

Generalization of appetitive conditioned responses

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Abstract

A stimulus (conditioned stimulus, CS) associated with an appetitive unconditioned stimulus (US) acquires positive properties and elicits appetitive conditioned responses (CR). Such associative learning has been examined extensively in animals with food as the US, and results are used to explain psychopathologies (e.g., substance-related disorders or obesity). Human studies on appetitive conditioning exist, too, but we still know little about generalization processes. Understanding these processes may explain why stimuli not associated with a drug, for instance, can elicit craving. Forty-seven hungry participants underwent an appetitive conditioning protocol during which one of two circles with different diameters (CS+) became associated with an appetitive US (chocolate or salty pretzel, according to participants' preference) but never the other circle (CS-). During generalization, US were delivered twice and the two CS were presented again plus four circles (generalization stimuli, GS) with gradually increasing diameters from CS- to CS+. We found successful appetitive conditioning as reflected in appetitive subjective ratings (positive valence, higher contingency) and physiological responses (startle attenuation and larger skin conductance responses) to CS+ versus CS-, and, importantly, both measures confirmed generalization as indicated by generalization gradients. Small changes in CS-US contingency during generalization may have weakened generalization processes on the physiological level. Considering that appetitive conditioned responses can be generalized to non-US-associated stimuli, a next important step would be to investigate risk factors that mediate overgeneralization.

KEYWORDS

appetitive conditioning, generalization, primary reinforcer, startle reflex

1 | INTRODUCTION

Avoidance of threats and discovery of food are crucial for organisms' survival, as is the reliable prediction of both threat and food sources. Classical conditioning (Pavlov, 1927) is a simple learning model that explains how organisms make associations between events in order to predict threats or food. In other words, biologically salient events (unconditioned stimuli, US) become associated with initially

irrelevant stimuli if such stimuli occur contiguous and contingent to the US (Rescorla, 1988). Through their association with the US, the irrelevant stimuli (now labeled conditioned stimuli, CS) acquire affective and predictive properties and are able to elicit specific responses (conditioned responses, CR) such as fear in the case of a threatening US (Andreatta & Pauli, 2015; Lipp, Sheridan, & Siddle, 1994; Sjouwerman, Niehaus, Kuhn, & Lonsdorf, 2016) or appetitive responses in the case of a rewarding US (Andreatta & Pauli, 2015;

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Blechert, Testa, Georgii, Klimesch, & Wilhelm, 2016; Kim, Shimojo, & O'Doherty, 2011; Klucken et al., 2015; Kruse, Leon, Stark, & Klucken, 2017; Myers et al., 2017; van den Akker, Nederkoorn, & Jansen, 2017).

Following such learning processes, survival is further optimized by generalization toward other stimuli that have never been associated with the US but which share properties with the CS (Hearst, 1960; Pearce, 1987). Notably, the similarity of the so-called generalization stimuli (GS) to the CS can be perceptual but also conceptual (Dunsmoor & Murphy, 2015; Dymond, Dunsmoor, Vervliet, Roche, & Hermans, 2015). Specifically, human studies found strong fear responses such as startle potentiation (Lissek et al., 2008, 2010) or a greater skin conductance response (SCR, Ahrens et al., 2016; Onat & Buchel, 2015; Schiele et al., 2016) to both the CS predictive of an aversive US (e.g., an electric shock or a desperate female scream) and GS that were perceptually similar to the CS. The generalization gradient describes the magnitude of such generalization with steep and shallow gradients indicating weak and strong generalization, respectively. Notably, people suffering from anxiety disorders were found to have quite shallow generalization gradients and therefore to show fear responses to a broader number of GS than healthy controls (Dymond et al., 2015; Struyf, Zaman, Vervliet, & Van Diest, 2015). This overgeneralization of fear responses is discussed as important for the development and maintenance of the disorders (Ahrens et al., 2016; Lissek et al., 2010).

According to animal studies, appetitive CR generalize as well. A study in monkeys (Hearst, 1960) demonstrated a strong generalization gradient for the rewarded behavior. Specifically, monkeys learned to avoid an electric shock (i.e., aversive US) by pressing a lever and to pull a chain in order to receive a food pellet (i.e., appetitive US). During learning, a light (CS) with a certain intensity was turned on. During the test, the intensity of the light was gradually modified, and the animals' rewarded response gradually increased with the similarity between the test light and the learning light. In a more recent study in humans (FeldmanHall et al., 2018), a similar generalization gradient of appetitive responses was found. Thus, participants preferred to play (prosocial behavior) with individuals who resembled an individual previously experienced as trustworthy. In other words, prosocial behavior was generalized to other similar players.

Aversive conditioning has been implicated in anxiety and stress-related disorders (Craske, Hermans, & Vervliet, 2018; Mineka & Oehlberg, 2008), and appetitive conditioning seems to play a crucial role in the etiology and maintenance of substance-related, addictive, and eating disorders (Martin-Soelch, Linthicum, & Ernst, 2007; Sanchis-Segura & Spanagel, 2006). Strikingly, a recent study found reduced discriminative verbal responses in overweight women to a food-associated as compared to a nonassociated CS (van den Akker, Schyns, & Jansen, 2017). This observation is in line with findings in

anxiety patients, who seem to have a reduced capacity to differentiate between threat and safety signals (Duits et al., 2015, 2017). Such reduced discrimination between food and nonfood signals by overweight individuals might be—in parallel to anxiety patients—associated with generalization of appetitive conditioned responses (see also van den Akker, Schyns, & Jansen, 2017). Interestingly, a similar impaired discriminative appetitive learning with water as the US has been observed in major depression disorder (MDD, Kumar et al., 2008). Particularly, MDD patients unresponsive to antidepressants showed less discriminative activation in the ventral striatum (VS) to the CS as compared to healthy unmedicated controls, and a similar blunted discrimination in the VS was observed in healthy individuals who took selective serotonergic reuptake inhibitors.

With the goal to develop a paradigm examining generalization of appetitive conditioned responses in humans, we adapted the generalization protocol established by Lissek et al. (2008). During the acquisition phase, participants saw two circles differing in diameter and learned to associate a rewarding US with one circle (CS+) but never with the other circle (CS−). We expected positive valence for the CS+ as compared to CS− as indicated by the ratings (explicit index) as well as by the startle responses (implicit index). Moreover, CS+ should elicit stronger arousal on both the explicit (i.e., ratings) and physiological (i.e., SCR) level of responses. During the generalization phase, these two circles were presented again as well as four additional circles having a gradually changing diameter from CS+ to CS−. Based on previous findings for aversive conditioning, we expected a gradual decrease of appetitive responses (i.e., ratings, startle responses, and SCRs) from CS+ over GS to CS− describing generalization gradients.

2 | METHOD

2.1 | Participants

Fifty-eight volunteers participated in the study.¹ Exclusion criteria were history of psychiatric or neurological disorder, actual use of psychoactive drugs, chronic pain, pregnancy, and color blindness. Students of psychology were included only if they had not completed the second semester. This was to avoid possible confounding factors such as their knowledge on conditioning. Four participants interrupted the recording, and therefore they were not considered in the analysis. Also excluded were four participants who were nonresponders (mean startle amplitude < 5 μ V), six with too many artifacts (see Data reduction), and one participant because of moderate depressive scores (>28 in the Beck Depression Inventory, Hautzinger, Keller, & Kühner, 2006). After these exclusions, we considered 43 participants for the

¹For the sample sizes, we referred to Andreatta and Pauli (2015) and van den Akker, Schyns, and Jansen (2017). For future studies, a power analysis is strongly recommended.

analysis (10 male; mean age: 22.37 years, SD : 3.26; range: 18–30 years). Two participants were not native Germans, and seven were left-handed. Two participants were unaware of the CS-US associations after learning, while four were uncertain (see Procedure). We decided not to exclude these participants, because of normal responses, which did not affect results—meaning that the significant as well as the nonsignificant main and interaction effects remained constant. On average, participants presented normal weight ($M = 22.10$, $SD = 4.38$), but the body mass index ranged from 17.72 to 46.61.

2.2 | Material and apparatus

2.2.1 | Unconditioned stimulus

Two kinds of appetitive US were used, namely, chocolate (Smarties) or small salty pretzels. Participants could freely choose if they preferred the chocolate or the salty pretzels during the experiment. Thirty participants chose the chocolate and 13 the salty pretzels.

2.2.2 | Conditioned stimulus

Six white circles with different diameters were presented as CS. Thus, the smallest circle had 5 cm diameter, while the biggest circle had 10 cm diameter, and the other four circles had a gradually increasing diameter, that is, 6, 7, 8, and 9 cm. The visual stimuli were presented in the middle of a black computer screen for 8 s. The screen was approximately 60 cm from the participants' eyes.

2.2.3 | Startle probe

A white noise of 103 dB with duration of 50 ms was used as startle probe. The acoustic stimuli were presented binaurally over headphones and occurred randomly 4–6 s after onset of the shape.

2.2.4 | Questionnaires

Before the experiment, participants filled in the German version of the State-Trait Anxiety Inventory (STAI, Spielberger, Gorsuch, & Lushene, 1996), the Positive and Negative Affect Schedule (PANAS, Watson, Clark, & Tellegen, 1988), the Barratt Impulsiveness Scale (BIS-15, Spinella, 2007), and the Beck-Depression Inventory (BDI, Hautzinger et al., 2006).

The STAI assesses trait and state anxiety of the participants on two scales consisting of 20 items each. Trait anxiety of this sample ranged between 22 and 52 ($M = 36.72$, $SD = 8.05$). The PANAS is an index for positive and negative mood. Each item consists of an adjective, and participants

indicate on a scale ranging from 1 (*very slightly*) to 5 (*extremely*) to what extent the adjective reflects participants' feelings in that particular moment. The BIS-15 assesses the personality construct of impulsiveness, which ranged between 21 and 48 ($M = 31.53$, $SD = 6.53$). The BDI measure the severity of depressive symptoms in an individual. Scores ranging between 0 and 13 are considered minimal, between 14 and 19 indicate mild depression, between 20 and 28 indicate moderate depression, and scores higher than 29 indicate severe depression. According to these criteria, one participant in this sample presented high scores in the BDI and therefore was excluded from the analysis. In the end, BDI scores ranged from 0 to 22 ($M = 6.72$, $SD = 5.73$).

STAI state as well as PANAS were collected at the beginning and at the end of the experiment. Simple contrasts (α level was set at 0.05) revealed that participants' anxiety level at the end of the experiment ($M = 38.16$, $SD = 9.01$) was significantly higher, $F(1, 42) = 8.87$, $p = 0.005$, partial $\eta^2 = 0.174$, than at the beginning ($M = 33.88$, $SD = 5.75$). Paralleling the state anxiety, participants reported significantly higher negative mood ($M = 14.14$, $SD = 5.15$; $F(1, 42) = 6.62$, $p = 0.014$, partial $\eta^2 = 0.136$) and less positive mood ($M = 26.63$, $SD = 7.13$; $F(1, 42) = 15.62$, $p < 0.001$, partial $\eta^2 = 0.271$) at the end than at the beginning (negative mood: $M = 12.26$, $SD = 3.25$; positive mood: $M = 30.12$, $SD = 5.63$). Similar to previous studies (e.g., Andreatta & Pauli, 2015), we believe that these changes are due to the long protocol during which aversive startle probes were presented.

2.3 | Procedure

Participants were required to come to the laboratory in the morning without breakfast, in order to ensure that they were hungry (see Andreatta & Pauli, 2015). Upon the arrival in the laboratory, participants read and signed an informed consent approved by the ethics committee of the Department of Psychology of the University of Würzburg. They were informed that the experiment consists of presenting circles, loud noises, and that they will be allowed to eat pieces of chocolate or salty pretzels at specific times during the experiment. They were not informed about the contingency between CS and US. After having filled in the questionnaires, the electrodes were attached.

During the habituation phase, the smallest and the largest circles were presented twice. Neither the US nor startle probes were delivered during this phase. The intertrial interval (ITI, i.e., the time between one stimulus offset and the next stimulus onset) varied between 18 and 25 s (mean: 21.5 s).

In order to decrease the initial startle reactivity, seven startle probes were presented every 7–15 s before the first acquisition phase. The following two acquisition phases were identical. One circle (CS+) was paired with the appetitive US

but not the other circle (CS⁻). Shapes were counterbalanced among participants. During each acquisition phase, the CS⁺ and the CS⁻ were presented six times each (12 trials in total). Participants received the appetitive US circa 4 s after CS⁺ onset in four trials by the experimenter, who stood behind the participant and held out a jar with the US, remaining silent (i.e., without giving any instruction). Participants then took with the dominant hand one rewarding US and ate it. CS sequence was pseudorandomized such that the same stimulus was not presented more than twice in a row.

During the generalization phase, participants saw the CS⁺ and the CS⁻ again as well as four additional GS for six times, each leading to 36 trials in total. CS⁺ was still partially reinforced, but the US was delivered only two times. Again, a pseudorandom order was used (i.e., the same stimulus was not presented more than twice consecutively).

During acquisition and generalization phases, startle probes were presented randomly during three of the six stimulus presentations for each CS type with the restriction that trials with startle probes were not repeated more than three times consecutively. The ITI duration was the same as for the habituation phase, and three additional startle probes were delivered during the ITI randomly between 9 and 13 s after CS offset.

After each phase, participants viewed each circle for 1 s and then rated, by pressing a button on the keyboard, the valence (pleasantness: How pleasant or rather unpleasant is the stimulus?) and the arousal (intensity: How arousing is the stimulus?) of the CS using visual analog scales (VAS). The valence scale ranged from 1 (*very unpleasant*) to 9 (*very pleasant*), and for the arousal scale 1 (*calm*) and 9 (*exciting*). In addition, contingency awareness was assessed (How high is the probability that a reward was presented with the stimulus?) after the two acquisition phases and the generalization phase by asking participants whether the circle was associated with the chocolate or the salty pretzel on a VAS ranging from 0% (*never associated*) to 100% (*always associated*). We then considered contingency ratings after the second acquisition phase and calculated difference scores between CS⁺ and CS⁻. Participants were labeled as aware ($N = 37$) when such difference score was equal or higher than 70, uncertain ($N = 4$) when the score was between 69 and 50, and unaware ($N = 2$) when it was below 49. Importantly, during ratings the experimenter moved behind a shield.

In order to verify whether participants were hungry from the beginning until the end of the experiment, we asked them to rate their hunger (How hungry are you at this moment?) on a scale from 1 (*not hungry*) to 9 (*highly hungry*). Moreover, participants reported how much they liked the appetitive US (How much did you like the chocolate or pretzel?) on a VAS from 1 (*not at all*) to 9 (*a lot*). These ratings were collected before the habituation phase, after the second acquisition phase, and after the experiment.

2.4 | Data reduction

Physiological responses were recorded with a V-Amp 16 amplifier and Vision Recorder V-Amp Edition Software (Version 1.03.0004, Brain Products Inc., Munich, Germany). A sampling rate of 1000 and a 50 Hz notch filter were applied. The offline analyses of these responses were conducted with BrainVision Analyzer (Version 2.0; Brain Products Inc., Munich, Germany).

2.4.1 | Startle response

Startle response was measured by means of electromyography (EMG) at the left orbicularis oculi muscle with two 5 mm Ag/AgCl electrodes. According to the guidelines (Blumenthal et al., 2005), one electrode was positioned below the pupil and the second one 1 cm laterally. The ground and the reference electrodes were placed on the right and left mastoids, respectively. Before attaching the electrodes, the skin was slightly abraded and cleaned with alcohol in order to keep the impedance below 10 k Ω . The EMG signal was offline filtered with a 28 Hz low cutoff filter and a 400 Hz high cutoff filter. Then, the EMG signal was rectified, and a moving average of 50 ms was applied. As a baseline, we used the 50 ms before startle probe onset. Responses to startle probes were scored manually, and trials with excessive baseline shifts (5 μ V) or movement artifacts were excluded from further analysis. We excluded 3.90% of CS⁺ trials, 1.86% of CS⁻, 2.03% of GS, and 3.26% of ITI trials. Six participants were excluded from further analysis, because all three startle responses in one of the conditions were excluded due to the artifacts, leaving no startle responses for the analyses. Startle responses lower than 5 μ V were coded as zero and considered for the calculation of startle magnitude (Blumenthal et al., 2005). The peak amplitude was defined as the maximum peak relative to the baseline during the 20–120 ms time window after startle probe onset. If the mean startle magnitude throughout all conditions was lower than 5 μ V, participants were labeled as nonresponders and excluded from the analysis. The raw data were then within-participant transformed into T scores and averaged for each condition (CS⁺, CS⁻, GS1, GS2, GS3, GS4, and ITI) separately for the acquisition phases and the generalization phase.

2.4.2 | Skin conductance response

SCR was recorded using two 8 mm Ag/AgCl electrodes placed on the palm of the nondominant hand. The galvanic response was offline filtered with 1 Hz high cutoff filter. The SCR was defined as the difference (in μ S) between the first response onset (1–4 s after stimulus onset) and the first response peak following the responses onset (see the

guidelines, Boucsein et al., 2012). Trials containing startle probes were excluded from the analysis. Responses below $0.02 \mu\text{S}$ were coded as zero. Four additional participants were considered as nonresponders (mean SCR $< 0.02 \mu\text{S}$) and excluded only for these analyses (final $N = 39$). We then applied a range correction transformation considering the maximum SCR among the conditions throughout all phases, in order to reduce interindividual difference in this response. Afterward, scores were averaged for each condition, separately for the two acquisition phases (CS+, CS-) and the generalization phase (CS+, GS4, GS3, GS2, GS1, CS-).

2.5 | Statistical analyses

All data were analyzed with SPSS for Windows (Version 23.0, SPSS Inc.). For the verbal and physiological responses, separated analyses of variance (ANOVAs) were calculated. We performed two kinds of analysis. On the one hand, we tested the appetitive conditioned responses by calculating ANOVAs with stimulus (for the ratings and SCR: CS+, CS-; for the startle responses: CS+, CS-, ITI) and phase (for the valence and arousal ratings: habituation, Acquisition 1, Acquisition 2, generalization; for the contingency ratings and the physiological responses: Acquisition 1, Acquisition 2, generalization) as

within-subject factors. On the other hand, we tested the generalization gradient by calculating ANOVAs with stimulus (for the ratings and the SCR: CS+, GS4, GS3, GS2, GS1, CS-; for the startle responses: CS+, GS4, GS3, GS2, GS1, CS-, ITI) as the within-subject factor. Moreover, we recalculated the ANOVA for the startle response but then excluding the ITI from the within-subject factor stimulus in order to better detect generalization processes. Notably, for further analysis of the generalization gradient, we also reported the linear and quadratic trend analysis (see Lissek et al., 2010).

The alpha (α) level was set at 0.05 for all analyses. In case of violation of the sphericity assumption, the Greenhouse-Geisser (GG) correction was applied. The effect size is reported as partial η^2 as well as the confidence intervals around the estimated effect sizes (90% CI).

3 | RESULTS

3.1 | Manipulation check

Hunger ratings slightly decreased with time, $F(2, 82) = 3.02$, $p = 0.054$, partial $\eta^2 = 0.069$, 90% CI [0.000, 0.156] (Figure 1a), but there was no difference between those who received the chocolate from those who received the salty pretzel

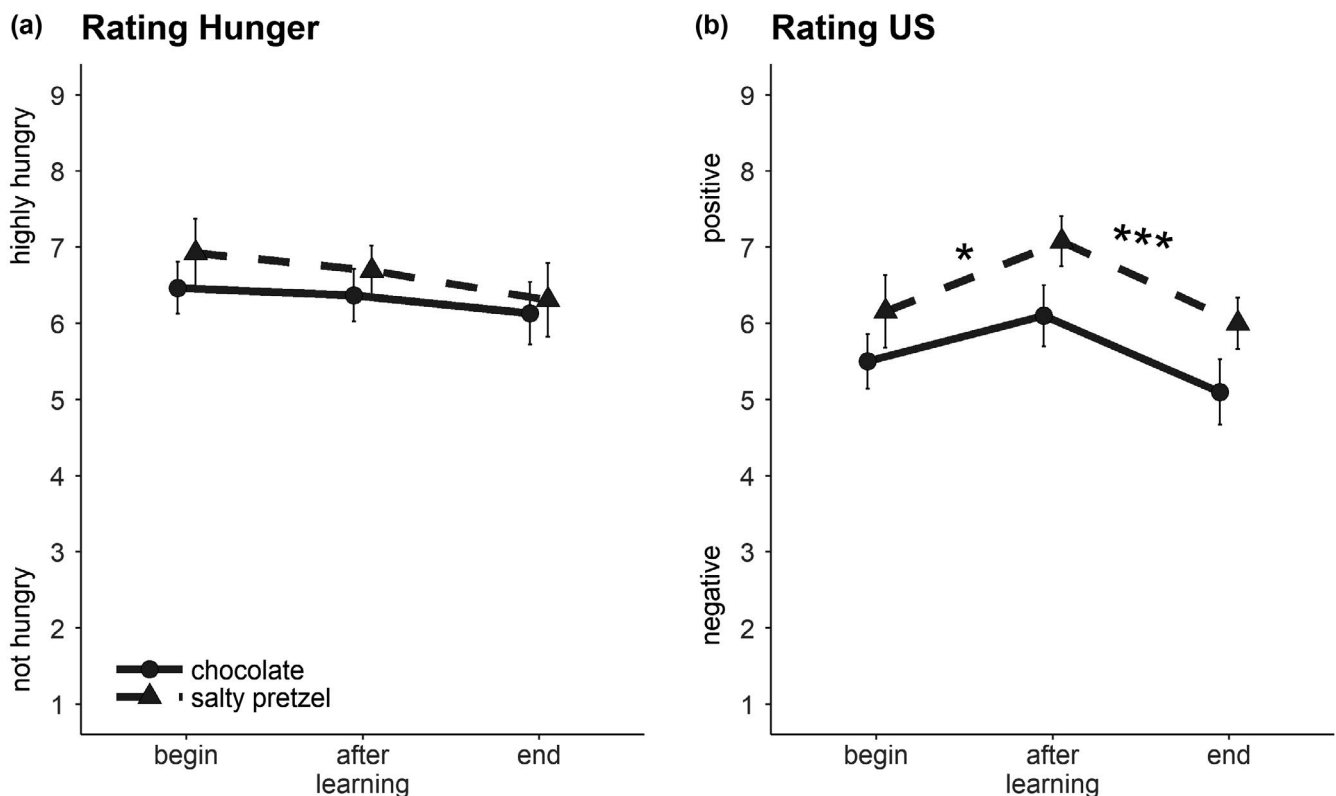


FIGURE 1 Hunger (a) and US ratings (b) at the beginning of the experiment, after the second acquisition phase (after learning), and at the end of the experiment. Hunger of the participants slightly decreased through the experiment (left). US were rated as appetitive throughout the experiment (right). Such ratings were significantly higher after the learning phases as compared to the beginning or the end of the experiment. Comparisons were Bonferroni corrected ($p < 0.017$). * $p < 0.02$; *** $p < 0.001$

(main effect US Group: $F(1, 41) = 0.30, p = 0.588$, partial $\eta^2 = 0.007$, 90% CI [0.000, 0.098]; Interaction Time \times US Group: $F(2, 82) = 0.26, p = 0.772$, partial $\eta^2 = 0.006$, 90% CI [0.000, 0.039]). At the beginning, participants rated their hunger at 6.60 ($SD = 1.79$) indicating that they followed the dietary protocol. Participants indicated that they were slightly less hungry after learning or at the end of the experiment.

We found significant changes in the valence ratings for the appetitive US through the experiment, $F(2, 82) = 7.73, p < 0.001$, partial $\eta^2 = 0.159$, 90% CI [0.046, 0.265] (Figure 1b). Again, no difference was revealed between participants who received the chocolate or the salty pretzel (main effect US group: $F(1, 41) = 2.13, p = 0.152$, partial $\eta^2 = 0.049$, 90% CI [0.000, 0.184]; Interaction Time \times US Group: $F(2, 82) = 0.19, p = 0.827$, partial $\eta^2 = 0.005$, 90% CI [0.000, 0.031]). Surprisingly, post hoc simple contrasts (after Bonferroni correction, $p < 0.017$) indicated that the US was rated more positively after learning than at the beginning, $F(1, 41) = 6.95, p = 0.012$, partial $\eta^2 = 0.145$, 90% CI [0.019, 0.304], or at the end, $F(1, 41) = 18.04, p < 0.001$, partial $\eta^2 = 0.306$, 90% CI [0.119, 0.459]; no difference was found between the beginning and the end in the US ratings, $F(1,$

$41) = 0.94, p = 0.337$, partial $\eta^2 = 0.022$, 90% CI [0.000, 0.137].

3.2 | Analysis of the conditioned responses

3.2.1 | Ratings

The ANOVA for both valence (Figure 2a) and contingency ratings (Figure 2c) revealed significant main effects of stimulus (valence: $F(1, 42) = 28.23, p < 0.001$, partial $\eta^2 = 0.402$, 90% CI [0.207, 0.540]; contingency: $F(1, 42) = 321.44, p < 0.001$, partial $\eta^2 = 0.884$, 90% CI [0.822, 0.913]) and the interactions between stimulus and phase (valence: $F(3, 126) = 12.91, p < 0.001$, partial $\eta^2 = 0.235$, 90% CI [0.122, 0.321]; contingency: $F(2, 84) = 25.22, GG-\epsilon = 0.759, p < 0.001$, partial $\eta^2 = 0.375$, 90% CI [0.214, 0.492]). The main effect of phase was significant for contingency, $F(2, 84) = 65.25, GG-\epsilon = 0.786, p < 0.001$, partial $\eta^2 = 0.608$, 90% CI [0.474, 0.687], but not valence, $F(3, 126) = 2.19, GG-\epsilon = 0.711, p = 0.115$, partial $\eta^2 = 0.050$, 90% CI [0.000, 0.124], ratings.

Follow-up simple contrasts (Bonferroni corrected, for the valence ratings, $p < 0.013$; for the contingency ratings,

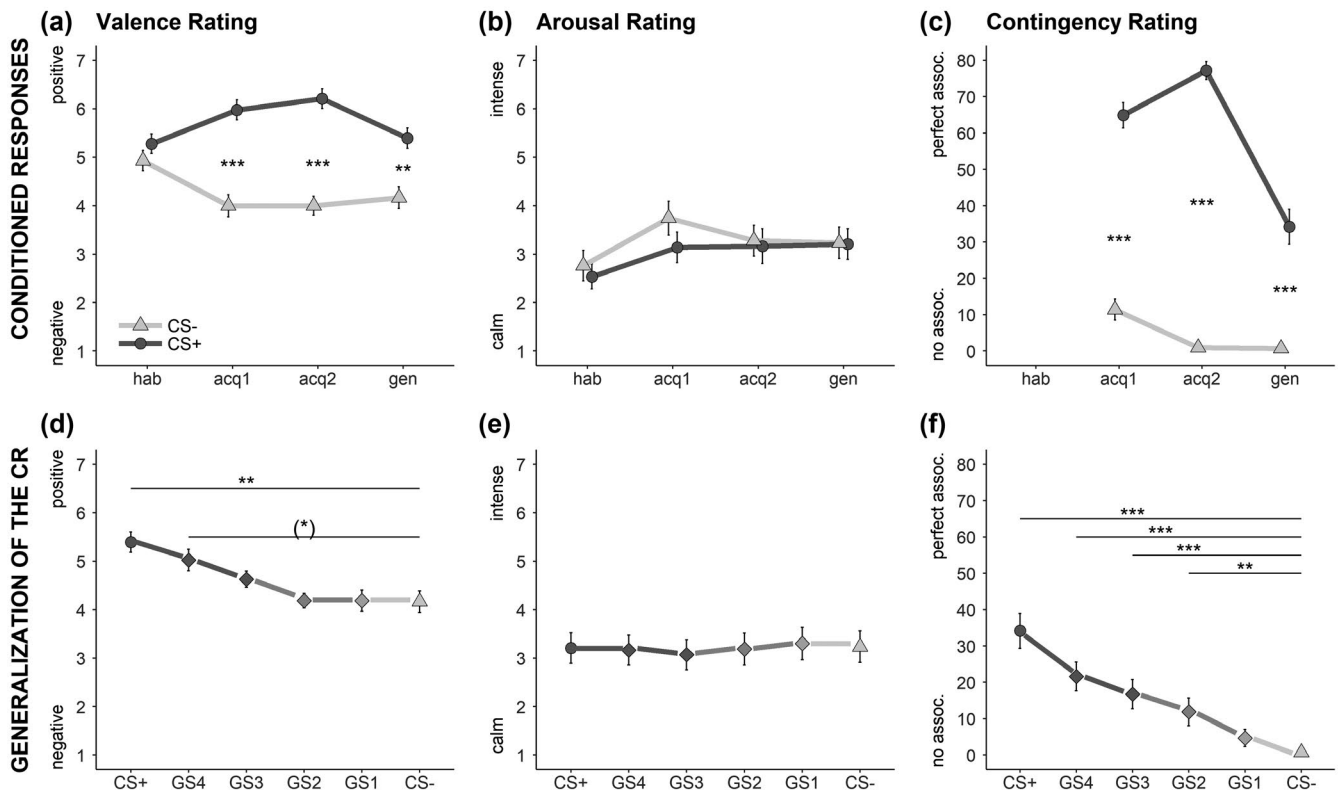


FIGURE 2 Valence (a,d), arousal (b,e), and contingency (c,f) ratings after habituation (hab), Acquisition 1 (acq1), Acquisition 2 (acq2), and generalization (gen) phase. Successful appetitive conditioning (upper) is indicated by more positive valence and higher association with the US for CS+ (dark gray lines with standard errors) compared to the CS- (light gray lines with standard errors). Generalization (lower) of appetitive conditioned responses is indicated by more positive valence and higher association with the US by GS versus CS-. Comparisons were Bonferroni corrected (for valence ratings, $p < 0.013$; for all other responses, $p < 0.017$). * $p < 0.05$; ** $p > 0.01$; *** $p < 0.001$

$p < 0.017$) indicated no significant difference for valence ratings of the two circles at the beginning of the experiment, $F(1, 42) = 1.25, p = 0.270$, partial $\eta^2 = 0.029$, 90% CI [0.000, 0.148]. After Acquisition 1 (valence: $F(1, 42) = 29.29, p < 0.001$, partial $\eta^2 = 0.411$, 90% CI [0.216, 0.547]; contingency: $F(1, 42) = 86.73, p < 0.001$, partial $\eta^2 = 0.674$, 90% CI [0.522, 0.754]), Acquisition 2 (valence: $F(1, 42) = 52.13, p < 0.001$, partial $\eta^2 = 0.213$, 90% CI [0.371, 0.662]; contingency: $F(1, 42) = 783.92, p < 0.001$, partial $\eta^2 = 0.949$, 90% CI [0.921, 0.962]), and generalization (valence: $F(1, 42) = 11.35, p = 0.002$, partial $\eta^2 = 0.213$, 90% CI [0.055, 0.372]; contingency: $F(1, 42) = 47.57, p < 0.001$, partial $\eta^2 = 0.531$, 90% CI [0.345, 0.644]), participants rated CS+ compared to CS- more positively and with a higher contingency.

The ANOVA for the arousal ratings (Figure 2b) only returned a significant main effect of phase, $F(3, 126) = 4.96$,

$GG-\epsilon = 0.618, p = 0.011$, partial $\eta^2 = 0.106$, 90% CI [0.014, 0.208], but no other significant effects (all $ps > 0.426$).

3.2.2 | Startle responses

The ANOVA returned significant main effects of stimulus, $F(2, 84) = 17.89, p < 0.001$, partial $\eta^2 = 0.299$, 90% CI [0.159, 0.406] (Figure 3a) and phase, $F(2, 84) = 13.95, p < 0.001$, partial $\eta^2 = 0.249$, 90% CI [0.115, 0.358], but no interaction effect, $F(4, 168) = 1.37, p = 0.252$, partial $\eta^2 = 0.032$, 90% CI [0.000, 0.065]. Simple contrasts (Bonferroni corrected $p < 0.017$) for the main effect of stimulus revealed successful appetitive conditioning as startle responses elicited during CS+ were more attenuated than during CS-, $F(1, 42) = 31.71, p < 0.001$, partial $\eta^2 = 0.430$, 90% CI [0.235, 0.563]. Startle magnitude elicited during ITI was comparable to CS+, $F(1, 42) = 1.49, p = 0.229$, partial $\eta^2 = 0.034$, 90%

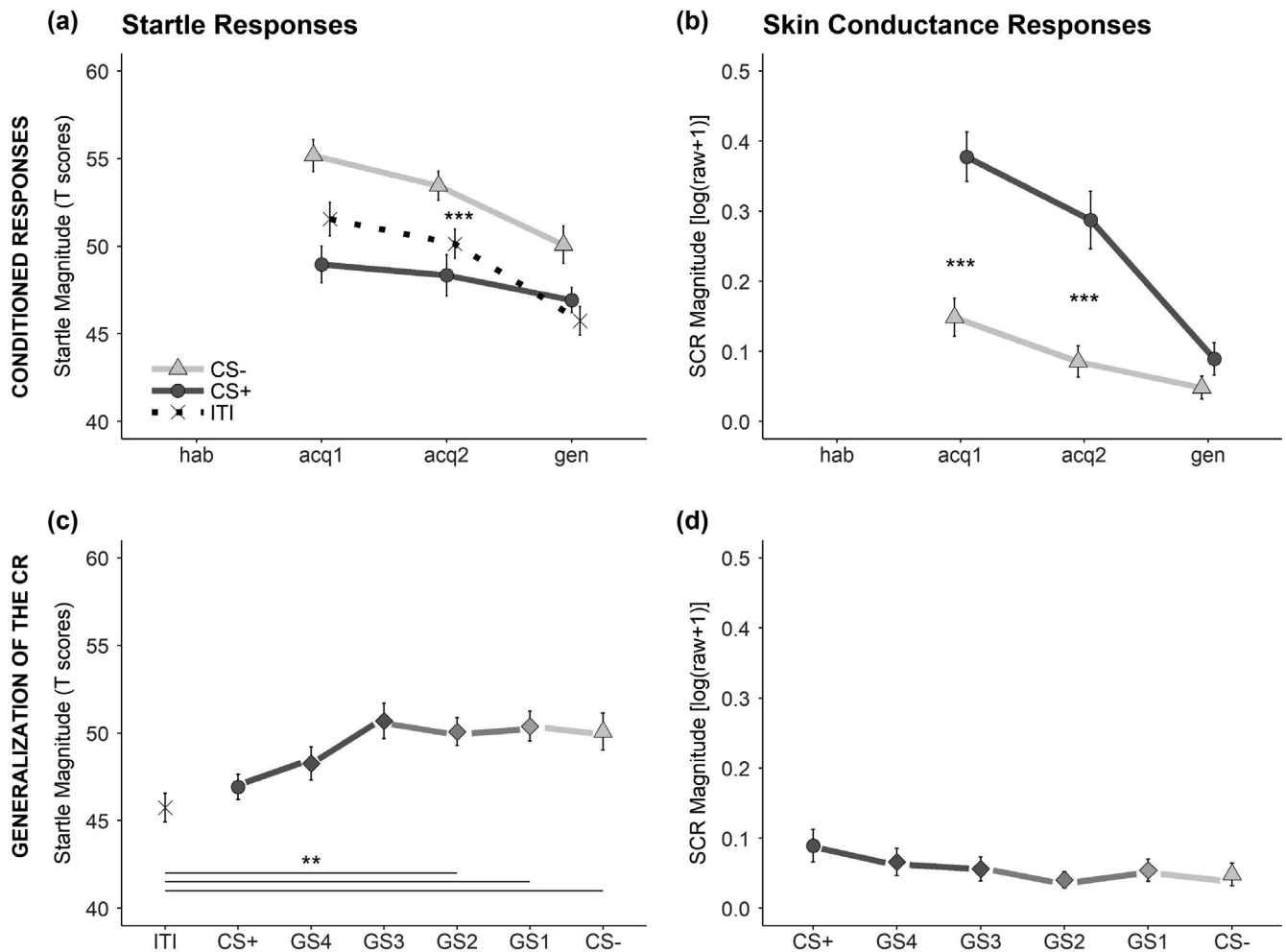


FIGURE 3 Startle (a,c) and skin conductance (b,d) responses during Acquisition 1 (acq1), Acquisition 2 (acq2), and generalization (gen) phase. Successful appetitive conditioning (upper) is indicated by startle attenuation and larger SCR to CS+ (dark gray lines with standard errors) as compared to the CS- (light gray lines with standard errors). Generalization (lower) of appetitive conditioned responses is indicated by significant linear trend for both startle responses and SCRs. Comparisons were Bonferroni corrected (for startle response, $p < 0.013$; for SCR, $p < 0.017$).

** $p > 0.01$; *** $p > 0.001$

CI [0.000, 0.158], and significantly lower than to CS–, $F(1, 42) = 21.54$, $p < 0.001$, partial $\eta^2 = 0.339$, 90% CI [0.149, 0.487].

3.2.3 | Skin conductance response

The ANOVA returned significant main effects for stimulus, $F(1, 38) = 57.74$, $p < 0.001$, partial $\eta^2 = 0.603$, 90% CI [0.420, 0.703], and phase, $F(2, 76) = 29.51$, $p < 0.001$, partial $\eta^2 = 0.437$, 90% CI [0.287, 0.535], as well as their interaction, $F(2, 76) = 6.88$, $p = 0.002$, partial $\eta^2 = 0.153$, 90% CI [0.039, 0.263] (Figure 3b). Post hoc simple contrasts (Bonferroni corrected, $p < 0.017$) for the interaction indicated significantly larger SCRs to CS+ compared to CS– during Acquisition 1, $F(1, 38) = 26.39$, $p < 0.001$, partial $\eta^2 = 0.410$, 90% CI [0.204, 0.552], and Acquisition 2, $F(1, 38) = 22.45$, $p < 0.001$, partial $\eta^2 = 0.371$, 90% CI [0.168, 0.520], but not during generalization, $F(1, 38) = 3.51$, $p = 0.069$, partial $\eta^2 = 0.084$, 90% CI [0.000, 0.239]. Hence, SCR indicates successful appetitive conditioning.

3.2.4 | Additional comparisons

We calculated 2 (Stimulus) \times 3 (Phase) \times 2 (US Type: chocolate, salty pretzel) ANOVAs in order to verify differences depending on the used US. No significant differences were found when comparing valence (all $ps > 0.140$), arousal (all $ps > 0.174$), and contingency (all $ps > 0.143$) ratings as well as startle responses (all $ps > 0.088$) and SCR (all $ps > 0.091$) to the CS+ associated with the chocolate versus the CS+ associated with the salty pretzel after each phase.

3.3 | Analysis of the generalization processes

3.3.1 | Ratings

ANOVAs for both valence, $F(5, 210) = 8.02$, $GG-\epsilon = 0.407$, $p = 0.001$, partial $\eta^2 = 0.160$, 90% CI [0.048, 0.265] (Figure 2a) and contingency, $F(5, 210) = 16.60$, $GG-\epsilon = 0.607$, $p < 0.001$, partial $\eta^2 = 0.283$, 90% CI [0.166, 0.368] (Figure 2c) ratings returned significant main effects for stimulus, but not the ANOVA for arousal ratings, $F(5, 210) = 0.20$, $GG-\epsilon = 0.489$, $p = 0.859$, partial $\eta^2 = 0.005$, 90% CI [0.000, 0.021] (Figure 2b).

Post hoc simple contrast (Bonferroni corrected, $p < 0.013$) comparing the CS– with all other stimuli indicated that participants generalized the appetitive conditioned valence to GS4 (i.e., the most similar to CS+; $F(1, 42) = 5.45$, $p = 0.024$, partial $\eta^2 = 0.115$, 90% CI [0.008, 0.269]), which, however, did not remain after the Bonferroni correction. No generalization of appetitive conditioned stimulus was found to the other GS (all $ps > 0.070$). Interestingly, participants broadly generalized their contingency responses, as contingency

ratings compared to the CS– were significantly increased for GS4, $F(1, 42) = 28.29$, $p < 0.001$, partial $\eta^2 = 0.403$, 90% CI [0.207, 0.540], GS3, $F(1, 42) = 17.08$, $p < 0.001$, partial $\eta^2 = 0.289$, 90% CI [0.108, 0.443], and GS2, $F(1, 42) = 9.52$, $p = 0.004$, partial $\eta^2 = 0.185$, 90% CI [0.039, 0.344], but not for GS1, $F(1, 42) = 3.80$, $p = 0.058$, partial $\eta^2 = 0.083$, 90% CI [0.000, 0.229].

Following Lissek et al. (2008, 2010), we also performed trend analyses to test the shape of the generalization gradients. These analyses revealed significant linear trends for both valence, $F(1, 42) = 11.09$, $p = 0.002$, partial $\eta^2 = 0.209$, 90% CI [0.053, 0.368], and contingency, $F(1, 42) = 41.53$, $p < 0.001$, partial $\eta^2 = 0.497$, 90% CI [0.306, 0.617], ratings, but not for arousal ratings, $F(1, 42) = 0.07$, $p = 0.796$, partial $\eta^2 = 0.002$, 90% CI [0.000, 0.062]. Moreover, we found a significant quadratic trend for valence, $F(1, 42) = 8.19$, $p = 0.007$, partial $\eta^2 = 0.163$, 90% CI [0.028, 0.322], but not for contingency or arousal ratings (all $ps > 0.287$).

3.3.2 | Startle responses

The ANOVA revealed a significant main effect for stimulus, $F(6, 252) = 4.53$, $p < 0.001$, partial $\eta^2 = 0.097$, 90% CI [0.031, 0.139] (Figure 3a), and this effect remained significant even after having excluded the ITI, $F(5, 210) = 2.54$, $p = 0.030$, partial $\eta^2 = 0.057$, 90% CI [0.003, 0.094]. However, post hoc tests (Bonferroni corrected, $p < 0.013$) revealed no significant difference in startle responses to CS– and the GS (all $ps > 0.188$).²

Trend analyses revealed a significant linear, $F(1, 42) = 25.22$, $p < 0.001$, partial $\eta^2 = 0.375$, 90% CI [0.181, 0.518], and quadratic, $F(1, 42) = 4.88$, $p = 0.033$, partial $\eta^2 = 0.104$, 90% CI [0.005, 0.256], trend. Additionally, by excluding the level ITI from the within-subject factor stimulus, the ANOVA returned a significant linear trend, $F(1, 42) = 9.01$, $p = 0.005$, partial $\eta^2 = 0.177$, 90% CI [0.035, 0.336], but not a quadratic trend, $F(1, 42) = 3.84$, $p = 0.057$, partial $\eta^2 = 0.084$, 90% CI [0.000, 0.231].

²Additional comparisons to ITI indicated that startle responses were stronger when elicited during GS1, $F(1, 42) = 18.82$, $p < 0.001$, partial $\eta^2 = 0.309$, 90% CI [0.124, 0.461]; GS2, $F(1, 42) = 17.07$, $p < 0.001$, partial $\eta^2 = 0.289$, 90% CI [0.108, 0.443]; and GS3, $F(1, 42) = 13.97$, $p = 0.001$, partial $\eta^2 = 0.250$, 90% CI [0.079, 0.407]; but not GS4, $F(1, 42) = 3.52$, $p = 0.067$, partial $\eta^2 = 0.077$, 90% CI [0.000, 0.222], and these comparisons survived after Bonferroni correction ($p < 0.013$). As suggested by a reviewer, we compared startle responses to the GS with startle responses to the CS+. We found after Bonferroni correction ($p < 0.013$) greater startle magnitude elicited during GS1, $F(1, 42) = 9.30$, $p = 0.004$, partial $\eta^2 = 0.116$, 90% CI [0.037, 0.340]; GS2, $F(1, 42) = 9.52$, $p = 0.004$, partial $\eta^2 = 0.185$, 90% CI [0.039, 0.344]; and GS3, $F(1, 42) = 7.19$, $p = 0.010$, partial $\eta^2 = 0.209$, 90% CI [0.020, 0.304]; but not GS4, $F(1, 42) = 1.22$, $p = 0.277$, partial $\eta^2 = 0.028$, 90% CI [0.000, 0.147]. These effects suggest discriminative responses between CS– and all GS except GS4, the stimulus most resembling the CS+.

3.3.3 | Skin conductance response

SCR did not show generalization, as the ANOVA returned no significant main effect for the stimulus, $F(5, 190) = 1.43$, $GG-\epsilon = 0.684$, $p = 0.233$, partial $\eta^2 = 0.036$, 90% CI [0.000, 0.065] (Figure 3b). Again, we calculated trend analyses for the SCR generalization gradient and found a significant linear trend, $F(1, 38) = 4.49$, $p = 0.041$, partial $\eta^2 = 0.106$, 90% CI [0.002, 0.265], but not quadratic trend, $F(1, 38) = 1.45$, $p = 0.235$, partial $\eta^2 = 0.037$, 90% CI [0.000, 0.169].

3.3.4 | Additional analyses

Similar to the analysis for the conditioned responses, we calculated 5 (Stimulus) \times 2 (US Type: chocolate, salty pretzel) ANOVAs in order to verify differences depending on the used US (see online supporting information, Figure S1). We found no significant differences in the generalization gradients for arousal (all $ps > 0.173$) and contingency (all $ps > 0.444$) ratings as well as for startle responses (all $ps > 0.436$) and SCR (all $ps > 0.191$). However, we found a significant main effect of US type for the valence ratings, $F(1, 41) = 5.07$, $p = 0.030$, partial $\eta^2 = 0.110$, 90% CI [0.006, 0.265], meaning that stimuli were rated more positively in participants who received the salty pretzels (valence: $M = 4.96$, $SD = 0.54$) compared to participants who received the chocolate (valence: $M = 4.44$, $SD = 0.75$). However, the interaction Stimulus \times US Type did not reach the significance level (valence: $F(5, 205) = 0.61$, $GG-\epsilon = 0.406$, $p = 0.546$, partial $\eta^2 = 0.015$, 90% CI [0.000, 0.064]).

4 | DISCUSSION

In this study, we investigated appetitive conditioned responses as well as subsequent generalization gradients in humans. During acquisition, one visual stimulus (CS+, a circle) was paired with a reward (either chocolate or salty pretzel according to participants' preference), while another circle (CS-, circle of different size) was never followed by the reward. We found successful acquisition of conditioned appetitive responses in all dependent variables, except for arousal ratings. Specifically, after the two learning phases, the reward signal (CS+) was rated more positively and strongly associated with the reward than the CS-. The lack of effects on arousal ratings is in line with previous studies and may be due to the low arousal of the US (for a broader discussion, see Andreatta & Pauli, 2015). As previously found (Andreatta & Pauli, 2015), SCR dissociated from arousal ratings, meaning that this response was significantly stronger to CS+ as compared to CS-. Conceivably, participants needed physiological activation in order to perform the approach movement and reach the

appetitive US, while on the verbal-reflective level, participants might not have found the appetitive US particularly arousing as a threatening event (Andreatta & Pauli, 2015). Moreover, the CS+ compared to the CS- elicited significantly attenuated startle responses. According to animal studies (Koch, 1999), startle attenuation is induced by the inhibitory projections from the nucleus accumbens (NAcc) to the caudal pontine reticular nucleus. Notably, NAcc is part of the striatum and mainly involved in processing of rewarding events (Bromberg-Martin, Matsumoto, & Hikosaka, 2010). Therefore, our results suggest that the presentation of the appetitive CS might have activated the NAcc, which consequently inhibited the startle response.

On the other hand, large SCRs indicate strong physiological activation, possibly necessary for initiating a behavioral response (Lang, Davis, & Öhman, 2000). Notably, startle response could be attenuated and SCR increased by the performance of a movement (Löw, Weymar, & Hamm, 2015). However, startle potentiation during a CS+ associated with either an aversive US or a nonaversive US was facilitated by behavioral responses (Lipp, Siddle, & Dall, 2003). Thus, although our paradigm does not allow disentangling the effects of an appetitive conditioned response from those of a mere movement (or its preparation) and future studies should disentangle these two components, it seems more likely that startle attenuation elicited during CS+ resulted from genuine appetitive learning rather than from mere movement preparation. Notably, startle responses elicited during CS- were stronger than startle responses elicited by the noise alone (i.e., during the ITI). Considering that CS- in aversive paradigms becomes a safety signal (Lissek et al., 2005; Seligman & Binik, 1971), the startle potentiation during CS- in this appetitive paradigm might indicate that this stimulus becomes an aversive signal, possibly of frustration for the missing reward.

We conclude on the basis of the registered verbal and physiological indices of learning that the realized appetitive conditioning paradigm caused the CS+ to elicit not only positive valence and reward expectancy but also appetitive behavioral responses. Importantly, these findings are in line with previous studies, which applied an appetitive conditioning protocol using either a primary reward such as food (Andreatta & Pauli, 2015; Blechert et al., 2016; Kim et al., 2011; van den Akker, Nederkoorn, & Jansen, 2017; van den Akker, Schyns, & Jansen, 2017), odors (Stussi, Delplanque, Coraj, Pourtois, & Sander, 2018), erotic pictures (Klucken et al., 2015), or a secondary reward such as money (Kruse et al., 2017; Tapia León et al. 2018). However, it should be kept in mind that the delivery of the US was not automatized, and, although the experimenter strictly followed a protocol, this was a limitation when considering the importance of temporal relation in classical conditioning (Rescorla, 1988).

During generalization, both CS+ and CS− were presented as well as four additional generalization stimuli, of which similarity gradually changed to the conditioned stimuli. We found generalization for appetitive conditioned responses in valence and contingency ratings but not in arousal ratings. In other words, our participants generalized the positive valence of the CS+ to the next most similar generalization stimulus (i.e., GS4) and exhibited an increased expectancy for the US for all GS except for the most dissimilar with the CS+ (i.e., GS1). Although the effect of positive valence generalization to the GS4 should be considered with caution because it was no longer statistically significant after Bonferroni correction, it is interesting to note that similar differences in generalization gradients have been observed for conditioned fear, that is, relatively strong generalization for contingency ratings and weaker generalization for valence ratings (Ahrens et al., 2016; Lissek et al., 2008, 2010).

On the physiological level, we observed a rather weak generalization for startle responses and SCRs. In particular, both startle responses and SCRs showed a significant linear trend indicating linear decrease of the startle responses from CS− to CS+, but no direct evidence that these responses generalized to the GS. Therefore, this effect needs to be replicated. Although rather weak, such generalization in startle responses corresponds quite well to comparable findings of fear generalization in healthy participants with aversive US (Lissek et al., 2008, 2010; Onat & Buchel, 2015; Schiele et al., 2016).

The lack of discriminative SCRs during generalization phase may be due to strong habituation processes that characterize this responses (Boucsein et al., 2012; Stussi et al., 2018). In other words, studies on generalization of conditioned fear indicate that only anxiety patients overgeneralize their fear responses, while healthy individuals limit their fear to stimuli strongly resembling the threat signal (Ahrens et al., 2016; Lissek et al., 2010). In parallel, individuals of this study limited their generalization of the appetitive conditioned verbal responses to the stimulus that mostly resembled the signal of a reward. Considering that aversive conditioning is a good model for anxiety disorders (Craske et al., 2018; Mineka & Oehlberg, 2008) and that appetitive conditioning models eating disorders as well as substance-related disorders (Martin-Soelch et al., 2007; Sanchis-Segura & Spanagel, 2006), it would be interesting to verify whether patients with eating or substance-related disorders show an overgeneralization of their appetitive conditioned responses. As for anxiety disorders, overgeneralization of appetitive responses may be a risk factor for disorders characterized by appetitive behavior. This is even more conceivable if one considers that overweight women (van den Akker, Schyns, & Jansen, 2017) and high impulsivity individuals (van den Akker, Jansen, Frentz, & Havermans, 2013; but see Papachristou, Nederkoorn, Beunen, & Jansen, 2013) as well as depressed individuals

(Kumar et al., 2008) showed reduced discrimination between reward signal and nonreward signal.

We found no generalization for arousal ratings, which is not surprising considering that participants did not show conditioned responses for arousal. Although appetitive, the rewarding US was low arousing. As a potential consequence of these affective properties, we observed generalization of the valence-related measures (i.e., startle response, valence ratings) but not of the arousal-related measures (i.e., SCR, Lang et al., 2000). It is conceivable that more arousing appetitive US as erotic pictures (Klucken et al., 2015) or money (Delgado, Jou, & Phelps, 2011; Kruse et al., 2017) may have been more effective in eliciting a generalization gradient for arousal measures as well.

The pattern of startle response generalization of conditioned appetitive responses in humans revealed by this study resembles the pattern of generalization of conditioned aversive responses (Dunsmoor & Murphy, 2015; Dymond et al., 2015; Struyf et al., 2015). This conclusion, however, has to consider that appetitive conditioned physiological responses, unlike aversive conditioned physiological responses, are strongly modulated by any minimal change in CS-US contingency, meaning that as soon as no rewarding US is delivered, no appetitive physiological response is evident anymore (Andreatta & Pauli, 2015; van den Akker et al., 2013; van den Akker, Nederkoorn, & Jansen, 2017; van den Akker, Schyns, & Jansen, 2017). We were aware of such an influence of the CS-US contingency on the appetitive CR and therefore decided to deliver the rewarding US during the generalization phase. Considering that such delivery was halved, this change in CS-US contingency could have been enough to weaken the appetitive conditioned responses and consequently the generalization gradient as seen in startle responses and SCRs. One possible reason for the sensitivity to CS-US contingency changes is that for an organism's survival it is highly adaptive to continue avoiding a source of danger and to change this behavior only after considerable verification that the signal does not predict the threat any longer (Craske et al., 2018). While a signal does not reliably predict a source of food anymore, it may be more adaptive for the organism to change behavior quickly and search for other sources of nourishment.

In summary, we found successful appetitive conditioning as well as generalization of appetitive conditioned responses. Thus, the CS predicting a rewarding US was rated more positively and elicited stronger physiological arousal as well as attenuation of startle responses. Participants generalized appetitive learning by showing gradually increasing appetitive responses (i.e., contingency and valence ratings as well as startle responses and SCR) as the generalization stimuli became more similar to the CS+. Overall, the observed patterns of generalization were similar to those found for aversive conditioning. However,

the appetitive generalization gradients were weaker than the threatening generalization gradient as observed in the physiological and startle responses. This may be related to the different predictive meanings of these two learnings regarding organisms' survival.

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CONFLICT OF INTEREST

No conflicting interests are declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1

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