforced) was randomised across all phases of the spatial cueing task.

***1.2.2.1.3 Description of spatial cueing task*.**

The spatial cueing task started with a short practice phase of 16 trials. Participants were instructed about trial course at the beginning of this phase. Within the practice phase, participants had to attain an accuracy rate of 85% to be able to proceed. Otherwise, the practice phase had to be repeated. The data from this phase were not analysed.

The practice phase was followed by a baseline phase, which consisted of 112 trials: 52 CS+ trials (13 invalid and 39 valid), 52 CS- trials (13 invalid and 39 valid), four catch and four digit trials. Catch trials, on which no target was presented, were used to prevent automatic response tendencies. Digit trials were included to keep participants' attention allocated to the middle of the screen. These trials started with a centrally presented digit that appeared for 200 ms. Participants were instructed to respond to this digit as fast as possible by pressing a central response box key.

After the baseline phase, participants were informed that, from now on, a loud white noise could follow their responses but that they had to continue the task as before. The acquisition phase consisted of 238 trials. At first, 6 buffer trials were presented, including four reinforced CS+ trials and two CS- trials. Thereafter, participants were presented with two blocks of 116 trials, with each block containing six catch trials and six digit trials. Furthermore, each block consisted of 104 regular trials, including 52 CS+ (13 invalid, 39 valid) and 52 CS- trials (13 invalid, 39 valid) trials. There was a 2:1 reinforcement rate during both blocks, which means that half of the CS+'s was followed by a US.

1.2.2.2 Awareness measurement.

1.2.2.2.1 Contingency awareness.

Immediately after the spatial cueing task, participants were asked (1) if they had noticed anything particular about the experiment; (2) if they had seen anything particular about the neutral face; (3) if they had noticed any pictures preceding the neutral face. These questions appeared on the computer screen and participants had to answer 'yes' or 'no' by pressing one out of two response box keys. Participants who answered ‘yes’ on the last question, were asked to elaborate further on their answer by describing in detail what they had seen to the experimenter.

1.2.2.2.2 Perceptual awareness.

The perceptual awareness measurement involved a 60-trial forced-choice task that was similar in appearance to the SCT, apart from the fact that no targets were presented. Participants were instructed that this task consisted of trials involving either only the masking face, one of the (masked) CS faces or another (masked) angry face. They were told that their task would be to discriminate between these three trial types.

On every trial, participants were presented with a blank screen, followed by a fixation cross and a cue. In 15 out of the 60 trials, this cue was a single presentation of the neutral masking face (mask-only trials). On the remaining 45 trials, the cue consisted of a 16.7 ms angry face that was masked by the neutral masking face (masked face trials). Fifteen of these 45 trials involved the presentation of a masked angry face that had not been used in the spatial cueing task. The remaining 30 trials were masked presentations of the CS+ (15) and the CS- (15).

After each trial, participants were asked (1) whether they had noticed a face preceding the neutral masking face or not. If they answered yes to this question, they were asked (2) whether the face they had seen was a face that had been presented during the spatial cueing task or whether it was a new face. If they chose the first response option, they were asked (3) whether they had seen the CS+ or the CS- face. Cardboard pictures of the CS faces were handed over to the participants and were positioned left and right of the computer screen. Participants indicated which of the faces they had seen by pressing one of two keyboard buttons.

The data for the first question (mask-only or masked face trials) were analysed using mean accuracy rates (above chance performance if more than 36 answers are correct) and signal detection measures (*d'*). Correct identifications of masked face trials were regarded as hits, while incorrect responses to mask-only trials were taken as false alarms. By subtracting the *z*-score that corresponds to the false-alarm rate from the *z*-score that corresponds to the hit rate, *d'* was calculated, with larger positive values indicating greater sensitivity.

The data for the second question (CS face or a new angry face) could be analysed for 18 participants only. For the other participants there was no sufficient amount of correctly identified masked face trials (< 10) to analyse these data. For the third question (CS+ or CS- face), we only had data available for 7 participants (i.e., only those participants who had enough correct identifications of the CS faces). These data were analysed using accuracy rates.

1.2.2.3 US ratings.

Participants completed US valence and painfulness ratings using paper and pencil. The anchored ratings scales ranged from *0* (not aversive at all; not painful at all) to *10* (very aversive; very painful).

1.2.2.4 Heartbeat detection task.

1.2.2.4.1 Apparatus and stimuli.

For the electrocardiogram (ECG), three Ag/AgCL electrodes were positioned on the left and right lower rib cage and on the right clavicle. A Lablinc V Coulbourn served to record heart rate. We used a Labmaster PRO card (Scientific Solutions) and customized software (A. De Clercq) to detect R-waves from the ECG and to generate 100 ms tones of 800 Hz in the HDT. Instructions for the task appeared on a PC that used Inquisit software. Responses were made using two keys of an AZERTY keyboard ("A"/"P").

***1.2.2.4.2 Task description*.**

We used a sample ECG to explain the heartbeat detection task to the participants. On this ECG, blue lines represented the presentation of the tones at either 100 ms or 500 ms after the actual R wave. Each participant was presented with several trials and the experimenter made sure that the participants understood the procedure and the goal of the task. After these instructions, participants were seated in front of a computer screen and electrodes were attached. The HDT consisted of 56 trials, including a 6-trial practice phase. This phase was included to ensure that participants understood the procedure well. The data of this phase were not analysed. After the practice phase, there were 5 blocks of 10 trials. Participants could take a break in between each pair of blocks.

Each trial consisted of 10 tones which were presented either 100 ms (no-delay trials) or 500 ms (delay trials) from the participant's actual R-waves. At the end of each trial, a written instruction appeared on the computer screen, asking participants to indicate with a button press (keyboard) whether the tones had been presented with a delay or not

According to the binomial distribution, participants scoring 32 or more answers correctly perform significantly above chance level (α = .05). These participants were considered good heartbeat detectors. Only six (17.1%) of our 34 subjects were good heartbeat detectors following this criterion.

1.2.3 Procedure

Upon arrival, participants were informed that they would participate in two unrelated experiments. After giving their informed consent, participants were asked to complete the state version of the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Then, half of the participants started with the conditioning task (spatial cueing task), immediately followed by the awareness measurement and US ratings. After this, they completed the HDT. The other participants completed the HDT first, followed by the spatial cueing task, awareness measurement and US ratings. The tasks were performed in adjacent rooms.

1.3 Results

1.3.1 Participant characteristics

The order of the conditioning task and the HDT was counterbalanced. Sixteen participants (one male) started with the HDT (HDT group) while 18 participants (six male) started with the conditioning task (control group). The amount of good heartbeat detectors did not differ significantly between the groups (n = 4 versus n = 2), Fisher's Exact Test, p = .39. Also, the total amount of correct responses on the HDT was similar across both groups (M = 28.31, SD = 5.84; and M = 27.44, SD = 5.64), $t < 1$. Mean STAI state score was 34.50 (SD = 5.99). STAI state scores did not differ significantly between the group that first participated in the HDT (M = 35.81, SD = 6.28) and the control group (M = 33.33, SD = 6.28), $t(32) = 1.21$, *ns*.

1.3.2 Response times on the spatial cueing task

1.3.2.1 Data preparation.

Practice and buffer trials were not included in the response time analyses and all error trials (2.75%) were omitted. Outliers were calculated for each trial type (valid/invalid and CS+/CS-) within each subject. Response times that deviated more than 3 SD from the mean were excluded.

"(Figure 2 about here)"

1.3.2.2 Baseline.

We performed a 2 (CS: CS+/CS-) x 2 (Validity: valid/invalid) x 2 (Group: HDT group/control group) mixed ANOVA on the baseline data. CS and validity were included as within-subjects factors and group served as a between-subjects factor. This analysis yielded a significant main effect of validity, $F(1, 32) = 100.41$, $p < .001$, partial $\eta^2 = .76$, with faster response times on valid trials (*M* = 266.92, *SD* = 26.60) than on invalid trials (*M* = 306.37, *SD* = 24.74) (see Figure 2). No other main or interaction effects reached significance, *F*'s < 1.20.

1.3.2.3 First block of acquisition.

The 2 (CS) x 2 (Validity) x 2 (Group) analysis yielded a significant main effect of validity, $F(1, 32) = 88.86$, $p < .001$, partial $\eta^2 = .74$. As shown in Figure 2, participants responded faster on valid trials (*M* = 254.06, *SD* = 25.13) than on invalid trials (*M* = 292.17, *SD* = 29.53). The CS x Group interaction effect also reached significance, $F(1, 32) = 4.41$, $p < .05$, partial $\eta^2 = .12$. Follow-up of this interaction by analysing the effect of CS in each group separately, however, showed that these differences were not significant, *t*'s < 1.63, *ns*. No other main or interaction effects reached significance, *F*'s < 1.95.

1.3.2.4 Second block of acquisition.

The same 2 (CS) x 2 (Validity) x 2 (Group) analysis was performed. The main effect of validity was replicated within this block, $F(1, 32) = 97.83$, $p < .001$, partial $\eta^2 = .75$, with faster responding on valid trials (*M* = 252.61, *SD* = 22.70) than on invalid trials (*M* = 296.63, *SD* = 26.18). The CS x Group interaction effect was marginally significant, $F(1, 32) = 2.87$, $p = .10$. Most importantly, the three-way interaction of CS, Validity and Group reached a significant value, $F(1, 32) = 8.17$, $p < .01$, partial $\eta^2 = .20$, indicating a between-group difference in the cue validity effect for the CS+ relative to the CS-. We followed up on this interaction by performing CS x Validity ANOVA's per group. In the HDT group, the two-way interaction reached significance, $F(1, 15) = 5.45$, $p < .05$, partial $\eta^2 = .27$. As Figure 2 suggests, there was no significant difference in response times between valid CS+ (*M* = 250.55, *SD* = 23.41) and valid CS- trials (*M* = 252.91, *SD* = 25.67), $t < 1$, but response times on invalid CS+ trials (*M* = 304.91, *SD* = 27.79) were slower than those on invalid CS- trials (*M* = 294.52, *SD* = 28.14), $t(15) = 2.11$, $p = .05$. In the control group, the interaction did not reach significance, $F(1, 17) = 2.97$, *ns*. No other main or interaction effects reached significance, *F*'s < 1[1].

1.3.3 Awareness data

1.3.3.1 Contingency awareness.

None of the participants reported having seen other faces preceding the neutral (mask) face during the spatial cueing task, nor was any of the participants able to verbalise the contingencies.

1.3.3.2 Perceptual awareness.

Participants were well able to correctly identify mask-only trials (90% correct answers). On the other hand, most masked angry faces were incorrectly identified as mask-only trials too (28% correct answers). Across all 60 trials of the perceptual awareness task, mean signal detection for the first question (masked-only verses masked faces trials) ($d' = 0.61$, $SD = .86$) was significantly different from zero, $t(33) = 4.10$, $p < .001$. This indicates that at least some participants were able to discriminate mask-only trials from masked angry face trials. Indeed, seven participants (20.6%) performed above chance level. Furthermore, two out of these seven participants succeeded in discriminating the angry faces that had been used in the task (CS+ and CS-) from another angry face above chance level (64% and 67% correct answers respectively) (second question). However, neither of them was able to discriminate between the CS+ and the CS- face at an above-chance level (third question).

"(Figure 3 about here)"

For the participants that had first participated in the HDT, a regression analysis was performed to exclude that the significant conditioning effects that were detected in the spatial cueing task were driven by differences in perceptual awareness (cf. Greenwald, Klinger, & Schuh, 1995). The detection sensitivity measure (*d'*) was regressed on the main conditioning effect (difference in cue validity between the CS+ and the CS-). This regression yielded a statistically significant intercept, *a* = 15.30 (*SE*= 6.93), $t(15)=2.21$, $p < .05$ (see Figure 3). This indicates that differential responding to the CS+ and CS- during the spatial cueing task was reliable, in the sense that this effect was also present in complete absence of awareness (*d'* = 0).

1.3.4 US ratings

The participants considered the US unpleasant (*M* = 7.94, *SD* = 1.18) but not as particularly painful (*M* = 4.24, *SD* = 2.79). Between-samples *t*-tests were used to examine the effect of group on US valence and pain ratings. No group differences were detected, *t*'s < 1.16.

1.4 Discussion

Although a lot of evidence suggests that fear conditioning can be achieved in the absence of contingency awareness (e.g., Knight et al., 2003; Raes et al., 2010; Smith et al., 2005; Tabbert et al., 2010) or in dissociation with conscious expectancies (Clark, Manns, & Squire, 2001; Perruchet, 1985; Perruchet, Cleeremans, & Destrebecqz, 2006), there is still ample debate on this issue (Lipp & Purkis, 2005; Mitchell, De Houwer, & Lovibond, 2009).

It is our suggestion that attentional processes might be crucially implicated in unaware fear conditioning. More specifically, we surmise that tuning participants' top-down attentional set to processing the relevant stimulus relations might increase the likelihood of unaware conditioning effects. In unaware fear conditioning, conditioning effects are largely established through affective learning. This means conditioning develops in a context of aversive external and internal sensations, rather than through a change in conscious expectancy of the US. Our hypothesis is that increasing participants' attention to their own autonomous signals might enhance conditioning effects through intensifying the experience of the aversive sensations that are paired with the US (UR). Previous studies have already demonstrated that interoceptive awareness is associated with the intensity of emotional experience (Barrett et al., 2004; Wiens et al., 2000). Moreover, (very) early studies of Mowrer (1939) already indicated that the power of the UR affects the strength of the conditioned response.

Overall, our findings support the notion that visceral self-perception is associated with enhanced conditioning effects in the context of subliminal conditioning. In half the participants, interoceptive awareness was stimulated before conditioning through participation in a HDT, whereas the other participants started with the conditioning task, which was embedded in an emotionally modified spatial cueing paradigm (cf. Raes et al., 2010). Only the first group of participants showed conditioned modulation of spatial cueing. More specifically, these participants showed an enhanced cue validity effect for the CS+ compared with the CS- during the second block of acquisition. This result is in line with previous studies that used a spatial cueing task to index conditioning and indicates enhanced attentional processing of the CS+ (Koster et al., 2004, 2005; Van Damme et al., 2006). Nonetheless, we detected conditioned responding on invalid trials only, whereas previous studies also found effects on valid trials (Koster et al., 2004, 2005; Van Damme et al., 2006). Still, in several other studies using an emotional version of the spatial cueing task, the effects were restricted to invalid trials, which is indicative of difficulties to disengage from threat (e.g., Cisler & Koster, 2010; Fox, Russo, Bowles, & Dutton, 2001; Yiend & Mathews, 2001).

To exclude the possibility that the detected conditioning effects were due to perceptual awareness of the CSs, the perceptual sensitivity measure (*d’*) was regressed on the conditioning effect. This analysis showed that, even at the point where perceptual awareness was absent, the conditioning effect remained significant. Therefore, the present results convincingly demonstrate unaware conditioning effects in a group of participants in whom interoceptive awareness was stimulated. These results are in line with findings from the implicit learning literature. Several studies have demonstrated that the inclusion of a seemingly unrelated task before the crucial task can greatly influence the learning and/or storing of associations afterwards, through unaware goal induction (Eitam et al., 2009) or preparing top-down attention to process a specific kind of stimulus relations (Custers & Aarts, 2010). The present results are also in line with a study of Critchley, Mathias and Dolan (2002). They demonstrated that patients with peripheral autonomic denervation (i.e., reduced perceptual awareness) showed less activity in both the insula and the amygdala during fear conditioning, which indicates that the experience of autonomic arousal is crucial for the development of conditioned fear. This is consistent with our finding of an association between enhanced attention to interoceptive sensations and increased conditioned responding.

Still, it is challenging to render a straightforward interpretation of the present results. Although we assume that HDT participation produces enhanced conditioning effects through intensifying the aversive interoceptive experience of the CS, alternative explanations are possible. That is, during the HDT, participants are encouraged to pay close attention to signals that usually stay undetected (i.e., their own heartbeat). It is possible that this effect generalises to other tasks and stimuli and that, therefore, the underlying mechanism consists of a general increase in attention, rather than a specific increase in interoceptive attention. Another possibility is that HDT participation increased the visibility of the CSs through installing the (unconscious) goal to attend to stimuli that are difficult to detect. As indicated earlier, Eitam et al. (2009) showed that unconscious goals that are installed in one task can transfer to and influence performance on unrelated tasks. However, given the results on our awareness measurement, it is unlikely that this explanation can account for the current results.

A further point of discussion relates to the use of the term ‘interoceptive awareness’. It is important to delineate that in the present study, we experimentally manipulated the level to which participants focus *attention* on their own interoceptive functioning, rather than studying the effect of trait interoceptive awareness. However, we believe that investigating whether individual differences in trait interoceptive awareness influence conditioning effects could be a fruitful avenue for future research.

It is noteworthy that conditioning effects were significant only in the second block of acquisition. It can be assumed that unaware conditioning effects are small and more difficult to accomplish than supraliminal conditioning effects (Raes et al., 2010) because they rely solely on trial-by-trial affective learning. By contrast, in regular conditioning experiments, conditioned responding can develop very quickly via expectancy learning, induced even through mere instructions or through observational learning (e.g., Olsson & Phelps, 2004).

Our original hypothesis was that conditioning effects would be more pronounced in the participants that performed the HDT before conditioning than in control participants. Therefore, the lack of any significant conditioning effects in the control group was not expected. However, we already mentioned that unaware conditioning effects are generally small and difficult to detect. More work is needed to mark out which set of variables determines whether or not unaware conditioning effects can develop and can be assessed.

A possible option would be to use a different approach to study affective learning. Instead of selectively suppressing conscious awareness, it is also possible to use paradigms in which affective learning can be dissociated from cognitive expectancy learning (e.g., Perruchet, 1985). These paradigms seem to yield consistent results (e.g., Clark et al., 2001; Perruchet et al., 2006), showing that affective learning can occur separately from cognitive learning (Weidemann, Tangen, Lovibond, & Mitchell, 2009). Future studies should certainly consider to use these paradigms to further investigate the mechanisms that drive affective learning.

1.5 Conclusion

In sum, in the present study, encouraging participants to attend to their bodily signals increased their level of subsequent conditioned responding, which suggests that attention to interoception is related to unaware conditioning. Future studies should further investigate the underlying mechanisms of this association and should examine whether differences in trait interoceptive awareness can also affect conditioning. As interoceptive awareness is crucially related to anxiety (Domschke et al., 2010), this might be one pathway to study vulnerability to developing fear and anxiety.

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References

Barrett, L. F., Quigley, K. S., Bliss-Moreau, E., & Aronson, K. R. (2004). Interoceptive sensitivity and self-reports of emotional experience. Journal of personality and social psychology, 87(5), 684-697.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double Dissociation of Conditioning and Declarative Knowledge Relative to the Amygdala and Hippocampus in Humans. Science, 269(5227), 1115-1118.

Bliss-Moreau, E., & Barrett, L. F. (2009). What's reason got to do with it? Affect as the foundation of learning. Behavioral and Brain Sciences, 32(2), 201-+.

Bliss-Moreau, E., Barrett, L. F., & Wright, C. I. (2008). Individual differences in learning the affective value of others under minimal conditions. Emotion, 8(4), 479-493.

Cisler, J. M., & Koster, E. H. W. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. Clinical Psychology Review, 30(2), 203-216.

Clark, R. E., Manns, J. R., & Squire, L. R. (2001). Trace and delay eyeblink conditioning: Contrasting phenomena of declarative and nondeclarative memory. Psychological Science, 12(4), 304-308.

Clark, R. E., & Squire, L. R. (1998). Classical conditioning and brain systems: The role of awareness. Science, 280(5360), 77-81.

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. Nature reviews. Neuroscience, 3(8), 655-666.

Critchley, H. D., Mathias, C. J.., & Dolan, R. J. (2002). Fear conditioning in humans: The influence of awareness and autonomic arousal on functional neuroanatomy. Neuron, 33(4), 653-663.

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. Nature Neuroscience, 7(2), 189-195.

Custers, R., & Aarts, H. (2010). Learning of predictive relations between events depends on attention, not on awareness. Consciousness and Cognition.

Dawson, M. E., Rissling, A. J., Schell, A. M., & Wilcox, R. (2007). Under what conditions can human affective conditioning occur without contingency awareness? Test of the evaluative conditioning paradigm. Emotion, 7(4), 755-766.

Domschke, K., Stevens, S., Pfleiderer, B., & Gerlach, A. L. (2010). Interoceptive sensitivity in anxiety and anxiety disorders: An overview and integration of neurobiological findings. Clinical Psychology Review, 30(1), 1-11.

Eitam, B., Schul, Y., & Hassin, R. R. (2009). Goal relevance and artificial grammar learning. Quarterly journal of experimental psychology, 62(2), 228-238.

Fifer, W. P., Byrd, D. L., Kaku, M., Eigsti, I. M., Isler, J. R., Grose-Fifer, J., et al. (2010). Newborn infants learn during sleep. Proceedings of the National Academy of Sciences of the United States of America, 107(22), 10320-10323.

Fox, E., Russo, R., Bowles, R., & Dutton, K. (2001). Do threatening stimuli draw or hold visual attention in subclinical anxiety? Journal of Experimental Psychology-General, 130(4), 681-700.

Greenwald, A. G., Klinger, M. R., & Schuh, E. S. (1995). Activation by marginally perceptible ("subliminal") stimuli: dissociation of unconscious from conscious cognition. Journal of experimental psychology. General, 124(1), 22-42.

Junge, J. A., Scholl, B. J., & Chun, M. M. (2007). How is spatial context learning integrated over signal versus noise? A primacy effect in contextual cueing. Visual Cognition, 15(1), 1-11.

Katkin, E. S., Wiens, S., & Ohman, A. (2001). Nonconscious fear conditioning, visceral perception, and the development of gut feelings. Psychological science : a journal of the American Psychological Society / APS, 12(5), 366-370.

Khalsa, S. S., Rudrauf, D., Feinstein, J. S., & Tranel, D. (2009). The pathways of interoceptive awareness. Nature Neuroscience, 12(12), 1494-1496.

Klucken, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., & Stark, R. (2009). Neural, Electrodermal and Behavioral Response Patterns in Contingency Aware and Unaware Subjects during a Picture-Picture Conditioning Paradigm. Neuroscience, 158(2), 721-731.

Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2003). Expression of conditional fear with and without awareness. Proceedings of the National Academy of Sciences of the United States of America, 100(25), 15280-15283.

Knight, D. C., Nguyen, H. T., & Bandettini, P. A. (2006). The role of awareness in delay and trace fear conditioning in humans. Cognitive Affective & Behavioral Neuroscience, 6(2), 157-162.

Koster, E. H. W., Crombez, G., Van Damme, S., Verschuere, B., & De Houwer, J. (2004). Does imminent threat capture and hold attention? Emotion, 4(3), 312-317.

Koster, E. H. W., Crombez, G., Van Damme, S., Verschuere, B., & De Houwer, J. (2005). Signals for threat modulate attentional capture and holding: Fear-conditioning and extinction during the exogenous cueing task. Cognition & Emotion, 19(5), 771-780.

Koster, E. H. W., Crombez, G., Verschuere, B., Vanvolsem, P., & De Houwer, J. (2007). A time-course analysis of attentional cueing by threatening scenes. Experimental Psychology, 54(2), 161-171.

Lipp, O. V., & Purkis, H. M. (2005). No support for dual process accounts of human affective learning in simple Pavlovian conditioning. Cognition & Emotion, 19(2), 269-282.

Lovibond, P. F., & Shanks, D. R. (2002). The role of awareness in Pavlovian conditioning: empirical evidence and theoretical implications. Journal of experimental psychology. Animal behavior processes, 28(1), 3-26.

Lundqvist, D., Flykt, A., &Öhman, A. (1998). *The Karolinska Directed Emotional Faces (KDEF)*. Department of Neurosciences, Karolinska Hospital, Stockholm.

Mitchell, C. J., De Houwer, J., & Lovibond, P. F. (2009). The propositional nature of human associative learning. The Behavioral and brain sciences, 32(2), 183-198; discussion 198-246.

Mowrer, O. H. (1939). A stimulus-response analysis of anxiety and its role as a reinforcing agent. Psychological Review, 46, 553-565.

Ohman, A., & Soares, J. J. (1998). Emotional conditioning to masked stimuli: expectancies for aversive outcomes following nonrecognized fear-relevant stimuli. Journal of experimental psychology. General, 127(1), 69-82.

Olsson, A., & Phelps, E. A. (2004). Learned fear of "unseen" faces after Pavlovian, observational, and instructed fear. Psychological science : a journal of the American Psychological Society / APS, 15(12), 822-828.

Perruchet, P. (1985). A pitfall for the expectancy theory of human eyelid conditioning. The Pavlovian journal of biological science, 20(4), 163-170.

Perruchet, P., Cleeremans, A., & Destrebecqz, A. (2006). Dissociating the effects of automatic activation and explicit expectancy on reaction times in a simple associative learning task. Journal of experimental psychology. Learning, memory, and cognition, 32(5), 955-965.

Pollatos, O., Traut-Mattausch, E., Schroeder, H., & Schandry, R. (2007). Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. Journal of anxiety disorders, 21(7), 931-943.

Posner, M. I. (1980). Orienting of attention. The Quarterly journal of experimental psychology, 32(1), 3-25.

Purkis, H. M., & Lipp, O. V. (2001). Does affective learning exist in the absence of contingency awareness? Learning and Motivation, 32(1), 84-99.

Raes, A. K., Koster, E. H., Van Damme, S., Fias, W., & De Raedt, R. (2010). Aversive conditioning under conditions of restricted awareness: Effects on spatial cueing. Quarterly journal of experimental psychology, 1-23.

Schultz, D. H., & Helmstetter, F. J. (2010). Classical conditioning of autonomic fear responses is independent of contingency awareness. Journal of experimental psychology. Animal behavior processes, 36(4), 495-500.

Smith, C. N., Clark, R. E., Manns, J. R., & Squire, L. R. (2005). Acquisition of differential delay eyeblink classical conditioning is independent of awareness. Behavioral Neuroscience, 119(1), 78-86.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R. & Jacobs, G. A. (1983). *Manual for the State Trait Anxiety Inventory*. Palto Alto, CA: Consulting Psychologists Tests.

Tabbert, K., Merz, C. J., Klucken, T., Schweckendiek, J., Vaitl, D., Wolf, O. T., et al. (2010). Influence of contingency awareness on neural, electrodermal and evaluative responses during fear conditioning. Social Cognitive and Affective Neuroscience.

Tabbert, K., Stark, R., Kirsch, P., & Vaitl, D. (2006). Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. Neuroimage, 32(2), 761-770.

Van Boxtel, J. J., Tsuchiya, N., & Koch, C. (2010). Consciousness and Attention: On sufficiency and necessity. Frontiers in Psychology, 1:217, 1-13.

Van Damme, S., Crombez, G., Hermans, D., Koster, E. H., & Eccleston, C. (2006). The role of extinction and reinstatement in attentional bias to threat: a conditioning approach. Behaviour research and therapy, 44(11), 1555-1563.

Vuilleumier, P., & Driver, J. (2007). Modulation of visual processing by attention and emotion: windows on causal interactions between human brain regions. Philosophical Transactions of the Royal Society B-Biological Sciences, 362(1481), 837-855.

Weidemann, G., Tangen, J. M., Lovibond, P. F., & Mitchell, C. J. (2009). Is Perruchet's dissociation between eyeblink conditioned responding and outcome expectancy evidence for two learning systems? Journal of experimental psychology. Animal behavior processes, 35(2), 169-176.

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. Psychophysiology, 44(1), 170-180.

Wiens, S., Mezzacappa, E. S., & Katkin, E. S. (2000). Heartbeat detection and the experience of emotions. Cognition & Emotion, 14(3), 417-427.

Yiend, J., & Mathews, A. (2001). Anxiety and attention to threatening pictures. Quarterly Journal of Experimental Psychology Section a-Human Experimental Psychology, 54(3), 665-681.

Footnotes

[1] All analyses were also performed with HDT performance as a covariate. During baseline, there were no effects of this covariate. During the first block of acquisition, a marginally significant interaction between Validity and HDT performance emerged, $F(1, 30) = 3.31$, $p=.08$. This interaction effect reached significance during the second block of acquisition, $F(1, 30) = 9.28$, $p<.01$, partial $\eta^2 = .24$. Follow-up tests indicated a negative association between the cue validity effect (i.e., difference in response times between invalid and valid trials) and HDT performance. That is, the better the performance on the HDT, the smaller the validity effect.

Figure Captions

Figure 1. Overview of an invalid reinforced CS+ trial (above) and a valid CS- trial (below) in the modified version of the spatial cueing task.

Figure 2. Response times across phase (baseline/acquisition block 1 and 2), CS (CS+/CS-) and validity (valid/invalid) for participants that participated in the heartbeat detection task before conditioning (HDT) and the control group (CG)

Figure 3. Regression analysis in which conditioned spatial cueing effects (CVE of the CS- subtracted from the CVE of the CS+) are predicted from the detection sensitivity measure (*d'*). Each dot represents a participant.

Figure 1.

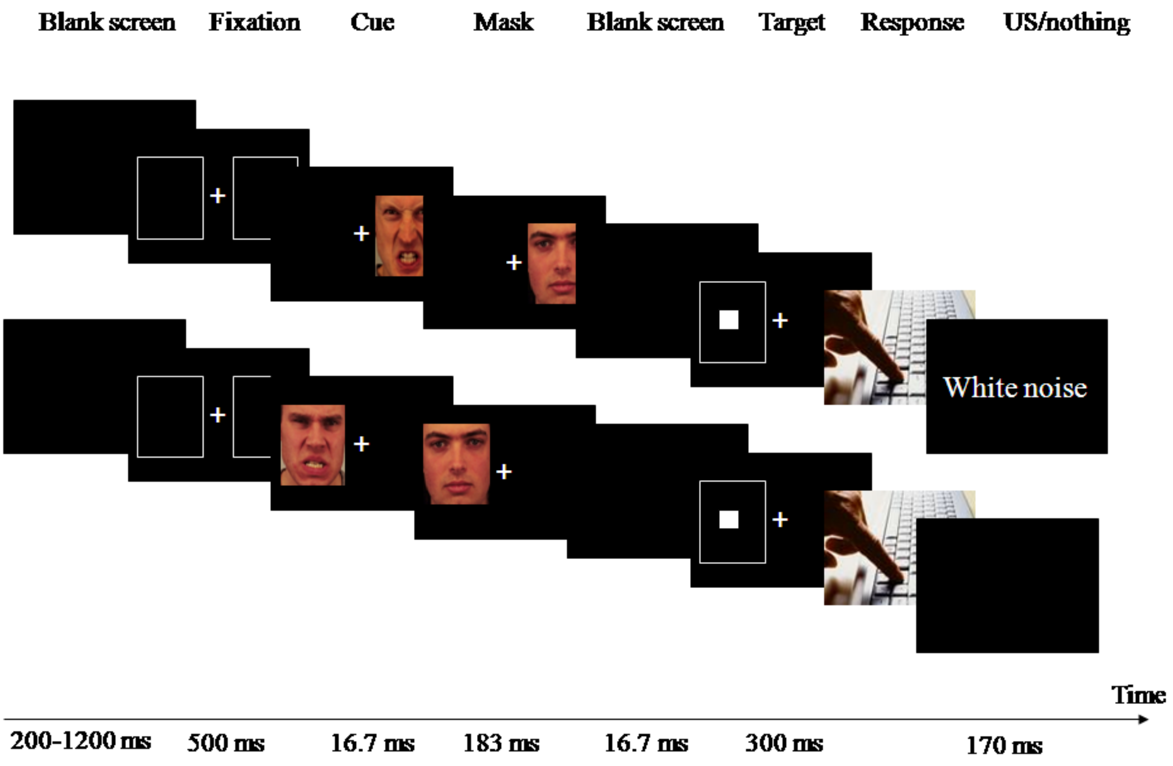
Blank screen
Fixation
Cue
Mask
Blank screen
Target
Response
US/nothing
White noise
Time
200-1200 ms
500 ms
16.7 ms
183 ms
16.7 ms
300 ms
170 ms

Figure 2.

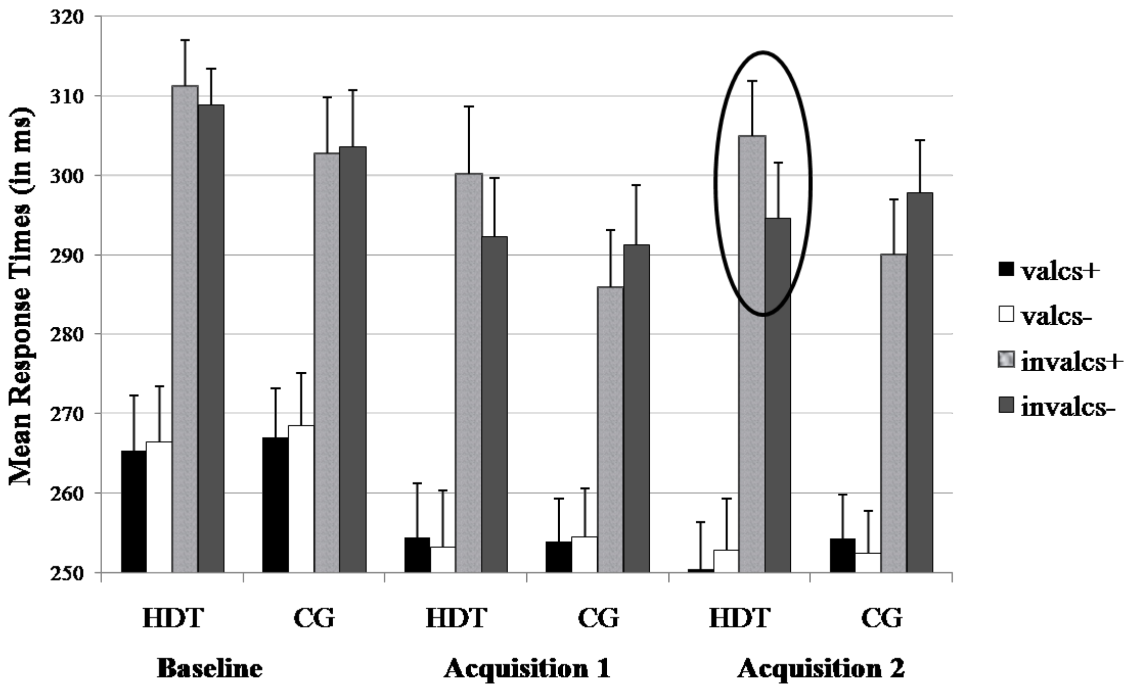
320
310
300
290
280
270
260
250
Mean Response Times (in ms)
HDT
CG
HDT
CG
HDT
CG
Baseline
Acquisition 1
Acquisition 2
valcs+
valcs-
invalcs+
invalcs-

Figure 3.