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Earlier studies provided preliminary support for the role of classical conditioning as a pathway of disgust learning, yet this evidence has been limited to self-report. This study included facial electromyographical (EMG) measurements (corrugator and levator muscles) and a behavioural approach task to assess participants' motivation-to-eat the actual food items (conditioned stimuli, CS). Food items served as CS and film excerpts of a woman vomiting served as unconditioned stimuli (US). Following acquisition the CS+ (neutral CS paired with US disgust) was rated as more disgusting and less positive. Notably, the conditioned response was transferred to the actual food items as evidenced by participants' reported lowered willingness-to-eat. Participants also showed heightened EMG activity in response to the CS+ which seemed driven by the corrugator indexing a global negative affect. These findings suggest that classical conditioning as a pathway of disgust learning can be reliably observed in subjective but not in disgust-specific physiological responding.

There is general agreement that various stimuli, such as rotten food, readily elicit a disgust response. These specific disgust-eliciting stimuli are typically potent in their ability to transmit infectious diseases, which is consistent with the widely accepted notion that disgust evolved with the central function of facilitating avoidance of contaminants (Curtis, de Barra, & Aunger, 2011; de Jong, 2013). This function of disgust has been described as a disease-avoidance mechanism (Oaten, Stevenson, & Case, 2009) highlighting the adaptive role played by this universal emotion. Despite this adaptive function, there is ample evidence implicating disgust in the aetiology and

maintenance of various psychopathologies (Borg, de Jong, & Weijmar Schultz, 2010).

In spite of this growing evidence of the involvement of disgust in various psychopathologies, there remains a paucity of research that has adequately examined how disgust is (un)learned (for review see de Jong, 2013). A better understanding of the mechanism of disgust learning may not only improve our theoretical understanding of the functional properties of disgust, but may also have important implications for the prevention and treatment of disgust-based disorders (Olatunji & McKay, 2009).

From a disease-avoidance framework, it would be adaptive if novel or initially neutral stimuli would rapidly acquire a disgust eliciting status when these stimuli are contingently present in time and space with stimuli that are somehow associated with the negative consequences of contamination (classical conditioning). Such a disgust conditioning pathway would be robust and evolutionarily adaptive, but may also confer risk for some disorders when rigid and inflexible (Olatunji, Forsyth, & Feldner, 2007).

The first study that tested a disgust conditioning pathway under controlled conditions used a classical conditioning procedure that was originally designed to investigate the influence of pairing a neutral picture with a negative or positive picture on the global affective appreciation of the originally neutral picture, an effect also known as evaluative conditioning (e.g., De Houwer, 2007). Participants were presented with 54 neutral pictorial conditional stimuli (CS; e.g., vest/jacket) that were either followed by a disgusting picture, a pleasant picture or a neutral picture as the unconditional stimulus (US; Schienle, Stark, & Vaitl, 2001). Although the disgusting USs reliably elicited disgust responses as indexed by self-reports and facial electromyographical (EMG) activity of the levator muscles, the conditioning procedure was neither effective in changing the global affective ratings, nor the more specific disgust ratings of the 18 CSs that were paired with the disgusting USs. However, one explanation for the absence of a conditioning effect might be that

there was no straightforward contingency between a particular CS and a particular US in line with this, only few participants were able to indicate post-experimentally the CS–US contingencies.

A subsequent experimental study used a similar but less complex classical conditioning paradigm comprising one CS that was never paired with a disgusting pictorial US (CS−) and one CS (neutral word) that was always paired with a disgusting US (CS+; Olatunji, Forsyth, & Cherian, 2007). The results showed that during acquisition, the CS+ elicited stronger subjective disgust and stronger physiological arousal than the CS−. Using similar differential conditioning procedures three subsequent studies successfully replicated the basic finding that contingent pairing of a neutral face (Engelhard, Leer, Lange, & Olatunji, 2014; Mason & Richardson, 2010) or a neutral word (Olatunji, Tomarken, & Puncochar, 2013) with a disgusting picture resulted in heightened subjective disgust ratings of the CS+.

Together these studies provided evidence supporting the view that disgust responses may be learned through a classical conditioning procedure. However, this research is not without important limitations that prevent strong inferences to be made. The assessment of the conditioned disgust response in these studies was largely restricted to self-report measures. Yet, there is increasing evidence pointing to the relevance of differentiating between automatic reflexive responses and deliberate reflective responses in the context of individual's attitudes towards particular stimuli (e.g., Gawronski & Bodenhausen, 2006). Although the reflective responses can be well captured by self-report measures, it is critical to include implicit measures that do not rely on conscious considerations such as psychophysiological responses.

Moreover, exclusive reliance on subjective reports of disgust makes it difficult to rule out demand effects or a more general negativity bias as the mechanism for the conditioned response (CR). Consistent with this view, a recent study found that in addition to disgust participants also reported significantly more anxiety, anger and sadness towards the CS+ following acquisition

(Olatunji et al., 2013). This suggests that previous findings may be most parsimoniously explained as reflecting a change in the global affective evaluation of the original neutral CSs (i.e., evaluative conditioning effect) rather than as a more specific effect of disgust-learning.

In an attempt to extend beyond self-report, some researchers have used a behavioural measure of the CR, such as visual avoidance (Armstrong, McClenahan, Kittle, & Olatunji, 2014; Engelhard et al., 2014; Mason & Richardson, 2010). Although these studies have found visual avoidance of the CS+ following a conditioning procedure, it is unclear if this is mediated by learned disgust specifically or negativity in general. Other researchers have assessed skin conductance (SC) response to the CS as a supplement to subjective reports of the CR (e.g., Olatunji, Forsyth, & Cherian, 2007). However, SC is not specific to disgust, but rather a measure of general arousal.

In light of these important limitations, the present study aimed to replicate and extend the available literature on disgust conditioning in several critical ways. First, the present study complemented self-report measures of disgust with the assessment of a more reflexive index of disgust. More specifically, we measured EMG activity of the facial levator labii muscle as a unique physiological marker of disgust (Vrana, 1993). To examine whether the current conditioning effects would be restricted to changing the global affective appreciation of the CSs we also measured EMG activity of the corrugator muscle as a more general index of negative affect (Vrana, 1993).

Second, the current study used disgust-relevant stimuli as the CS, as disgust-relevant stimuli are probably more susceptible to disgust conditioning than disgust-irrelevant stimuli. Stimuli that were used in previous studies (e.g., pictures of neutral faces) are theoretically distant from the original function of disgust, and previous research has shown that some CS–US combinations are more easily learned than other combinations. In their seminal work, Garcia and Koelling (1966), for example, found that it is easier to associate taste

with illness than with electric shock (*belongingness*). Extensions of this research have shown that a-priori belongingness renders stimuli selectively conditionable, by either enhancing or inhibiting visceral response associations (Hamm, Vaitl, & Lang, 1989). This research suggests that also disgust conditioning will be probably facilitated if the initially neutral CS "belongs" to the contingently occurring disgust-related US.

Third, the pictures of disgusting stimuli that were previously used might not accurately represent the disgust-relevant catastrophic outcome. From a disease-avoidance perspective it would be adaptive if a CS would elicit disgust to motivate avoidance of potentially disease-inflicting substances. In other words, classical conditioning would help one to learn that a particular initially neutral stimulus may represent a risk of contamination. Thus, the US should somehow represent the aversive outcome or threat of becoming contaminated. In most aversive-conditioning procedures electro-cutaneous stimulation is used as the aversive outcome (e.g., Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2007). However, experiencing aversive electrical stimulations more closely mimics a threat of getting physically harmed than of becoming contaminated. Therefore, in the present study we selected a film clip of a woman vomiting as the US. Such a film is a better representation of the negative consequences of contamination as well as a stronger disgust-elicitor than the disgusting pictures that were typically used as the US in previous disgust-conditioning studies. In an attempt to make the storyline of the US more consistent and thus more intense, each conditioning trial consisted of eight CS–US pairings presented without inter stimulus interval (ISI).

To enhance the sensitivity as well as the external validity of our design, we used images of neutral food items as CS instead of disgust-irrelevant stimuli. The selection of food items as CS and vomiting as the US also ensures relatively strong a-priori belongingness. As an additional dimension to the subjective and disgust-specific

EMG assessment of the CR, the present study employed a behavioural approach task (BAT) to index conditioned avoidance. More specifically, we assessed participants' willingness-to-eat the real food versions of the CS+ and CS−, and examined if participants actually took a real "bite" from the food items presented. Indeed, a major limitation of previous research is a failure to examine the effects of CR on behavioural outcomes.

Previous research that relied on self-report measures provided evidence that once subjective disgust is acquired, it is relatively insensitive to a CS-only extinction procedure (e.g., Olatunji, Forsyth, & Cherian, 2007). This is again consistent with the view that perhaps previous findings can be best interpreted as reflecting a change in global valence. There is abundant evidence that conditioned affective evaluations are relatively resistant to extinction procedures (De Houwer, Thomas, & Baeyens, 2001). Therefore, the current study also included an extinction procedure to test whether the resistance to extinction effects would be restricted to self-report measures of disgust or would also be evident for the psychophysiological and behavioural indices of disgust learning.

Consistent with prior research, it was predicted that following acquisition the CS+ would be subjectively rated as more disgusting and less positive than before acquisition. It was also predicted that participants would show stronger EMG activation of both the levator and corrugator muscle to the CS+ after acquisition. Furthermore, we predicted that following acquisition participants would report less willingness-to-eat and would more often refuse to take an actual bite of the CS+ than of the CS−. Finally, we explored to what extent the various types of CRs are sensitive to extinction (i.e., exposure to CS-only presentations).

METHOD

Participants

First year psychology students (N = 66, women, M_{age} = 19.41, SD = 1.72 years) were recruited via an internal university system. Eight participants were excluded post hoc from the initially recruited sample (N = 74, power = .80, α = .05, N = 68) due to poor compliance or problems with data acquisition. This study was approved by the local Ethical Committee of Psychology.

Materials

Conditioned stimulus

The CS consisted of two types of neutral food items (i.e., cheese and a bruschetta wrap), each pictured from two different angles. The two food items were counterbalanced (i.e., both items were equally often served as CS− and CS+).

Unconditioned stimulus

The disgust-relevant US was a sound-attenuated film clip depicting a woman vomiting (Borg et al., 2010; de Jong, Peters, & Vanderhallen, 2002). As a neutral outcome we used a neutral sound-attenuated film clip depicted the making of handmade glass (Borg et al., 2010). Both types of clips consisted of 5 seconds sequential film excerpts (i.e., resulting in 40 seconds storyline).

Validation of the CS and US

To select the optimal stimuli, a validation test was done prior to the experiment (N = 17, all women, M_{age} = 19.35, SD = 1.06). For the CSs, we used visual analogue scales (VASs) to measure "disgust" *very disgusting* (= 0) to *not at all disgusting* (= 100), "willingness-to-eat" *not at all willing* to (= 0) to *very willing* (= 100). In line with previous fear-conditioning research we additionally measured the more general affective "valence" *negative* (= 0) to *positive* (= 100). Both items used as CS were considered neutral (i.e., rated between 40 and 60, on all three VAS dimensions), and were not significantly different from each other.

The $US_{Disgust}$ and $US_{Neutral}$ clip were validated on the dimension of "disgust" and "valence". The $US_{Disgust}$ was rated as very disgusting (M = 7.65, SD = 23.53), whereas the $US_{Neutral}$

was rated as very low on disgust [M = 84.12, SD = 30.23; $F (1, 16)$ = 41.08, $p < .001$, η_p^2 = .720]. In a similar vein the US$_{\text{Disgust}}$ was rated more negative (M = 15.29, SD = 20.02) than the US$_{\text{Neutral}}$ [M = 77.82, SD = 22.09; $F (1, 16)$ = 88.14, $p < .001$, η_p^2 = .864].

Measures

The *Disgust Propensity and Sensitivity Scale Revised* (DPSS-R; van Overveld, de Jong, Peters, Cavanagh, & Davey, 2006) is a 12-item measure of disgust propensity (i.e., how frequently someone feels disgusted) and disgust sensitivity (i.e., how negative someone interprets the feeling of disgust). In this study Cronbach's α for the DPSS-R propensity scale was .68 and .72 for the sensitivity scale.

The *Vancouver Obsessive Compulsive Inventory—Contamination Fear Subscale* (VOCI; Geres, Barelds, & Meesters, 2012; Thordarson et al., 2004) is a 12-item measure of contamination obsessions and washing compulsions. The Cronbach's α for the subscale was .88 in the present study.

The *Hunger Scale* (HS; Grand, 1968) is a four-item questionnaire that measures how much participants could eat of their favourite food and the time passed from their last meal. In this study we used one item from the HS (see Data Reduction and Analysis).

The VAS was used to index the conditioning effect on the CSs at the subjective level on three dimensions (same VASs and scales as used in the validation of the CS and US).

Psychophysiological measurement

EMG activity was measured with PortiLab2 (hardware: Porti5-16/ASD) with pairs of Ag-AgCI electrodes (disc shaped, diameter of gel bed = 6.5 mm, diameter of electrode = 1.8 mm), placed on the left side of the face according to standard recommendations (Fridlund & Cacioppo, 1986). Data were recorded from the levator alesque nasii muscle as a unique marker of disgust relative to other negative emotions. Additionally, the musculus corrugator supercilii (corrugator) was selected as an index of general negative emotions (Wolf et al., 2005). The two disc electrodes for each of the two muscle types (i.e., levator and corrugator) had an approximate 1 cm inter-electrode distance. Fz functioned as the reference point and the EMG signal was sampled at 2000 Hz.

Behavioural approach task

A BAT was used following acquisition and extinction. The real CS− and CS+ food items were placed on separate plates that were covered and numbered. Participants were asked to uncover the indicated plate, observe the item (*step 1*) and rate this item (*step 2*) on two dimensions (i.e., *how tasteful*, on a scale ranging from 0 = negative to 100 = positive; and *how much they felt like eating it*, on a scale ranging from 0 = not at all to 100 = a lot). In *step 3*, participants were asked to actually take a bite of the food item presented, while they were aware they could just skip this step. Finally in *step 4*, participants indicated whether they had indeed eaten from the food item on a binary scale. The order of stimulus presentation was randomised.

Procedure

On arrival at the lab participants were provided with written information about the study and the informed consent. Participants then completed a set of demographic questions and the HS. This initial administration of the HS was done to check for possible pre-existing group differences and the second HS was aimed to assess whether participants changed their level of hunger during the experiment.

Participants were instructed to make themselves comfortable before attaching the electrodes. The skin was then cleaned with NuPrep scrub to optimise the conduction of the EMG signal, and the electrodes were filled with Medelec electrode conductivity gel. Participants were asked to remain focused on the computer screen and were informed that they could communicate with the researcher via a microphone. The task was separated into three different phases.

During *habituation*, all four CS images were presented at random. These four images later served as the CS+ and CS− in the acquisition and extinction phase. Each CS was presented for 6 seconds, followed by VAS ratings for baseline measurements. After each CS an inter-trial interval (ITI) followed that had a mean duration of 10 seconds, fluctuating between 8 and 12 seconds. During the ITI a fixation cross (+) was presented on the screen.

During *acquisition* the CS was always presented for 6 seconds. The CS+ was always followed by the disgust-relevant US clip (5 seconds), whereas the CS− was always followed by the neutral clip (5 seconds). A trial consisted of eight pairs of CS−US presentations (without ISI) followed by an ITI. Both types of trials (disgust, neutral) were randomly presented 10 times, resulting in a total of 20 trials (i.e., 10 times eight pairings for CS+ $US_{Disgust}$, and 10 times eight pairings for CS− $US_{Neutral}$). Acquisition had a total duration of 37 minutes. At the end of the acquisition, participants rated both CSs and both images again.

To assess the effect of acquisition on approaching the CS+/CS−, half of the participants performed the BAT immediately following the acquisition stage (i.e., group labelled as "post-acquisition"). To test the influence of extinction, the other half of the participants directly continued with the extinction phase following the post-acquisition CS-rating procedure, and carried out the BAT only following *extinction* (group labelled as "post-extinction"). In order to examine the effects of prior BAT experience on BAT performance following extinction, the post-acquisition group repeated the BAT following extinction.

During *extinction*, the CSs were presented for 6 seconds followed by an ITI. Each CS was shown 10 times, resulting in 40 trials. Following the 15-minute extinction phase, participants again rated the CSs on the VASs. Both groups continued with the BAT. Electrodes were removed before participants were asked to complete the questionnaires. The experiment was concluded with a debriefing session.

Data reduction

For the DPSS-R, we computed two subscales, disgust propensity and disgust sensitivity. For the VOCI we used the sum of all the items. We only used one item from the HS ("How hungry are you at this moment?"). The EMG data were processed offline and filtered (high-pass: 10 Hz; low-pass: 500 Hz). Data were visually inspected with Aphys (version 2.1.2.0, Ruiter, 2013); missing trials or trials containing artefacts were replaced with the mean (root-mean-square) EMG activity expressed in microvolts. From the total number of trials (N = 10,920), 176 trials (i.e., 1.16%) were either missing or contained artefacts. For each muscle the mean EMG activity was calculated over the whole duration of the stimulus presentation (i.e., for the CS the mean over 6 seconds and for the US the mean over 5 seconds). A pre-stimulus baseline of 100 ms was used. For habituation and extinction each stimulus presentation had its own 100 ms baseline. Due to the atypical set-up used in acquisition to enhance the intensity of the US, it was impossible to have a neutral baseline interval for all of the presented stimuli; therefore we only used the first CS presented in each of the 10 acquisition trials. Furthermore, to index EMG responding during habituation we included all trials of habituation, plus the first trial of acquisition given that participants' were still naive about the US. Acquisition was indexed by all of the remaining acquisition trials, plus the first trial of extinction, because the CS was still predictive for the US. For extinction we included all of the remaining trials.

To test whether disgust can be learned, VAS data were subjected to a 2 CS (CS− vs. CS+) × 2 Phase (habituation vs. acquisition) analysis of variance (ANOVA) with both being within-subjects factors. In addition, EMG data were analysed via a 2 CS (CS− vs. CS+) × 2 Muscle (levator vs. corrugator) × 2 Phase (habituation vs. acquisition) repeated measures (RM)-ANOVA. Second, we tested whether disgust is sensitive to extinction via CS-only exposure. Therefore, VAS scores were subjected to 2 CS (CS+ vs. CS−) × 2 Phase (acquisition vs. extinction) × 2 Group (BAT post-acquisition vs. BAT post-extinction) with only the latter factor being a

between-subjects. Group was included to control for the influence of the post-acquisition-BAT on extinction. With regards to the EMG data, the multivariate part of the RM-ANOVA was reported. Finally, to assess whether there were significant differences between pre- and post-extinction in the actual approach task, we calculated the percentages of participants eating the CS+, the CS−, and participants not eating any of the two food items, and subjected these scores to a χ^2 test.

In this study we report the number of missing or artefactual data and how we dealt with them, all manipulations and all measures in the study. All data collected in this study are reported, with the exception of SC data, which were excluded due to noise and technical problems.

RESULTS

Manipulation checks for pre-existing group differences

Both groups showed similar DPSS-R scores with mean scores on the propensity scale being 19.63 (SD = 2.93) for the post-acquisition group and 19.79 (SD = 2.79) for the post-extinction group [$F(1, 64) = .06, p = .811$], and mean scores on the sensitivity scale being 14.69 (SD = 3.69) and 14.00 (SD = 3.62) [$F(1, 64) = .59, p = .447$], respectively. Also the VOCI scores were similar for the post-acquisition and post-extinction group with mean scores of 5.94 (SD = 6.85) and 6.53 (SD = 5.80), respectively [$F(1, 64) = .14, p = .705$].

The groups did also not differ on their level of hunger as measured by the HS [$F(1, 64) = .53, p = .469$]. Moreover, level of hunger did not change from the start to the end of the experiment in the

post-acquisition group [$M = 2.91$, SD = 1.73 to $M = 3.21$, SD = 1.61; $t(31) = .96, p = .347$] or the post-extinction group [$M = 3.16$, SD = 1.55 to $M = 3.38$, SD = 1.83; $t(33) = .67, p = .510$]. Thus, hunger level was virtually the same for both groups and remained stable throughout the experiment.

Can disgust be learned as evidenced by subjective ratings?

In Table 1, the means (M) and standard deviations (SDs) for each dimension are provided as a function of experiment phase and type of CS.[1]

Disgust ratings for the CS+ increased following acquisition as evidenced by a CS × Phase interaction [$F(1, 131) = 76.71, p < .001, \eta_p^2 = .369$] (see Table 1). In a similar vein, willingness-to-eat the CS+ declined following acquisition as evidenced by a CS × Phase interaction, [$F(1, 131) = 81.98, p < .001, \eta_p^2 = .385$]. A similar CS × Phase interaction, [$F(1, 131) = 96.38, p < .001, \eta_p^2 = .424$] was also found for valence, indicating that the CS+ became more negative following acquisition.

Subsequent paired comparisons showed that in *habituation* there were no significant differences for willingness-to-eat and valence between the CS+ and CS− ($ps > .533$). For disgust the CS+ was rated significantly less disgusting compared to the CS− [$t(131) = 2.07, p = .040$], given that this effect was the opposite direction of what was expected after conditioning, we did not consider it as problematic. Following *acquisition* the difference between the CS+ and CS− was significant for all three dimensions i.e., for disgust [$t(131) = 8.22, p < .001$], willingness-to-eat [$t(131) = 10.33, p < .001$] and valence [$t(131) = 9.74, p < .001$].

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[1] *Manipulation checks for the CS* — we conducted three separate one-way ANOVAs on *valence, willingness-to-eat* and *disgust*. This analysis was based on the habituation scores, with the means collapsed over both pictures of each category. The analysis showed that the bruschetta wrap (M = 66.81, SD = 19.68) was evaluated more positively than the cheese [M = 51.93, SD = 24.80; $F(1, 286) = 31.81, p < .001, \eta_p^2 = .100$]. Also the willingness-to-eat ratings were higher for the wrap (M = 66.06, SD = 22.42) than for cheese [M = 48.94, SD = 27.62; $F(1, 286) = 33.34, p < .001, \eta_p^2 = .104$]. The wrap was also rated as less disgusting (M = 69.95, SD = 28.87) than cheese [M = 58.85, SD = 28.79; $F(1, 286) = 10.68, p = .001, \eta_p^2 = .036$]. This was not in line with expectations given the validation of the food items (see Method). However, this difference in appraisal of the CS was not considered problematic because the two food items were counterbalanced in the design of the experiment.

Can disgust be learned as evidenced by differential EMG activity?

Table 2 provides the M and SDs separately for the CS+ and CS− for all phases of the experiment per muscle[2] (Figures 1 and 2).

EMG magnitudes were subjected to a 2 CS × 2 Phase × 2 Muscle RM-ANOVA. Most important for the present context was that the CS × Phase interaction reached significance, [$F (1, 64) = 5.71$, $p = .020$, $\eta_p^2 = .082$], indicating that following acquisition the CS+ elicited stronger EMG activity than the CS− (see Table 2). This effect appeared similar for the corrugator and levator as the three-way interaction did not approach significance, [$F (1, 64) = 1.12$, $p = .295$, $\eta_p^2 = .017$]. To test whether the interaction effect was significant for both muscles, the RM-ANOVA was repeated for the corrugator and levator muscles separately. When the analysis was restricted to the corrugator the CS × Phase effect remained [$F (1, 64) = 4.30$, $p = .042$, $\eta_p^2 = .063$], whereas for the levator the effect fell just short of the conventional level of significance [$F (1, 64) = 3.88$, $p = .053$, $\eta_p^2 = .057$]. The effect size was very similar for both muscles; it should, however, be acknowledged, that the two-way CS × Phase interaction for the levator seemed at least partly carried by a relatively strong muscular response towards the CS− during habituation, that attenuated during acquisition, whereas the opposite was true for the CS+.

Accordingly, subsequent paired comparisons for the levator indicated that neither during habituation [$t (64) = 1.46$, $p = .147$], nor during acquisition [$t (64) = −1.27$, $p = .208$] the difference between CS+ and CS− reached the conventional level of significance. For the corrugator, there were no differences between the CS+ and CS− during habituation [$t (64) = .254$, $p = .805$], while differential responding was observed in acquisition [$t (64) = −2.18$, $p = .033$]. Thus although the critical interaction was evident for both muscles, only for the corrugator this resulted in a significant difference between CS+ and CS− during acquisition (Figures 3 and 4).

Can disgust-based avoidance be learned?

Table 3 illustrates the M and SDs for the CS+ and CS− per VAS as a function of group.

Following acquisition individuals' appreciation of the actual CS+ food item in terms of tastiness was lower than for the CS− [$t (31) = 2.13$, $p =.042$]. The same yields for how much participants felt like eating the CS+ during the BAT compared to the CS− [$t (31) = 2.84$, $p = .008$].

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[2] *Manipulation checks for physiological data* — To examine whether there might have been a priori differences between the responsivity to the CS− and the CS+, we subjected the EMG activity during habituation to a 2 CS− × 2 Muscle within-subject RM-ANOVA. There were no main effects of CS, [$F (1, 64), = 1.10$, $p = .299$, $\eta_p^2 = .017$], Muscle [$F (1, 64) = .28$, $p =. 596$, $\eta_p^2 = .004$], nor a CS × Muscle interaction [$F (1, 64) = .52$, $p = .473$, $\eta_p^2 = .008$], indicating that there were no meaningful differences between both CS and both Muscles before conditioning with regard to their EMG responsivity. A 2 US (US$_{Disgust}$, US$_{Neutral}$) × 2 Muscle (corrugator, levator) × Block (1–10) RM-ANOVA showed a significant main effect of US [$F (1, 64) = 17.16$, $p < .001$, $\eta_p^2 = .211$], indicating that the US$_{Disgust}$ elicited stronger EMG activity than the neutral clip, means being $M = 0.051$ (SE = 0.015) and $M = −0.006$ (SE = 0.005), respectively. The absolute increase in EMG activity was stronger for the corrugator (US$_{Neutral}$: $M = −0.004$, SE = 0.004; US$_{Disgust}$: $M = 0.070$, SE = 0.019) than for the levator muscle (US$_{Neutral}$: $M = −0.008$, SE = 0.008; US$_{Disgust}$: $M = 0.033$, SE = 0.014) as was reflected in a significant US × Muscle interaction [$F (1, 64) = 5.60$, $p = .021$, $\eta_p^2 = .080$]. Subsequent t tests (for each separate muscle) between the US$_{Disgust}$ and US$_{Neutral}$ indicated that for both muscles the responsivity was larger for the disgusting US than for the neutral film outcome [corrugator $t (64) = 3.67$, $p < .001$; levator $t (64) = 3.73$, $p < .001$]. Furthermore the main effect of block approached the conventional level of statistical significance [$F (9, 56) = 1.88$, $p = .075$, $\eta_p^2 = .232$]. To test for habituation over these 10 blocks, we computed a simple contrast with the first block used as the reference category. All of these contrasts reached significance ($p < .030$), clearly indicating that following the first viewing habituation occurred.

Table 1. *Subjective evaluation for each of the three phases (i.e., habituation, acquisition, extinction) on the three dimensions (i.e., disgust, willingness-to-eat, and valence) as measured on the VASs per group (i.e., post-acquisition, post-extinction)*

Dimensions	Group	Habituation		Acquisition		Extinction	
		CS− M (SD)	CS+ M (SD)	CS− M (SD)	CS+ M (SD)	CS− M (SD)	CS+ M (SD)
Disgust	Post-acquisition	67.66 (31.29)	71.25 (25.30)	67.39 (28.87)[a]	45.11 (31.12)[b]	62.56 (31.99)	56.45 (29.44)
	Post-extinction	59.16 (28.51)	65.50 (27.68)	59.76 (22.77)[a]	37.59 (28.25)[b]	59.69 (24.12)[a]	46.03 (26.95)[b]
	Total	63.28 (30.08)	68.29 (26.61)	63.46 (26.09)[a]	41.23 (29.80)[b]	61.08 (28.14)[a]	51.08 (28.56)[b]
Willingness to eat	Post-acquisition	61.58 (29.56)	61.22 (25.48)	58.83 (30.22)[a]	39.44 (28.69)[b]	54.31 (30.48)	48.77 (28.32)
	Post-extinction	53.65 (26.59)	56.91 (23.67)	51.38 (22.19)[a]	28.15 (23.73)[b]	50.97 (24.20)[a]	34.12 (23.62)[b]
	Total	57.49 (28.25)	59.01 (24.57)	54.99 (26.55)[a]	33.62 (26.75)[b]	52.59 (27.37)[a]	41.22 (26.93)[b]
Valence	Post-acquisition	61.97 (27.65)	62.80 (22.26)	60.92 (28.03)[a]	43.75 (27.52)[b]	58.72 (29.03)[c]	52.16 (25.76)[d]
	Post-extinction	58.87 (22.49)	59.03 (21.57)	59.46 (17.91)[a]	35.22 (23.96)[b]	57.62 (18.18)[a]	41.63 (22.26)[b]
	Total	60.37 (25.08)	60.986 (21.91)	60.17 (23.29)[a]	39.36 (26.00)[b]	58.15 (23.97)[a]	46.73 (24.50)[b]

Note: Different letters in superscript indicate significant differences across rows: [a,b] at $p \leq .001$ and [c,d] at $p = .0270$.

Table 2. *Mean EMG activity in microvolt for muscle type (corrugator, levator) as a function of phase (habituation, acquisition, extinction) and type of CS per group (post-acquisition, post-extinction)*

Muscle Type	Group	Habituation		Acquisition		Extinction	
		CS− M (SD)	CS+ M (SD)	CS− M (SD)	CS+ M (SD)	CS− M (SD)	CS+ M (SD)
Corrugator	Post-acquisition	0.019 (0.038)	0.011 (0.046)	0.001 (0.038)	0.047 (0.123)	0.020 (0.041)	0.014 (0.033)
	Post-extinction	0.012 (0.032)	0.015 (0.053)	0.006 (0.021)	0.015 (0.065)	0.007 (0.022)	0.008 (0.013)
	Total	0.015 (0.035)	0.013 (0.049)	0.004 (0.024)	0.030 (0.100)	0.013 (0.032)	0.011 (0.025)
Levator	Post-acquisition	0.011 (0.045)	0.007 (0.039)	0.005 (0.023)	0.022 (0.041)	0.006 (0.042)	0.007 (0.027)
	Post-extinction	0.019 (0.048)	0.007 (0.042)	0.010 (0.028)	0.009 (0.035)	0.009 (0.024)	0.016 (0.026)
	Total	0.015 (0.046)	0.007 (0.040)	0.008 (0.026)	0.015 (0.038)	0.007 (0.034)	0.012 (0.026)

Note: Superscripts [a] and [b] indicate significant difference across rows ($p < .001$). The total in the second column represents the overall activity independent of group.
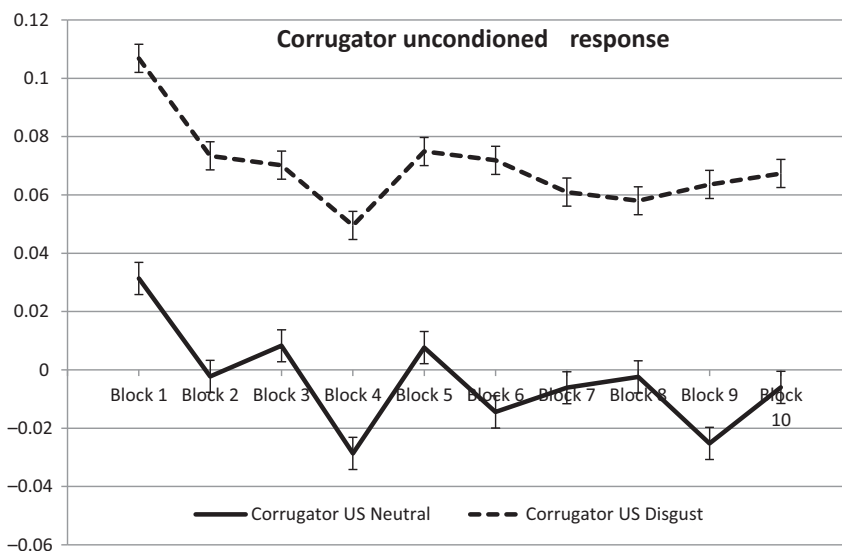
Figure 1. *Mean unconditioned response per block of the corrugator towards US$_{Disgust}$ and US$_{Neutral}$.*

Is conditioned disgust sensitive to extinction as evidenced on self-reports (as a function of group)?

To test the influence of extinction, each of the three VASs were subjected to 2 CS × 2 Phase × 2 Group RM-ANOVA. Most critical for the current question, for disgust there was a significant CS × Phase interaction [$F (1, 130) = 33.02$, $p < .001$, $\eta_{p}^{2} = .203$], showing that conditioned disgust is sensitive to extinction (see Table 1). The
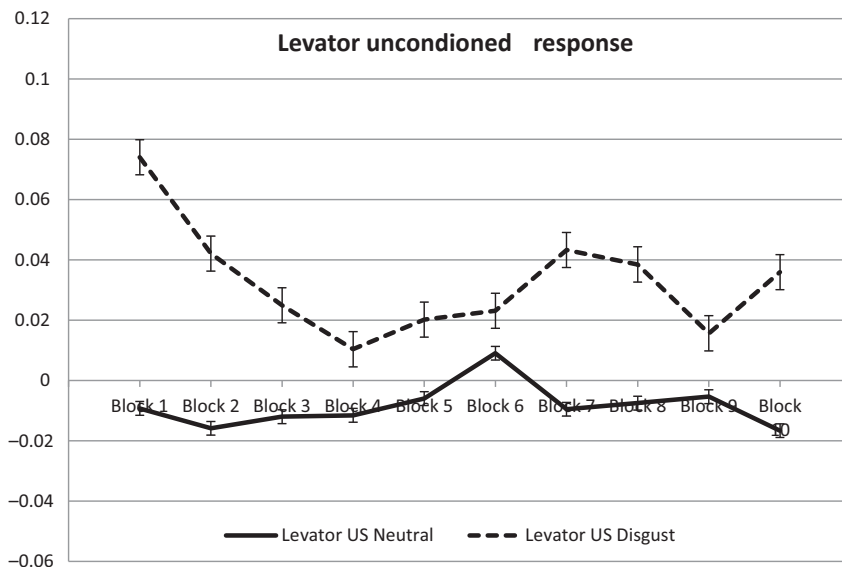

Figure 2. *Mean unconditioned response per block of the levator towards US$_{Disgust}$ and US$_{Neutral}$.*
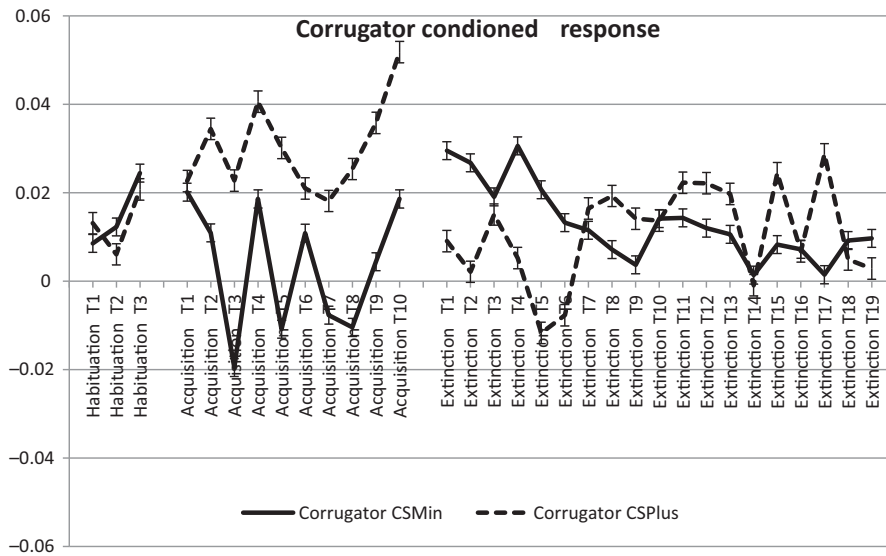
Figure 3. *Mean response in the different phases (habituation, acquisition, extinction) of the corrugator towards the CS+ and CS−.*

effect of extinction seemed most pronounced for the post-acquisition group (see Table 1), yet the CS-type × Phase × Group interaction did not reach the conventional level of significance, $[F (1, 130) = 3.18, p = .081, \eta_p^2 = .024]$. Although the difference in disgust responding between the CS+ and CS−

overall declined following extinction, the difference remained significant, $[t (131) = 3.18, p < .001]$.

For willingness-to-eat there was also a significant CS × Phase interaction, $[F (1, 130) = 26.71, p < .001, \eta_p^2 = .170]$, indicating that the reported willingness-to-eat the CS+ heightened again
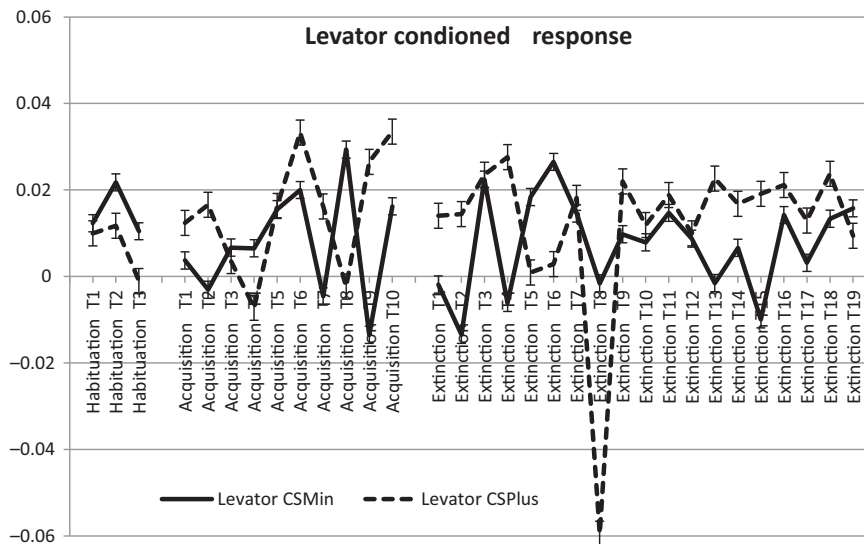

Figure 4. *Mean response in the different phases (habituation, acquisition, extinction) of the corrugator towards the CS+ and CS−.*

following extinction. The three-way interaction did not reach significance, [F (1, 130) = 3.64, p = .059, η_p^2 = .027], but there was a trend suggesting that willingness-to-eat increased most for the group that completed the BAT before extinction (see Table 1). Furthermore, also following extinction, the reported willingness-to-eat the CS+ remained significantly lower from the willingness-to-eat the CS−, [t (131) = 4.97, p < .001] (see Table 1).

Finally, also for valence there was a significant CS × Phase interaction, [F (1, 130) = 30.29, p < .001, η_p^2 = .189], indicating that the differential responding to the CS+ and CS− was reduced again following extinction. The three-way CS × Phase × Group interaction did not approach significance, [F (1, 130) = .47, p = .492, η_p^2 = .003]. Just as for disgust and willingness-to-eat, the CS+ ratings remained significantly more negative than the ratings of the CS−, [t (131) = 5.52, p < .001] (see Table 1).

Is learnt disgust sensitive to extinction as evidenced by the physiological disgust response?

EMG data were subjected to a 2 CS × 2 Phase × 2 Muscle × 2 Group RM-ANOVA. The three-way CS × Phase × Muscle interaction reached the conventional level of significance [F (1, 63) = 4.58, p = .036, η_p^2 = .068]. This pattern was similar for both groups, as evidenced by the absence of a CS × Phase × Muscle × Group interaction [F (1, 63) = .49, p = .488, η_p^2 = .008]. To decompose the three-way CS × Phase × Muscle interaction, we tested the CS × Phase interaction for the two muscles separately: for the corrugator, the interaction reached significance [F (1, 63) = 5.16, p = .026, η_p^2 = .075], whereas the interaction did not approach significance for the levator [F (1, 63) = .10, p = .759, η_p^2 = .001]. However, this differential pattern did not seem to reflect a qualitative difference in sensitivity to extinction, but seemed mainly due to the weaker differential responding during acquisition of the levator compared to the corrugator (see Table 2). In line with this, the difference between the CS+ and CS− was no

longer significant during extinction for both the corrugator [t (64) = .79, p = .435] and the levator [t (64) = .86, p = .395]. This suggests that differential (conditioned) responding disappeared during extinction.

Can disgust-based avoidance be unlearned via extinction?

To test the influence of extinction (at their first encounter with the actual food items), we subjected both types of scores (i.e., tastiness, feel-like-eating) to a 2 CS × Phase RM-ANOVA with the latter being a between-subjects factor (note that only half of the participants were exposed to an extra BAT at post-acquisition). For tastiness, there was a main effect of CS [F (1, 64) = 10.47, p < .002, η_p^2 = .141], indicating that overall participants reported lower tastiness ratings for the CS+ than for the CS−. This pattern was not affected by the extinction procedure as was evidenced by the absence of a CS × Phase interaction [F (1, 64) = .05, p = .827, η_p^2 = .001] (see also Table 3).

The same pattern emerged for the ratings regarding feel-like-eating. There was a significant main effect of CS [F (1, 64) = 15.38, p < .001, η_p^2 = .194], indicating that participants reported less motivation to eat the CS+ than the CS−. These ratings remained unaffected by the extinction procedure as again there was no CS × Phase interaction, [F (1, 64) = .12, p = .729, η_p^2 = .002].

To examine the influence of prior BAT exposure to the appreciation of the CSs following extinction, we conducted a 2 CS × 2 Group ANOVA with the latter being a between-group factor. No statistically significant CS × Group interaction was observed for tastiness [F (1, 64) = 2.73, p = .103, η_p^2 = .041], nor for feel-like-eating [F (1, 64) = 2.00, p = .162, η_p^2 = .030], although there was a trend suggesting that the differential responding was most pronounced in the post-extinction group. Accordingly, within-group analyses indicated that participants of the post-extinction group considered the CS+ compared to the CS− significantly less tasty [t (33) = 2.53, p = .016] and felt less like eating it [t (33) = 2.69, p = .011], while these differences

Table 3. *Subjective evaluations of real food items on VAS tastiness and VAS feel-like-eating*

Group	Post-acquisition		Post-extinction		Post-acquisition	
BAT	Post-acquisition		Post-extinction		Post-extinction (second encounter)	
VAS	CS−	CS+	CS−	CS+	CS−	CS+
Tastiness	55.16 (29.73)[a]	42.63 (24.87)[b]	52.06 (21.54)[a]	41.12 (21.95)[b]	52.59 (28.71)	52.63 (25.06)
Feel-like-eating	47.66 (32.19)[a]	31.84 (24.99)[b]	41.76 (28.92)[a]	28.53 (26.16)[b]	42.53 (28.70)	39.09 (28.04)

Note: Different letters in superscript ([a,b]) indicate significant differences across rows. For example if we take VAS tastiness the "[a]" on *BAT post-extinction* for CS− indicates that the mean is significantly different from that of the CS+ (as denoted with a "[b]"). The first two columns illustrate the ratings for the CSs for the two groups (post-acquisition, post-extinction) at their initial contact with BAT. The third column represents the post-acquisition group during their second BAT ratings that took place following extinction.

were not observed in the post-acquisition group during their performance of the BAT after extinction [tastiness: $t(31) = .01$, $p = .995$; feel-like-eating: $t(31) = .70$, $p = .492$].

BAT eating behaviour

Table 4, illustrates the percentages of participants actually approaching the food stimuli provided to them.

A McNemar test within the post-acquisition group yielded no difference between the CS+ and CS− at post-acquisition ($p = .791$). Also for the post-extinction group there was no significant differential eating ($p = .989$) between the CS+ and CS−. Thus the post-acquisition group and the post-extinction group showed no differences in the percentage of people eating the CS− and those eating the CS+. Subsequent between-groups analyses provided complementary evidence that

the pattern of eating the CS+/CS− was unaffected by the exposure to CS-only trials. Most important, the percentage of participants that ate the CS+ [χ^2 (1, $n = 66$) = 1.03, $p = .952$] following extinction was similar to the percentage of participants that ate the CS+ following acquisition. For the CS−, there was a non-significant tendency indicating that a larger percentage took a bite of it following extinction rather than before this procedure took place [χ^2 (1, $n = 66$) = 3.47, $p = .060$] (see also Table 4). As can be seen in Table 4, extinction did not have an effect on percentage of participants who ate the actual food items for CS+ nor for CS−.

A McNemar test within the post-acquisition group indicated that the percentage of participants who ate the CS− was not significantly different from the percentage who ate the CS− during the second (post-extinction) BAT ($p = .224$). For the food items representing the CS+ the percentage of

Table 4. *The percentage of participants that actually eat the "real" food items which were represented as CS+ or CS−*

Group	Post-acquisition group	Post-extinction group	Post-acquisition group
BAT	Post-acquisition	Post-extinction	Post-extinction (second encounter)
Ate CS+	71.88	82.35	40.63
Ate CS−	65.63	85.29	53.13
Ate nothing	9.78	8.82	40.63

Note: For specificity the "Ate CS+" includes participants that ate the CS+ only as well as participants that eat both the CS+ and the CS−. The third row is the percentage of participants that eat nothing. The third column represents the post-acquisition group during their second BAT that took place following extinction.

participants who took a bite from the CS+ was even lower post-extinction than post-acquisition (p < .008). This seems to reflect the general tendency for participants to eat less during the second BAT which is also reflected in the much larger percentage of participants who ate nothing during the second encounter (see Table 4). Also within the post-acquisition group a McNemar test confirmed that there was no difference between the CS+ or CS− regarding the percentage of participants taking a bite during the second (post-extinction) BAT (p = .287).

DISCUSSION

The main findings can be summarised as follows: (1) Following *acquisition* the CS+ food pictures were rated as more disgusting and less positive, and similarly participants reported reduced willingness-to-eat the CS+; (2) During the BAT, participants rated the concrete CS+ food items as less tasty than the CS− and gave lower ratings on the "feel-like-eating" scale, but complied with the instruction to take a bite from the CS+ at a similar rate as the CS−; (3) The conditioning procedure also resulted in increased facial EMG activity upon presentation of the CS+ mainly driven by the global negative-affect-corrugator muscle (rather than by the disgust specific levator muscle), which disappeared during extinction; (4) Although subjective disgust ratings of the CS+ also declined, differential responding remained significant following extinction, whereas the lowered willingness-to-eat the CS+ food-item during the BAT remained fully unaffected by the extinction procedure.

Disgust learning (acquisition)

Consistent with earlier disgust conditioning research, the current study indicates that the disgust response towards the US was transferred to the CS+ as evidenced by the subjectively reported increased disgust and reduced positive affect towards the CS+. In addition, the current study showed that the effect of the conditioning

procedure can also extend to the behavioural level as shown in the lowered willingness-to-eat food items representing the CS+. However, there was no convincing evidence that the classical conditioning procedure also resulted in reflexive disgust-specific levator activity upon presentation of the CS+.

Attesting to the validity of the US material, participants responded with an increase of the EMG activity with both the facial levator and corrugator muscles when they watched the disgust-relevant US. Most important for the present context, participants also showed heightened activation of the facial muscular activity when presented with the CS+ during *acquisition*. However, despite maximising belongingness of the CS to the US, the use of a highly disgusting film clip (vs. pictures) that represented the actual consequences of contamination threat, and the large number of CS−US pairings, the CR as expressed in facial EMG provided no convincing support for CR that is disgust-specific.

Analyses within the levator muscle provided no strong evidence for differential activity during acquisition. This suggests that it may be difficult to learn a disgust response following a classical conditioning procedure that is also expressed at the more reflexive, physiological level. However, the enhanced responding of the corrugator muscle indicates that the conditioning procedure was not restricted to self-reports (e.g., Engelhard et al., 2014; Mason & Richardson, 2010; Olatunji, Forsyth, & Cherian, 2007; Olatunji et al., 2013), but seems to exert their influence also on the more reflexive facial expressions related to global negative effect (Olatunji et al., 2013).

Notably, the effect of disgust conditioning was strong enough to transfer to the actual food items, as evidenced by the less intrinsic motivation to eat the real food items representing the CS+. This decline in motivation was demonstrated by lower scores on feeling-like-eating and tastiness of the actual (CS+) food item during the BAT. This finding may have important implications for the development of disgust-induced food avoidance. It should be acknowledged though, that this difference did not result in differential eating. That is, the disgust properties of the CS+ were

not sufficiently strong to refuse the request to take a bite. Perhaps avoidance might be evident in a context without external pressure, or if outside the safe-lab-context. In the latter exemplar the quality of the food items probably remains more ambiguous (and thus less safe).

These findings suggest that pairing an initially neutral stimulus with a disgust-relevant US changes the subjective appraisal of the CS+ as well as its tendency to elicit facial EMG responses of the corrugator muscle. This pattern of findings suggests that the conditioning effects may represent changes in global affect rather than more specific changes in disgust. In a similar vein, most of the disgust conditioning studies conducted to-date may not have demonstrated disgust acquisition per se but rather changes in the general affective evaluation of the CS. If only disgust is measured in the context of a disgust conditioning procedure, disgust would naturally become the salient (negative) emotion. However, the previous finding that participants also reported significantly more anxiety, anger and sadness towards the CS+ following disgust acquisition (Olatunji et al., 2013) may reflect a change in global affective evaluation of the original neutral CSs (i.e., evaluative conditioning effect) rather than as a more specific effect of disgust-learning.

Unlearning the conditioned disgust response (extinction)

In line with previous research, the strength of the subjective conditioned disgust responses declined following a series of CS only presentations. This extinction effect seemed most pronounced for participants who were exposed to the BAT between acquisition and extinction. Though replication is needed to confirm the robustness of this effect, these results seem to suggest that just looking at (pictures of) the CS+ was less effective as a strategy to reduce disgust compared to actual confrontation with the CS+ as was done during the BAT. In line with the notion that merely looking at representations of the CS+ may not be sufficient to reduce its disgusting properties, we found that both the feeling-like-eating and tastiness of the

CS+ were fully unaffected by the extinction procedure. Relevant here is the trend that participants who also performed the BAT post-acquisition, in contrast to participants who only conducted the BAT after extinction, showed an increase in their subjective valence ratings of the CS+ following the extinction procedure. Even though the appreciation of the CS+ increased, the CS+ was still appraised more negative than the CS−. The lingering conditioning effect indicates that the changed liking of the CS+ was robust against extinction at the subjective level even with additional exposure (Engelhard et al., 2014; Mason & Richardson, 2010; Olatunji, Forsyth, & Cherian, 2007).

What remains unclear is whether this relatively larger shift in the appraisal of the CS+ for the post-acquisition group was mainly due to the knowledge that participants received from physical contact with the actual food items (e.g., not smelling like rotten food, expected texture, etc.). This additional safety information could have served to neutralise the signal value of the food item as a potential contagious agent. Yet, it cannot be ruled out that this was a mere effect of time. The post-acquisition group did not look at the computer for a few minutes in order to complete the BAT before continuing with *extinction*. Thus this group compared to the post-extinction group had more exposure to the actual stimuli and more time between phases which may have decreased the negative value attached to the CS+. Future research is clearly needed to test the robustness of these trends and if confirmed, to examine whether additional exposure to the images is sufficient to increase the relative liking of the stimulus or if providing more concrete safety information would be more effective. If the impact of the BAT on extinction represents a robust phenomenon, it may have clinical value in light of the consistent observation that traditional exposure treatment is typically not very effective in reducing disgust (Mason & Richardson, 2012; Smits, Telch, & Randall, 2002).

Although the subjective ratings were relatively insensitive to the CS-only presentations, CRs at the physiological level were readily eliminated during extinction. Thus in spite of the continued differential CR at the subjective level the facial

muscular activity no longer differentiated between the CS+ and CS−. This discrepancy between the EMG results and the subjective ratings might reflect differential stimulus habituation effects (Thompson & Spencer, 1966; Lang, Greenwald, Bradley, & Hamm, 1993). Alternatively, it could also be that contingent pairing may be especially effective in modifying the reflective appreciation of the CS whereas a robust change in reflexive responding might require more intense or more extensive procedures (i.e., multiple conditioning series).

De-synchrony was also observed between the subjective appraisal as an indication of the participants' internal motivation (i.e., VAS feel-like-eating) of the food items and the BAT (i.e., to actually have a bite). This discrepancy might be related to the absolute ratings of the CSs. The range in which stimuli were considered neutral was a VAS score between 40 and 60. In *habituation* both the CS+ and CS− were scored at the high end or above this range (maximum of 71 for the CS+ on the dimension of disgust). So even though we found that the CS+ was appreciated significantly less than the CS−, when looking at absolute numbers the ratings on valence, disgust and willingness-to-eat remain within or just under the neutral range. This observation suggests that a larger learned disgust response at the subjective level may be necessary to trigger avoidance, which may partially explain the absence of a robust facial levator activity. Alternatively, different mechanisms may moderate the subjective appreciation of a stimulus as opposed to the actual behaviour. This would be consistent with the finding that participants did indicate that they did not really feel-like-eating the food, yet this did not result in actual avoidance.

In addition the different appraisal of the CSs, it needs to be acknowledged that the fact that the CSs were rated as slightly positive might have influenced the effect of conditioning. On the one hand a slightly positive appraisal may allow for greater shifts towards negative and/or disgusting evaluations following the conditioning procedure. Yet, on the other hand, the slightly positive appraisal might also have lowered the CS susceptibility to the current conditioning procedure. It should also be

acknowledged that we restricted our sample to women. Because women consistently show higher disgust sensitivity than men (e.g., Haidt, McCauley, & Rozin, 1994), we anticipated that relying on female participants would enhance the sensitivity of our design. However, this strategy of course also implies that it remains to be tested whether similar effects can be found in men. Finally, it needs mention here that although the relevance of the CS−US belongingness is well documented in the literature, the possibility that a US that is not related to the behaviour may also leads to learned aversions remains. Thus for disgust research this important issue is open for empirical scrutiny.

In conclusion, the current findings convincingly showed that a disgust-relevant aversive conditioning procedure resulted in heightened disgust ratings and a lowered willingness-to-eat the food item that was used as the CS+, which were both highly resistant to extinction. Although the conditioning procedure did also result in heightened EMG activity of the corrugator muscle in response to the CS+, there was no strong evidence for enhanced disgust-specific (levator) EMG responding. The overall pattern of findings seems therefore best explained as reflecting a conditioned change in global affect rather than as a change in disgust per se.

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