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Can you learn to starve yourself? Inducing food avoidance in the laboratory

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ABSTRACT

The restriction of energy intake is a central and persistent symptom of anorexia nervosa. Recent models of the disorder suggest that food restrictions are learned avoidance behaviours, which are acquired and maintained by classical and operant conditioning. The present study aims to test this learning model of food restriction. It investigates whether introducing negative consequences for the intake of tasty high-calorie food and introducing positive consequences for its avoidance can create food avoidance, increase fear of food, and decrease eating desires in healthy individuals. 104 women were randomly assigned to an experimental or control condition and completed an appetitive conditioning and avoidance learning task. While the experimental condition received money after *avoiding* the tasty high-calorie food item and heard an aversive sound after *not avoiding* food intake, the control condition never received these consequences. In the extinction phase, reward and punishment discontinued for both conditions. We measured avoidance frequency, mouse movements, fear, eating desires and stimulus liking. Participants in the experimental condition avoided the food more often than controls and showed increased fear, reduced eating desires and less liking for cues associated with food intake. These results support the notion that food avoidance behaviours, reduced eating desires and fear of food can be learned via classical and operant conditioning. Conditioning paradigms might be a useful tool to study the development and maintenance of food restriction in anorexia nervosa.

1. Introduction

Anorexia nervosa is a severe mental disorder that is marked by an intense fear of gaining weight, dysfunctional beliefs related to body, weight and shape, and restriction in calorie intake relative to energy needs, resulting in low body weight (American Psychiatric Association, 2013). Anorexia nervosa is one of the most lethal mental illnesses (Steinglass et al., 2011) with a crude mortality rate of 5.1% over 10 years (Fichter & Quadflieg, 2016). Treatment of anorexia nervosa is a real challenge: treatment dropout rates are high (~20–46%), many patients do not recover (~50%), relapse rates are high (~35–40%) and specialized treatments recommended by international clinical guidelines do not perform better than control treatments or treatment as usual (Murray et al., 2019; Solmi et al., 2021; van den Berg et al., 2019). A better understanding of the mechanisms responsible for the development and maintenance of anorexia nervosa is needed to improve treatments (Glashouwer et al., 2020; Jansen, 2016).

The fear of weight gain and food restriction are core symptoms of anorexia nervosa: patients avoid high-calorie foods, limit the range of foods they eat and experience reduced eating desires (Schaumberg et al., 2021; Steinglass et al., 2015; Stoner et al., 1996). Restrictive eating has been rarely conceptualized as an avoidance behaviour resulting from the fear of weight gain. However, such a conceptualization would allow for the application of well-established theoretical models and experimental paradigms to the study of anorectic symptoms: learning theory and conditioning tasks have been successfully used to explain and examine fear and avoidance for over 100 years (De Houwer & Hughes, 2020; Meulders, 2020). Accordingly, a detailed knowledge of the learning processes underlying food avoidance might be helpful in understanding the processes perpetuating anorexia nervosa and developing better treatments for the disorder (Melles et al., 2021). Therefore, we will experimentally test whether and how specific learning processes affect the development and maintenance of food avoidance behaviours and reduced eating desires in healthy women.

For healthy individuals high-calorie foods, especially when containing fat and sugar, are inherently appetitive stimuli (Simon et al., 2015). Eating behaviors and food preferences can easily be learned by appetitive conditioning (Jansen, 1998; Jansen et al., 2015; Koskina

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et al., 2013) during which a neutral cue or context, for example a food-related accessory, time or location (conditioned stimulus, CS) is paired with rewarding food intake (unconditioned stimulus, US). As the CS becomes a predictor of food intake (see e.g., van den Akker et al., 2013; Van Gucht et al., 2010), it is perceived as more pleasant and triggers food cravings, as well as approach behaviors (appetitive conditioned responses, CRs) (Wardle et al., 2018). Thereby, food cues can direct eating behaviors and are able to override homeostatic signals, such as satiety (Jansen et al., 2016; Koskina et al., 2013; Petrovich, 2011). To explain why individuals with anorexia nervosa show reduced appetitive conditioned responses and avoidance towards formerly rewarding food and eating-related cues (Steinglass et al., 2015; Stoner et al., 1996), an important role for fear and fear learning has recently been suggested (Cardi et al., 2019; Garcia-Burgos et al., 2019; Levinson & Byrne, 2015; Levinson & Williams, 2020; Melles et al., 2021; Murray et al., 2018; Papalini et al., 2021; Schaumberg et al., 2021; Steinglass et al., 2011, 2012; Strober, 2004). Individuals with anorexia nervosa report a range of fears and concerns related to eating, weight and shape; they are typically afraid of gaining weight, feeling full and/or experiencing disgust after eating (Glashouwer & de Jong, 2020; Melles & Jansen, 2023; Murray et al., 2016; Schaumberg et al., 2021; Steinglass et al., 2012). Via classical conditioning food and eating-related cues (Conditioned stimuli; CSs) may become associated with said aversive emotional states and negative outcomes (unconditioned stimuli; USs) (Hildebrandt et al., 2015; Melles et al., 2021). As a result, food and eating related cues come to trigger aversive conditioned responses (CRs) including heightened autonomic arousal, feelings of fear and avoidance tendencies. Operant conditioning then determines whether (food) avoidance is maintained in the long run (Krypotos et al., 2015; LeDoux, 2016; Melles et al., 2021). During operant conditioning individuals learn about the consequences of their avoidance behaviour, which increases or decreases the likelihood that they will avoid again in the future (De Houwer & Hughes, 2020). By avoiding food intake, the chance to experience the negative consequences of eating is limited and consequently feelings of anxiety and disgust are reduced (negative reinforcement) (Cardi et al., 2019; Hildebrandt et al., 2015; Steinglass et al., 2012; Steinglass et al., 2011). Once in place, (food) avoidance behaviours are persistent (Sidman, 1955) and might hinder individuals with anorexia nervosa from correcting their faulty expectations regarding the consequences of eating (protection from extinction) (Cornwell et al., 2013; Lovibond et al., 2009; Morriss et al., 2018). By repeatedly restricting food intake a patient might not notice that eating a 'forbidden' food does not lead to a loss of control or immediate weight gain (Melles et al., 2021). Thus, avoidance behaviours cement threat expectancies, and threat expectancies trigger avoidance (Pittig et al., 2020) – a vicious cycle that might explain patients' persistent eating-related fears and food avoidance.

Additionally, food avoidance behaviours could be maintained by their rewarding consequences (positive reinforcement): initial weight loss might be complimented by significant others and successful dieting might increase one's self-confidence, pride and sense of control (Coniglio et al., 2017; Dignon et al., 2006; Marzola et al., 2015; Selby & Coniglio, 2020; Walsh, 2013). As individuals with anorexia nervosa show heightened reward responses towards low-calorie food stimuli on brain imagining, psychophysiological and self-report measures (for a detailed discussion see Haynos et al., 2020) it has been suggested that restrictive eating gets associated with its positive consequences; food avoidance itself becomes rewarding (Walsh, 2013). This might explain why patients continue to avoid food intake after initial rewards, such as compliments of significant others, have ceased (Walsh, 2013).

In sum, if food avoidance is the behavioural output of a classically conditioned relationship between food and an aversive outcome (Hildebrandt et al., 2015), the behaviour is maintained by a reduction in anorectic fears (Cardi et al., 2019; Garcia-Burgos et al., 2019; Levinson & Byrne, 2015) and by its (initially) rewarding consequences. Learning theory provides a useful framework for understanding the development and maintenance of anorectic food restriction (see Christian & Levinson, 2022; Melles et al., 2021; Murray et al., 2018; Schaumberg et al., 2021; Strober, 2004). However, empirical studies testing this model are lacking.

Therefore, the present study tests whether classical and operant conditioning can trigger food avoidance, increase fear of food, reduce eating desires, and lower the liking of high-calorie foods in healthy individuals. Additionally, we want to learn more about the effect of food avoidance behaviours on fear and eating desires. For this, healthy participants complete a conditioning task: first they learn to associate a simple geometric shape with a tasty high-calorie food; then they receive negative consequences for eating this food item and positive consequences for avoiding it. We expect participants to consistently avoid the food and to show increased fear, reduced eating desires and less liking for predictors of food intake. Additionally, they are expected to display less liking for the food itself. Based on the 'protection from extinction effect' (Lovibond et al., 2009; Pittig et al., 2020) we expect these changes to persist when reward and punishment are discontinued. As food avoidance might constitute a strategy to manage eating related anxiety, changes in participants' responses to the high calorie food should aggravate when avoidance is not possible anymore.

2. Methods

2.1. Study design

Participants were assigned to the experimental or control condition. During a conditioning task they first learned about the association between a cue and the delivery of a tasty high-calorie food. In the following avoidance learning phase, participants could avoid food intake by clicking on a response box on the computer screen. In the experimental condition, participants gained money in case they *avoided* food intake, while they heard a loud and aversive sound in case, they did *not avoid* food intake. The control condition did not receive any consequences for avoiding or not avoiding the food outcome. In the extinction phase, the consequences for food intake and food avoidance were dropped; participants in the experimental condition were not punished or rewarded anymore. Several test trials were included in the task, on which participants could not avoid food intake (and the accompanying aversive sound).

2.2. Participants

Participants were recruited via advertisements on social media and the university research participation board. To be included in the study participants needed to be at least 16 years old and like the taste of milkshake. The exclusion criteria were a current psychiatric diagnosis, the use of psychotropic medication, a known neurological condition, visual problems that were not corrected, chronic ear problems (e.g., tinnitus), allergies against the ingredients of the milkshake and being pregnant. As a reimbursement, participants received course credits or a voucher over €15. Participants in the experimental condition additionally received the money that they gained during the computer task. The study was approved by the local ethical committee.

2.3. Apparatus and stimuli

2.3.1. Unconditioned stimulus

A small portion of milkshake (3 ml) served as the unconditioned stimulus (US). Participants could choose their preferred milkshake flavour from strawberry, chocolate and (vegan) vanilla. To guarantee a precise and fast administration (within 2 s), the milkshake was given to participants using a mouthpiece, silicone tube (inner diameter 4 mm) and syringe pump (NE-4000-ES Aladdin dual syringe pump). For an image of the pump set up consult Supplementary material 1. The syringe pump and computer task were programmed using Presentation (Version

21.1).

2.3.2. Reward and punishment

As a reward for avoiding the milkshake, the picture of a 50-cent coin and the text 'You won 50 cents' were presented on the computer screen for 2s. As a punishment for drinking the milkshake, participants heard an aversive scream (92 dB) via headphones for 2s. Monetary rewards and aversive sounds have been used successfully in previous learning tasks (Dibbets & Evers, 2017; Pittig, 2018).

2.3.3. Conditioned stimuli

Two geometrical shapes (triangle and circle) served as the conditioned stimuli. One of the shapes was always followed by the US (CS+), while the other shape was never followed by it (CS-). The assignment of the geometrical shapes to CSs was counterbalanced across participants, so that both geometrical shapes were paired with the US. The CSs were presented in a semi-randomized order meaning that the same CS was not shown more than two times in a row.

2.3.4. Avoidance cue and response

When the avoidance response was available, the text "You can avoid now!" popped up at the bottom of the screen and the labels "Avoid" and "Not avoid" appeared in the two response boxes in the left and right corner of the screen. Participants had to mouse click on "Avoid" to prevent US delivery and on "Not avoid" to receive the US.

2.4. Measures

An overview of the assessment time-points for each outcome measure is provided in Fig. 1 and in the description of the Conditioning task.

2.4.1. Avoidance behaviours

We used two measures to assess food avoidance: the actual avoidance response (Avoid vs. Not Avoid) and mouse movements while selecting the response option. When looking at a definite time interval, motor movements can reflect simultaneously occurring cognitive processes (Freeman, 2018; Koop & Johnson, 2011; Spivey & Dale, 2006). Accordingly, mouse tracking constitutes a validated measure to capture the cognitive processes during a choice as it offers insights into the competing attractiveness of response options and changes in response preferences (Dshemuchadse et al., 2013; Georgii et al., 2022; Sullivan et al., 2015). By looking at the relative directedness of movements from the start position to the selected response option, mouse tracking can provide information on indecisiveness and ambivalence during a choice (Pittig & Scherbaum, 2020). More details on the collection of the mouse tracking data are presented in Supplementary material 1.

2.4.2. US-expectancy

To track learning of the CS – US relationship, US-expectancy was measured on every trial. Participants rated their expectancy for the food US on a visual analogue scale (VAS; When you see this shape, to what extent do you expect that a sip of milkshake will follow?) with the anchors 0 = "certainly not" and 100 = "certainly".

2.4.3. Fearfulness and desire

To measure aversive conditioned responses, we asked participants about their fearfulness when seeing the CS ("When you see this shape, how strong is your fear right now?"). To assess appetitive conditioned responses, participants rated their desire for the milkshake ("When you see this shape, how strong is your desire for milkshake right now?"). Both questions were answered on a VAS ranging from 0 = "not strong at all" to 100 = "very strong". The presentation order of the desire and fearfulness VAS was randomized per trial.

2.4.4. Valence

At baseline and at the end of each phase in the conditioning task, participants rated the pleasantness of the CSs ("How pleasant do you find this shape?") on a 7-point Likert ranging from −3 = "very unpleasant" to 3 = "very pleasant". The presentation order of CS+ and CS- valence ratings was randomized per assessment time-point. Additionally, participants indicated how much they liked the milkshake on a 7-point Likert scale (−3 = "Did not like it at all", 3 = "Liked it very much") at the end of the acquisition, second and third test phase.

2.4.5. Manipulation check

Participants in the experimental condition rated how much they liked hearing the scream and winning the money on a 7-point Likert scale (−3 = "Did not like it at all", 3 = "Liked it very much") at the end of the conditioning task.

2.4.6. Relief and frustration

We also assessed how relieved and frustrated participants felt after not receiving the milkshake. These measurements followed on every trial in which the US was not delivered, and the avoidance response was possible. To ensure the readability of the present paper we decided to present these data in another publication.

2.4.7. Facial electromyography (EMG)

By tracking muscle activity, facial EMG can be used to make inferences about an individual's affective state (van Boxtel, 2010). To track appetitive conditioned responses, we measured activation in the *zygomaticus major* muscle area (Winkler et al., 2011). Moreover, we assessed activation in the *corrugator supercilli* as an indicator of aversive conditioned responses (Hildebrandt et al., 2015) and the *levator labii* as an indicator of conditioned disgust (Borg et al., 2016). A detailed description of EMG data collection and preparation can be found in Supplementary material 1.

2.4.8. BMI

The experimenter measured participants' height and weight in street clothes. Their *Body Mass Index (BMI)* was calculated by dividing their weight in kilograms by their height in meters^2.

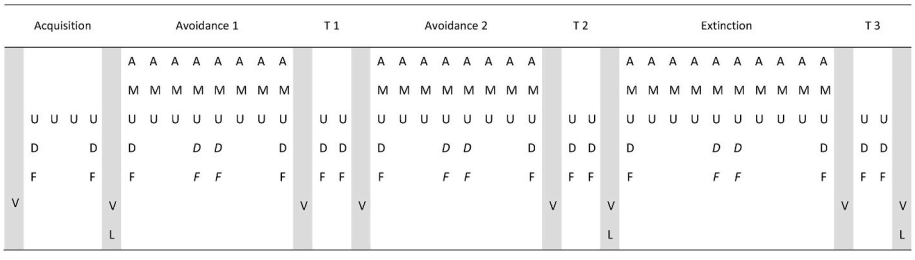

Fig. 1. Measurement Time Points across the Acquisition, Avoidance, Test and Extinction Phases for CS+ and CS- Trials.
Note. T = Test phase, A = Avoidance behaviour, M = mouse trajectory data, U=US-expectancy, D = Desire, F = Fearfulness, V=CS valence, L = Milkshake liking. Fearfulness and eating desires were measured in the middle of the avoidance and extinction phase. It was determined randomly whether these ratings took place on the 4th or 5th trial of the respective phase; these measurements are written in italics. The grey background indicates that measurements were obtained between trials.

2.4.9. Questionnaires

Participants completed several questionnaires on eating disorder symptoms, perfectionism, behavioural inhibition, intolerance of uncertainty, distress tolerance and disgust sensitivity at the end of the experiment. As analysing and discussing these data would go beyond the scope of the present paper, we decided to present them in another publication.

2.5. Procedure

Participants were tested individually between 10 a.m. and 18 p.m. They were instructed to not eat or drink anything besides water in the 2 h before visiting the laboratory. After their arrival, the experimenter briefly checked the inclusion and exclusion criteria. If participants were eligible, they received written and oral information about the study and signed the informed consent form. Next, participants practiced with the different components of the conditioning task: The experimental condition listened to the aversive sound once and could ask the experimenter to adjust the volume a bit (84 dB–90 dB), if it was perceived as painful or unbearable ($n = 9$); both conditions tested the pump set-up with water and rated the comfortableness of the procedure on a 7-point Likert scale (0 = "extremely uncomfortable"; 6 = "extremely comfortable"). Then, the experimenter cleaned participants' face and attached the EMG electrodes. Participants completed a short practice task (2 acquisition and 10 avoidance learning trials) with a neutral image (IAPS 7010) as the US and similar rating scales and avoidance cues as in the conditioning task. Before the start of the conditioning task, participants were reminded to keep their head still and to leave the mouse on the table. The conditioning task itself took approximately 30 min. Subsequently, participants answered several questions on the computer screen assessing their reasons for avoiding the milkshake (if applicable), the time of their last meal and whether they used medication on the day of the study. Then, they filled in the questionnaires. Lastly, the experimenter measured participants' height and weight, thanked them for their participation and handed out the reimbursement.

2.6. Conditioning task

At the start of the conditioning task participants were informed that from then on, they would repeatedly get to see two different pictures. One of these pictures would be followed by a sip of milkshake, while the other picture would not be followed by anything. The **acquisition phase** consisted of 4 CS+ and 4 CS- trials. An acquisition trial proceeded as follows: After the CS was presented on screen for 4 s, the VASs appeared under the stimulus. While the expectancy VAS followed on every trial, fearfulness and desire were only assessed on certain trials (see Fig. 1). Participants had 8 s per VAS to provide their answer. Once all ratings were made, the CS disappeared, and the US was delivered. During the 9 s inter-trial interval (ITI) a fixation cross was shown in the middle of the screen. One second before the start of the next trial the response boxes (without labels) appeared in the upper corners of the screen. Before the **avoidance phase** (16 CS+ and 16 CS- trials), participants were informed that from now on they could sometimes choose whether they wanted to drink the milkshake. The experimental condition was additionally notified that during the rest of the task they could sometimes hear the loud sound or win a small amount of money. By paying attention to the task, they should be able to predict these events. An avoidance trial proceeded as follows: One second before CS onset, the avoidance cue and response labels appeared on screen. There was no time restriction for clicking on one of the response boxes. After participants made their choice, the avoidance cue and response labels disappeared, while the CS remained on screen. After 2 s, the VASs appeared. On CS- trials, or CS + trials *with an avoidance response*, the relief and frustration scales immediately followed CS offset. From this point on, CS + trials proceeded differently for both conditions: After a 1.5 s break the experimental condition saw the picture of a 50-cent coin, while the control condition entered the ITI. On CS + trials *without an avoidance response*, the US was delivered immediately after CS + offset. In the experimental condition the aversive scream followed after a 1.5 s break; in the control condition the trial ended and the ITI started. During the 11 s ITI a fixation cross was shown in the middle of the screen. The timeline of avoidance trials is visualized in Fig. 2. In the middle and at the end of the avoidance phase, participants completed two **test phases** (2 CS+ and 2 CS- trials), in which the avoidance response was unavailable. Trials proceeded similarly to the acquisition phase besides that participants in the experimental condition heard the aversive scream 1.5 s after US delivery.

Next, participants entered an **extinction phase** (8 CS+ and 8 CS- trials). While nothing changed for the control condition, the consequences for (non-)avoidance were dropped in the experimental condition. Thus, participants in both conditions received the same treatment. Trials followed a similar course as in the avoidance phase. In the subsequent **third test** phase (2 CS+ and 2 CS- trials) the avoidance response was again unavailable. However, the aversive scream was not conveyed anymore irrespective of participants' condition. Trials proceeded similarly to the acquisition phase. The transition to the test and extinction phases was unannounced; participants needed to figure out the accompanying changes by themselves. An overview of the conditioning phases is presented in Fig. 3.

2.7. Preparation mouse tracking data

The mouse tracking data were processed in R using the *mousetrap* package (Wulff et al., 2021). Mouse trajectories were time normalized into 101 bins and rescaled so that every trajectory terminated in the top right corner. On 3.3% of the trials, participants needed more than 1.5 s to initiate movements and on 8.5% trials they took more than 3 s to reach one of the response boxes. Due to these low percentages, we expected no undue influence of these trials on the analyses and consequently excluded no data points. As an index of participants' decisiveness and ambivalence we calculated the maximum absolute deviation (MAD), which depicts the maximum deviation from the straight line between the start and end point of the trajectory (Kieslich et al., 2019). A smaller MAD reflects a more direct trajectory and thus, greater decisiveness and less ambivalence when (not) avoiding food intake.

2.8. Statistical analysis

The statistical analyses were conducted in R Studio (Version 4.1.2). To analyse the dichotomous avoidance response ('Avoid' vs. 'Not avoid') we ran generalized linear mixed model analyses (GLMMs). For the mouse-trajectory, EMG and self-report data (fear, desire, CS valence and milkshake liking) we conducted linear mixed model analyses (LMMs). The models were built using the *lme4* package (Bates et al., 2015). LMMs with fear and US expectancy ratings as the dependent variable violated the normality and homogeneity assumptions. To resolve this, we log transformed fear ratings and turned the continuous expectancy ratings into an ordinal variable with 5 levels (0–20, 21–40, 41–60 etc.). These ordinal data were analysed with cumulative link mixed models (CLMMs) using the *ordinal* package (Christensen, 2019). To identify the best fitting CLMM, we compared models based on their AIC and log likelihood. We report the simplest model with the lowest log likelihood. Each phase of the conditioning task was analysed separately. Only to test how conditioned responses were affected by the possibility to avoid, we analysed the last trial of the avoidance phases together with the respective test trials. Condition (Experimental vs. Control), CS (CS + vs. CS-), Trial (depending on the dependent variable and phase of the conditioning task) and their interactions were included into the models as fixed effects. Models slightly differed for US expectancies and mouse tracking data: here we also included participants' avoidance response (Avoid vs. Not avoid) and its interactions with the other predictors as

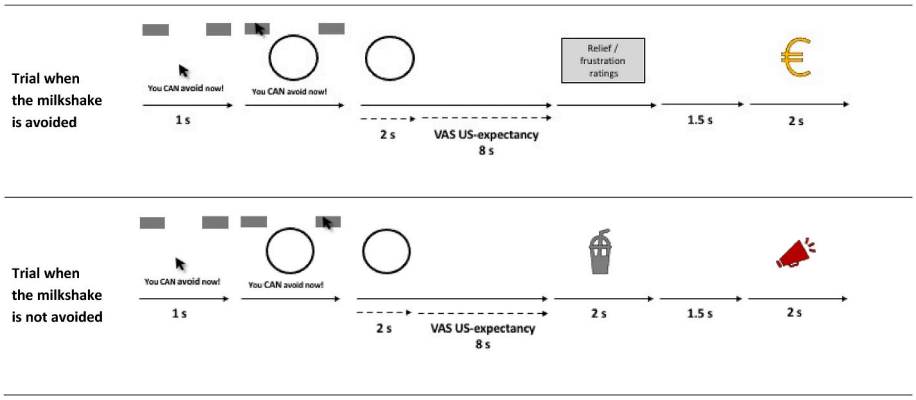

Fig. 2. Timeline of an avoidance trial in the experimental condition.

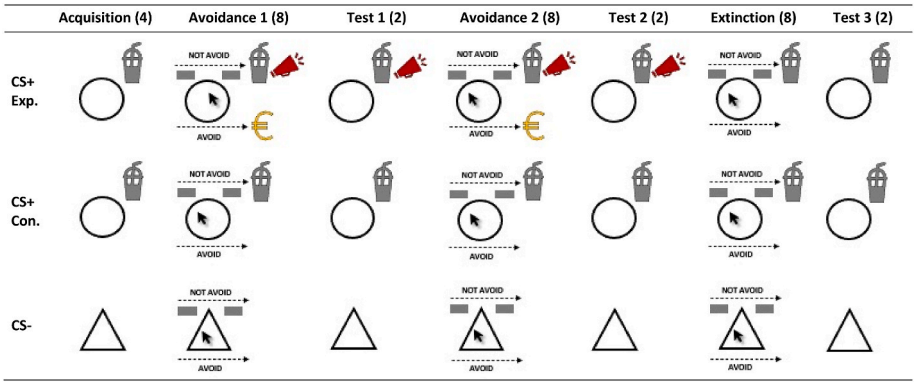

Fig. 3. Schema of the conditioning task.
Note. (…) = Number of trials per CS; Exp. = Experimental condition; Con. = Control Condition.

fixed effects. To keep our results interpretable, we only looked at CS + trials when analysing the mouse trajectory data. A random intercept per participant was included in all models. Random slopes for Condition, CS, Trial and Avoidance were added, in case they improved model fit as indicated by a significantly lower log likelihood. An overview of the estimated models per dependent variable and conditioning phase can be obtained from the Supplementary material 1. To verify whether reward and punishment successfully changed participants' responses to the high-calorie food we looked at the main effect of Condition and its interactions with the other predictors (Condition x CS, Condition x Avoidance etc.). We assessed the significance of fixed effects with the F-statistic for LMMs and with Wald's z for GLMMs and CLMMs. We followed up on significant findings with post-hoc comparisons using the *emmeans* package (Lenth et al., 2022) and controlled for multiple comparisons with Tukey's HSD. Cohen's d for the post-hoc comparisons was calculated with the *effect size* package (Ben-Shachar et al., 2020). We considered findings with a p-value lower than 0.05 as significant.

3. Results

3.1. Participants

104 women participated in the study and were randomly assigned to the experimental condition ($n = 52$) or control condition ($n = 52$). Mixed model analyses usually require a simulation-based power analyses (Brysbaert & Stevens, 2018), so that power estimates heavily depend on the accurate specification of the simulation (Kumle et al., 2021). As we could neither base out simulation on previously collected data nor on strong a-priori assumptions regarding the expected data structure and model parameters (Kumle et al., 2021), we based this sample size on previous avoidance learning studies (e.g., Morriss et al., 2018; Pittig, 2019; Pittig & Dehler, 2019; Pittig & Wong, 2021). Five participants dropped out of the study due to technical difficulties and extreme stress caused by the aversive scream or the milkshake delivery. Additionally, data from 4 participants were excluded from the analyses as they indicated at the end of the study that they did not understand the task, or they did not comply with the experimenters' instructions. Thus, the final analyses were conducted with 95 participants (experimental condition = 45; control condition = 50). For further information on sample characteristics consult Table 1.

3.2. Manipulation check

Participants perceived the reward and punishment as intended: they rated the loud scream as unpleasant and the monetary win as pleasant. Additional information on participants' liking for the different components of the conditioning task, including the pump set-up and the milkshake, can be found in Table 2.

Table 1
Participant characteristics.

	Experimental (n = 45)	Control (n = 50)	df	t	p
Age (Mean, SD)	23.84 (5.32)	22.58 (5.81)	92	−1.09	.278
BMI (Mean, SD)	22.86 (3.42)	22.39 (2.67)	83.06	−0.73	.467
Time since last meal in minutes (Mean, SD)	283 (233)	250 (227)	85	−0.66	.511

Table 2
Valence ratings for components of the conditioning task.

	Experimental (n = 45)	Control (n = 50)	df	t	p
Pump procedure (1–7)	5.42 (1.23)	5.18 (1.40)	93	−0.89	.377
Milkshake (-3 to 3)	2.64 (0.61)	2.46 (0.86)	88.19	−1.19	.228
Scream (-3 to 3)	−2.69 (0.94)	n.a.			
Monetary win (-3 to 3)	2.02 (1.48)	n.a.			

Note: Valence ratings for the milkshake refer to the first measurement after the acquisition phase.

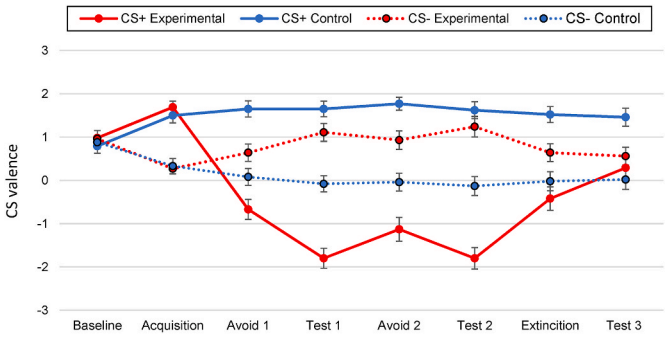

Fig. 5. Change in CS valence across the conditioning task.

3.3. Conditioning task

The frequency of avoidance behaviours, and the strength of US expectancy, desire and fear across the conditioning task are depicted in Fig. 4 Changes in participants' valence ratings for the two CSs are shown in Fig. 5. For an overview of the most relevant findings consult Table 4. (Standardized) regression coefficients per model are presented in Supplementary material 2.

3.3.1. Both conditions showed successful appetitive conditioning (acquisition phase)

During the acquisition phase US expectancy, desire and valence ratings increased for CS + trials and decreased for CS- trials (see Figs. 3 and 4). Consequently, participants indicated higher US expectancy, desire and pleasantness ratings on CS + compared with CS- trials at the end of the acquisition phase. Fear ratings remained low throughout the acquisition phase. Thus, participants in the experimental and the control condition displayed successful differential learning and appetitive conditioning. For more details on analyses and test statistics consult Supplementary material 1.

3.3.2. After reward and punishment were introduced, the experimental condition changed in food avoidance behaviours and other conditioned responses (avoidance phase 1)

Avoidance frequency. In the course of the avoidance phase participants in the experimental condition became more likely to avoid on CS + trials, Trial x CS × Condition interaction: Wald's $z = 5.71$, $p < .001$, while there was no change in avoidance frequency for CS + trials in the control condition ($p = .17$). Post-hoc comparisons showed that at the

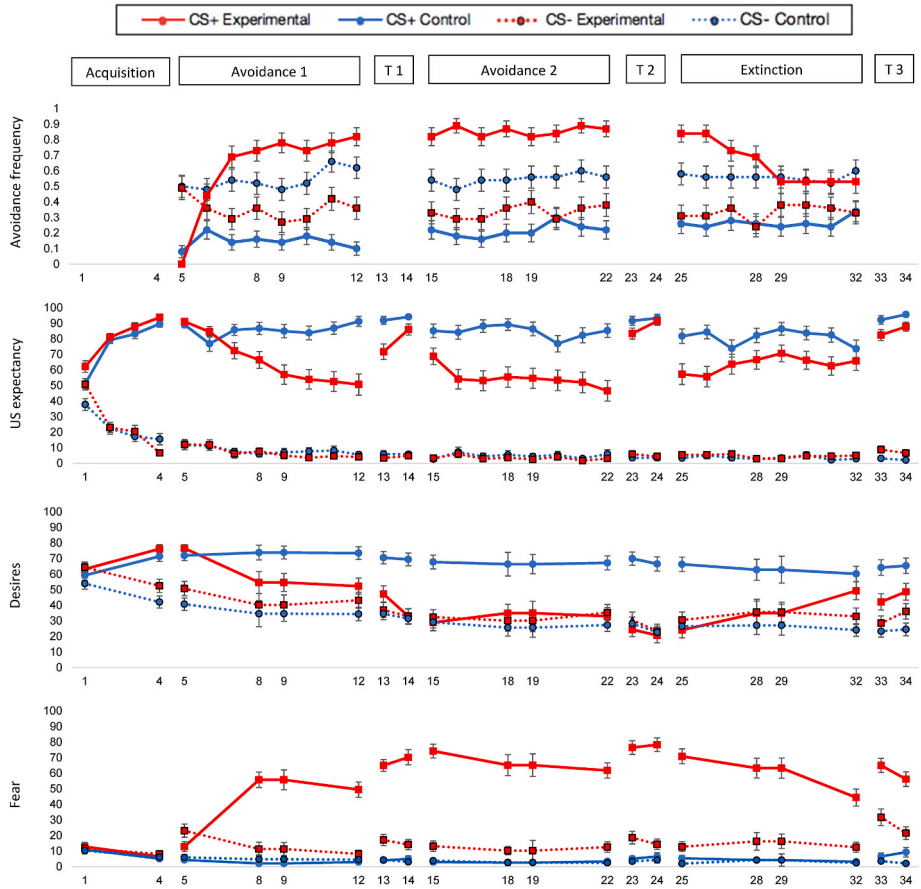

Fig. 4. Frequency of the avoidance response (first panel) and level of expectancy ratings (second panel), desire ratings (third panel) and fear ratings (fourth panel) across the conditioning task.
Note: T = Test phase; As desire and fear were measured randomly on trial 8 or 9, 18 or 19 and 28 or 29 their average is indicated at both time points.

end of the avoidance phase the experimental condition was significantly more likely to avoid on CS + trials ($OR = 0.01$, $SE = 0.005$, $z = -10.37$, $p < .001$, d = −2.13) and significantly less likely to avoid on CS- trial than the control condition ($OR = 3.81$, $SE = 1.29$, $z = 3.96$, $p < .001$, $d = 0.81$). Most participants in the experimental condition indicated that they avoided the milkshake because of the loud sound ($n = 33$). Fewer participants mentioned the monetary reward as a reason for avoiding the milkshake ($n = 8$). An overview of participants' reasons for avoiding the milkshake can be found in Table 3.

Maximum Absolute Deviation (MAD). During the first avoidance phase, the MAD reduced more in the experimental condition than in the control condition, Condition × Trial interaction: $F_{(1,\ 699.75)} = 4.06$, $p = .044$. In line with this, post-hoc comparisons showed that at the end of the avoidance phase the experimental condition had a significantly lower MAD when selecting the avoidance response compared with the control condition ($\beta = 254.2$, $SE = 73.6$, $t_{(561)} = 3.46$, $p < .001$, $d = 0.29$). Thus, the experimental condition was more decisive when avoiding the milkshake than the control condition. Changes in MAD across the conditioning task are presented in Fig. 6.

US expectancy. Participants in both conditions successfully learned how to use the response boxes. During the avoidance phase, in both conditions expectancy ratings reduced for CS + trials with an avoidance response, CS x Avoidance x Trial: $F_{(1,\ 1328.23)} = 37.51$, $p < .001$) and Condition x CS x Avoidance × Trial interaction: $F_{(1,\ 1328.23)} = 5.16$, $p < .023$). While participants still had higher expectancies on CS + compared with CS- trials, CS: $F_{(1,\ 194.28)} = 550.28$, $p < .001$ and CS × Condition interaction: $F_{(1,\ 194.28)} = 7.16$, $p = .008$, post-hoc comparisons showed that this difference was smaller on CS + trials with an avoidance response (Avoided: $\beta = -42.7$, $SE = 2.93$, $t_{(210)} = -14.58$, $p < .001$, $d = -1.01$ vs. Not-avoided: $\beta = -81.6$, $SE = 2.48$, $t_{(120)} = -32.87$, $p < .001$, $d = -3.00$). Fig. 4 shows participants' US expectancies without taking their avoidance responses into account. As the experimental condition avoided the US more frequently, they are presented here with a lower US expectancy on CS + trials than the control condition.

Desire. In the course of the avoidance phase CS + desire ratings reduced in the experimental condition, CS x TRIAL × Condition interaction: $F_{(2,\ 368.53)} = 7.41$, $p < .001$, while there was no change in desire ratings in the control condition ($p = .348$). Post-hoc comparisons showed that the experimental condition reported lower desires on CS + trials than the control condition at the end of the avoidance phase ($\beta = 21.11$, $SE = 5.47$, $t_{(178)} = 3.86$, $p < .001$, $d = 0.58$). Conditions did not significantly differ in their desires on CS- trials (all p's > 0.174). The experimental condition indicated similar desire levels for both CSs at trial 12 ($\beta = -8.76$, $SE = 4.94$, $t_{(227)} = -1.77$, $p < .079$, $d = -0.12$), while the control condition continued to give higher desire ratings on CS + compared with CS- trials throughout the avoidance phase, CS: $F_{(1,\ 93.47)} = 99.82$, $p < .001$.

Fear. Already at the beginning of the avoidance phase the experimental condition reported greater fear than the control condition on CS+ and CS- trials, CS × Condition interaction: $F_{(1,\ 93.08)} = 73.95$, $p < .001$ and Condition: $F_{(1,\ 92.95)} = 56.68$, $p < .001$. This have been caused by the additional information about the loud sound that participants in the experimental condition received before the start of the avoidance phase. Participants in the experimental condition became more fearful on CS + trials, Condition x CS × Trial interaction: $F_{(2,\ 370.64)} = 38.11$, $p < .001$, and less fearful on CS- trials, Condition × Trial interaction: $F_{(2,\ 370.64)} = 12.73$, $p < .001$, in the course of the avoidance phase. There was no change in fear ratings in the control condition (all p's > 0.679).

CS valence. In the experimental condition, CS + valence ratings decreased and CS- increased from trial 4 to trial 12, Condition x CS × Trial interaction: $F_{(1,\ 186)} = 65.56$, $p < .001$ and Condition × Trial interaction: $F_{(1,\ 186)} = 22.27$, $p < .001$. In the control condition valence

Table 3
Participants' self-reported reasons for avoiding the milkshake.

Reasons for avoidance	Number of times mentioned by participants	
	Experimental condition (n = 36)	Control condition (n = 24)
Punishment (aversive scream)	33	n.a.
Reward (monetary gain)	8	n.a.
Pump procedure	1	8
Dieting	1	3
Trying things out	1	5
Feeling full (satiated)	0	13
Taste of the milkshake	0	8

Note: This question was added to the study after testing the 5th participant.

Table 4
Overview of main statistical results per phase and CS.

Phase and measure	CS+	CS-
Avoidance 1		
Avoidance behavior	Experimental ↑ > Control ↔	Experimental ↔ < Control ↔
Fear	Experimental ↑ > Control ↔	Experimental ↓ = Control ↔
Desire	Experimental ↓ < Control ↔	Experimental ↔ = Control ↔
CS valence	Experimental ↓ < Control ↔	Experimental ↑ > Control ↓
Avoidance 2		
Avoidance behavior	Experimental ↔ > Control ↔	Experimental ↔ < Control ↔
Fear	Experimental ↔ > Control ↔	Experimental ↔ > Control ↔
Desire	Experimental ↔ < Control ↔	Experimental ↔ = Control ↔
CS valence	Experimental ↑ < Control ↔	Experimental ↔ > Control ↔
Test 1 and 2		
Fear	Experimental ↑ > Control ↔	Experimental ↑ = Control ↔
Desire	Experimental ↓↓ < Control ↔	Experimental ↓ = Control ↔
CS valence	Experimental ↓ < Control ↔	Experimental ↑ > Control ↔
Extinction		
Avoidance behavior	Experimental ↓ > Control ↔	Experimental ↔ < Control ↔
Fear	Experimental ↓ > Control ↔	Experimental ↔ > Control ↔
Desire	Experimental ↑ = Control ↓	Experimental ↔ = Control ↔
CS valence	Experimental ↑↑ < Control ↔	Experimental ↓ > Control ↔
Test 3		
Fear	Experimental ↓ > Control ↔	Experimental ↓ > Control ↔
Desire	Experimental ↑ < Control ↔	Experimental ↑ > Control ↔
CS valence	Experimental ↑ < Control ↔	Experimental ↔ > Control ↔

Note: Experimental/Control = Results for the experimental and control condition; Within-group effects: ↑ and ↓ = significant increase or decrease within condition, ↔ = no change within condition; Between-group effects: > and ≤ significantly larger or smaller in experimental compared to control condition at the end of the respective phase, " = " indicates no difference between conditions.

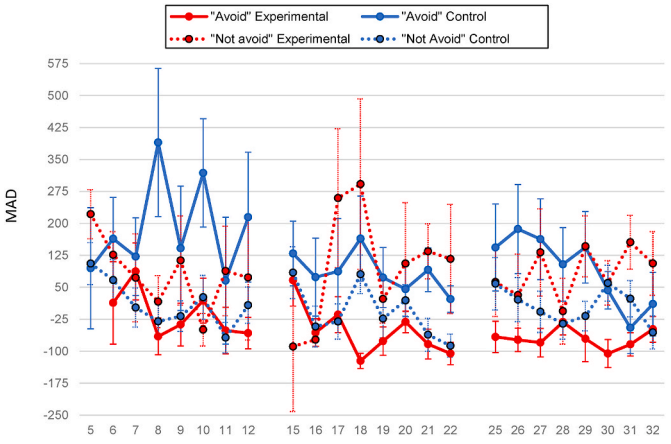

Fig. 6. Change in Maximum Absolute Deviation (MAD) for CS + trials with and without an avoidance response across the conditioning task
Note. Red error bars = SE for avoided trials in the experimental condition; Red dotted error bars = SE for not avoided trials in the experimental condition; Blue error bars = SE for avoided trials in the control condition; Blue dotted error bars = SE for not avoided trials in the control condition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ratings for the CS- decreased across the avoidance phase, Trial: $F_{(1, 186)} = 27.20$, $p < .001$. Post-hoc comparisons showed that the experimental condition reported higher CS + valence ratings ($\beta = 2.31$, $SE = 0.25$, $t_{(179)} = 9.09$, $p < .001$, $d = 1.36$) and lower CS- valence ratings ($\beta = -0.63$, $SE = 0.26$, $t_{(176)} = -2.43$, $p = .016$, $d = -0.37$) than the control condition at the end of the avoidance phase.

Summary. In the first avoidance phase, both conditions learned that they could avoid the US. In the experimental group reward and punishment successfully changed participants' responses to (predictors of) the milkshake: They were more likely to avoid on CS + trials and executed these avoidance responses with more direct mouse movements than the control condition. The increase in avoidance responses went along with more fear, lower desires and less CS liking on CS + trials.

3.3.3. Changes in avoidance behaviour and other conditioned responses were persistent over time (avoidance phase 2)

Avoidance frequency. The experimental condition was more likely to avoid on CS + trials compared with the control condition, CS × Condition interaction (Wald's $z = 16.28$, $p < .001$); the experimental condition was less likely to avoid on CS- trials than controls, Condition (Wald's $z = -2.84$, $p = .004$).

Maximum Absolute Deviation (MAD). The LMM analysis showed a significant Condition x Avoidance × Trial interaction ($F_{(1, 680.70)} = 4.83$, $p = .028$): Post-hoc comparisons indicated that in the course of the avoidance phase, the MAD reduced in the experimental condition on trials with an avoidance response ($\beta = 107.4$, $SE = 40.3$, $t_{(649)} = 2.67$, $p = .007$, $d = 0.10$) and in the control condition on trials without an avoidance response ($\beta = 106.8$, $SE = 40$, $t_{(644)} = 2.67$, $p = .007$, $d = 0.11$). At the end of the avoidance phase the experimental condition had a lower MAD for trials with an avoidance response than the control condition ($\beta = 144$, $SE = 56.3$, $t_{(291)} = 2.56$, $p = .011$, $d = 0.30$). Thus, participants in the experimental condition executed the avoidance response with more decisiveness and less ambivalence than controls.

US expectancy. The Condition + Trial + CS x Avoidance CLMM showed that participants in both conditions were less likely to expect the US on CS + trials with an avoidance response than on CS + trials without an avoidance response, CS × Avoidance interaction: Wald's $z = -6.40$, $p < .001$. While participants had greater odds to expect the US on CS + compared with CS- trials, CS: Wald's $z = 17.78$, $p < .001$, this difference was smaller for trials with an avoidance response (Avoided: $\beta = -9.71$, $SE = 0.55$, $z = -17.78$, $p < .001$, $d = -1.82$ vs. Not-avoided: $\beta = -5.50$, $SE = 0.44$, $z = -12.55$, $p < .001$, $d = -1.29$). There were no differences between conditions (all p's = 0.332).

Desire. Participants in the experimental condition reported lower desires on CS + trials compared with the control condition, CS × Condition interaction: $F_{(1, 93.07)} = 31.67$, $p < .001$. Conditions did not differ in desires on CS- trials ($p = .433$). While the control condition reported more desires on CS + compared to CS- trials throughout the second avoidance phase, CS: $F_{(1, 93.07)} = 31.59$, $p < .001$, this differences was absent in the experimental condition ($p = .996$).

Fear. In the second avoidance phase, the experimental condition reported greater fear than the control condition on CS+, CS × Condition interaction: $F_{(1, 93)} = 121.10$, $p < .001$, and CS- trials, Condition: $F_{(1, 93)} = 114.8$, $p < .001$. Post-hoc comparisons showed that the experimental condition was more fearful on CS + compared with CS- trials ($\beta = -2.57$, $SE = 0.17$, $t_{(93)} = -14.73$, $p < .001$, $d = -1.53$); this difference was absent in the control condition ($p = .642$).

CS valence. Valence ratings for the CS + increased in the experimental condition across the second avoidance phase, CS x Condition × Trial interaction: $F_{(1, 186)} = 6.56$, $p = .011$, while ratings remained stable in the control condition ($p = .421$). Still, the experimental condition rated the CS + as significantly less pleasant than the control condition, CS × Condition interaction: $F_{(1, 92.999)} = 100.94$, $p < .001$. Participants in the experimental condition also rated the CS- as significantly more pleasant than controls, Condition: $F_{(1, 93)} = 36.86$, $p < .001$. In line with this, post-hoc comparisons showed that the experimental condition gave lower valence ratings for CS + compared with CS- trials ($\beta = 2.53$, $SE = 0.31$, $t_{(93)} = 8.08$, $p < .001$, $d = 0.84$), while the control condition showed the opposite pattern ($\beta = -1.81$, $SE = 0.30$, $t_{(93)} = -6.08$, $p < .001$, $d = -0.63$).

Summary. The changed responses to (predictors of) the milkshake were persistent over time: Participants in the experimental condition were again more likely to avoid the US and executed these avoidance behaviours with more direct mouse movements than controls. The experimental condition continued to display more fear, lower desires and less CS liking on CS + trials compared to the control condition.

3.3.4. Changes in conditioned responses intensified when avoidance behaviour was not possible

3.3.4.1. Test phase 1. ***US expectancy.*** When avoidance was not possible anymore, US-expectancies quickly recovered: participants in the experimental condition had greater US expectancies on CS + trials at the end compared with the beginning of the first test phase, Condition x CS × Trial interaction: $F_{(1,183.665)} = 4.44$, $p = .036$. There was no change in expectancy ratings in the control condition (p's > 0.205).

Desire. In the experimental condition desires decreased from the last trial of the avoidance phase to the end of the first test phase, Condition × Trial interaction: $F_{(2, 365.88)} = 6.42$, $p = .001$. There was no significant change in desires in the control condition (p's > 0.369). Participants in the experimental condition expressed lower desires on CS + trials compared with the control condition at all time points, CS × Condition interaction: $F_{(1, 93.21)} = 24.53$, $p < .001$. Post-hoc comparisons showed no significant difference between conditions on CS- trials ($p > .175$).

Fear. In the experimental condition fear increased from the last avoidance trial to the first test trial, Condition × Trial interaction: $F_{(2, 371.36)} = 10.56$, $p < .001$. There was no significant change in fear levels in the control condition (p's > 0.578). The experimental condition was more fearful than the control condition on CS+ and CS- trials, Condition: $F_{(1, 93.01)} = 87.39$, $p < .001$. Thereby, participants in the experimental condition expressed greater fear on CS + compared with CS- trials, CS x Condition: $F_{(1, 93.11)} = 134.13$, $p < .001$, while fear ratings were similar for both CSs in the control condition ($p = .6$).

CS valence. The experimental condition reduced in CS + valence ratings from the end of the avoidance phase to the end of the test phase, Condition x CS × Trial interaction: $F_{(1, 186)} = 41.16$, $p < .001$. Post-hoc comparisons showed that the experimental condition simultaneously increased in valence ratings for the CS- ($\beta = -0.49$, $SE = 0.14$, $t_{(186)} = -3.39$, $p < .001$, $d = -0.25$). There was no significant change in valence ratings in the control condition (all p's > 0.19). Across all time-points, the experimental condition rated the CS + as less pleasant, CS x Condition: $F_{(1, 92.999)} = 89.34$, $p < .001$, and the CS- as more pleasant, Condition: $F_{(1, 93.001)} = 29.95$, $p < .001$, than the control condition.

3.3.4.2. Test phase 2. A similar pattern of results was present in test phase 2. Interested readers can find a detailed description in Supplementary material 1.

Summary. In the experimental condition fear increased and desires and CS-liking decreased, when avoidance was not possible. Thus, previously acquired changes in conditioned responses became stronger when the opportunity to avoid was removed.

3.3.5. Food avoidance and changes in other conditioned responses persisted in the absence of reward and punishment

3.3.5.1. Extinction phase. ***Avoidance frequency.*** During the extinction phase the likelihood to avoid on CS + trials reduced for participants in the experimental condition, CS x Condition × Trial interaction: Wald's $z = -3.73$, $p < .001$, while there was no change in avoidance frequency for the control condition ($p = .741$). Still, the experimental condition was more likely to avoid on CS + trials, CS × Condition interaction:

Wald's $z = 9.84$, $p < .001$, and less likely to avoid on CS- trials than the control condition, Condition: Wald's $z = -3.35$, $p < .001$. Accordingly, post-hoc comparisons showed that the experimental condition had greater odds to avoid the US on the last CS + trial of the extinction phase than the control condition ($OR = 0.41$, $SE = 0.16$, $z = -2.27$, $p = .02$, $d = 0.47$).

Maximum Absolute Deviation (MAD). At the beginning of the extinction phase participants in the experimental condition had a lower MAD on trials with an avoidance response than the control condition ($\beta = 258.6$, $SE = 58.9$, $t_{(192)} = 4.39$, $p < .001$, $d = 0.63$), Condition × Avoidance interaction: $F_{(1,\ 318.28)} = 13.3$, $p < .001$. The MAD did not change in the experimental condition ($p = .71$), but reduced in the control condition over the course of the extinction phase, Condition x Trial: $F_{(1,\ 658.37)} = 5.17$, $p = .023$. Post-hoc comparisons showed, that at the end of the extinction phase conditions did not differ anymore in the MAD for trials with an avoidance response ($p = .324$). However, the experimental condition had a higher MAD than the control condition for trials without an avoidance response ($\beta = -135.2$, $SE = 55.7$, $t_{(163)} = -2.43$, $p = .016$, $d = -0.38$), meaning that participants in the experimental condition were less decisive and more ambivalent than controls when not avoiding the US.

US expectancy. The Condition x CS x Avoidance + Trial (covariate) CLMM showed that both conditions were less likely to expect the US on CS + trials *with* an avoidance response than on CS + trials *without* an avoidance response, Avoidance: Wald's $z = -3.20$, $p = .001$, and Condition x CS × Avoidance interaction: Wald's $z = -2.21$, $p = .027$. Overall, participants in both conditions were more likely to expect the US to follow on CS + trials than on CS- trials, CS: Wald's $z = 15.35$, $p < .001$.

Desire. In the course of the extinction phase, desires increased on CS + trials in the experimental condition, CS x Trial × Condition interaction: $F_{(2,\ 370.29)} = 9.59$, $p < .001$, while ratings reduced in the control condition, CS × Trial interaction: $F_{(2,\ 370.29)} = 5.55$, $p = .004$.

Post-hoc comparisons showed that participants in the experimental condition reported lower desires for CS + trials than controls on trial 25 ($\beta = 43.30$, $SE = 7.11$, $t_{(120)} = 6.09$, $p < .001$, $d = 1.11$) and trial 29 ($\beta = 26.70$, $SE = 7.11$, $t_{(120)} = 3.75$, $p < .001$, $d = 0.68$); both conditions did not differ anymore in their desires at trial 32 ($p > .125$). Participants in both conditions indicated greater desires on CS + compared with CS- trials at the end of the extinction phase (experimental: $\beta = -15.58$, $SE = 5.70$, $t_{(145)} = -2.74$, $p = .007$, $d = -0.23$; control: $\beta = -35.32$, $SE = 5.39$, $t_{(144)} = -6.55$, $p < .001$, $d = -0.55$).

Fear. During the extinction phase, the experimental condition became less fearful on CS + trials, CS x Condition × Trial interaction: $F_{(2,\ 372)} = 3.83$, $p = .022$, while fear levels remained stable on CS- trials (all p's > 0.186). There was no change in fear ratings in the control condition (all p's > 0.186). Participants in the experimental condition were still more fearful than the control condition on both CS+ and CS- trials, Condition: $F_{(1,\ 93)} = 103.06$, $p < .001$. Thereby, they expressed greater fear on CS + compared with CS- trials, Condition × CS interaction: $F_{(1,\ 93)} = 79.17$, $p < .001$, while there was no such difference in the control condition ($p = .191$).

CS valence. In the extinction phase, valence ratings for the CS + increased and valence ratings for the CS- decreased in the experimental condition, CS x Condition × Trial interaction: $F_{(1,\ 186.001)} = 34.65$, $p < .001$ and Trial × Condition interaction: $F_{(1,\ 186.001)} = 4.89$, $p = .028$. There was no significant change in valence ratings for the control condition (p's > 0.518). Still, the experimental condition continued to rate the CS + as *less* and the CS- as *more* pleasant than the control condition, CS × Condition interaction: $F_{(1,\ 92.999)} = 69.43$, $p < .001$ and Condition: $F_{(1,\ 93)} = 23.06$, $p < .001$. Post-hoc comparisons showed that the experimental condition provided lower valence ratings for CS + compared with CS- trials at the end of the extinction phase ($\beta = 1.13$, $SE = 0.36$, $t_{(126)} = 3.15$, $p = .002$, $d = 0.28$, while the opposite pattern was present in the control condition ($\beta = -1.56$, $SE = 0.34$, $t_{(126)} = -4.57$, $p < .001$, $d = -0.41$).

3.3.5.2. Test phase 3. ***US expectancy.*** The Condition x CS + Trial CLMM showed that participants in the experimental condition were less likely to expect the US after CS + trials compared with the control condition, CS × Condition interaction: Wald's $z = -3.84$, $p < .001$. Besides, the experimental condition was more likely than the control condition to expect the US after CS- trials, Condition: Wald's $z = 2.39$, $p = .017$. Participants in both conditions reported a greater expectancy on CS + compared with CS- trials, CS: Wald's $z = 9.52$, $p < .001$.

Desire. In the experimental condition desires increased over the course of the third test phase, Trial × Condition interaction: $F_{(1,\ 184.284)} = 8.1$, $p = .004$, while there was no significant change in desire ratings in the control condition (all p's > 0.436). Still, participants in the experimental condition expressed lower desires on CS + trials than the control condition throughout the third test phase, CS × Condition interaction: $F_{(1,\ 92.995)} = 14.42$, $p < .001$. Post-hoc comparisons showed that while both conditions initially did not differ in their desires on CS- trials ($p = .297$), the experimental condition expressed greater desires at the end of test phase 3 ($\beta = -12.4$, $SE = 6.11$, $t_{(104)} = -2.03$, $p = .045$, $d = -0.40$). The difference in desires between CS+ and CS- trials was smaller in the experimental condition compared to the control condition (Experimental: $\beta = -12.5$, $SE = 5.14$, $t_{(93)} = -2.43$, $p = .017$, $d = -0.25$ vs. Control: $\beta = -39.4$, $SE = 4.88$, $t_{(93)} = -8.08$, $p < .001$, $d = -0.84$).

Fear. The experimental condition expressed less fear on trial 34 compared with trial 33, Condition × Trial interaction: $F_{(1,\ 186)} = 4.48$, $p = .035$, while there was no change in fear ratings in the control condition ($p > .169$). Across the third test phase, participants in the experimental condition were more fearful than the control condition on both CS+ and CS- trials, Condition: $F_{(1,\ 93.001)} = 96.09$, $p < .001$. Thereby, the experimental condition showed greater fear on CS + compared with CS- trials, CS × Condition interaction: $F_{(1,\ 93)} = 19.21$, $p < .001$.

CS valence. In the experimental condition CS + valence ratings increased from the end of the extinction phase to the end of the third test phase, Trial x Condition × CS interaction: $F_{(1,\ 186.001)} = 10.82$, $p = .001$; there was no change in valence ratings for the CS- ($p = .660$). Valence ratings for both CSs also remained stable in the control condition (all p's > 0.537). Still, the experimental condition continued to rate the CS + as less pleasant than the control condition, CS x Condition: $F_{(1,\ 92.999)} = 20.81$, $p < .001$. While the experimental condition rated the CS- as significantly more pleasant than the control condition at the end of the extinction phase, Condition: $F_{(1,\ 92.999)} = 5.79$, $p = .018$, post-hoc comparisons showed that there was only a marginally significant difference between conditions at the end of test phase 3 ($\beta = -0.62$, $SE = 0.32$, $t_{(114)} = -1.97$, $p = .051$, $d = -0.37$). Post-hoc comparisons also showed that at trial 34 valence ratings were similar for both CSs in the experimental condition ($p = .401$), while the control condition again rated the CS + as significantly more pleasant than the CS- ($\beta = -1.46$, $SE = 0.35$, $t_{(109)} = -4.17$, $p < .001$, $d = -0.40$).

Summary. After reward and punishment were stopped, differences between conditions reduced: participants in the experimental condition became less likely to avoid the milkshake; they stopped to execute avoidance behaviours with more direct mouse movements than controls and they indicated less fear, greater desires and more CS liking on CS + trials. However, avoidance frequency and conditioned responses did not reach the same level as in the control condition. Additionally, the experimental condition displayed *less* direct mouse movements when *not* avoiding the milkshake. Thus, changes in responses to (predictors of) the milkshake persisted after the offset of rewards and punishments.

3.3.6. Reward and punishment had a small effect on milkshake liking

At the end of the acquisition phase participants in both conditions rated the milkshake as tasty (see Table 1 and Fig. 7). In the experimental condition milkshake liking reduced from the end of the acquisition phase to the end of the second test phase, Condition × Trial interaction: $F_{(1,93)} = 11.98$, $p < .001$. Post-hoc comparisons showed that at the end of the second test phase the experimental condition rated the milkshake as less tasty than the control condition ($\beta = 0.74$, $SE = 0.24$, $t_{(93)} = 3.13$, p

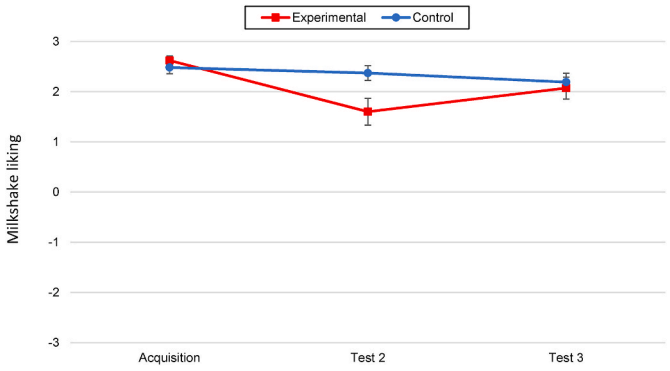

Fig. 7. Change in milkshake (US) liking across the conditioning task.

$= .002$, $d = 0.65$). From the end of the second test phase to the end of the third test phase, milkshake liking increased again in the experimental condition, Condition × Trial interaction: $F_{(1, 93)} = 9.79$, $p = .002$. Post-hoc comparisons showed that both conditions did not differ in their milkshake liking at the end of the task ($p = .805$). Across the conditioning task there was no significant change in milkshake liking for the control condition (all p's > 0.224). In sum, receiving rewards and punishments for (not) avoiding food intake reduced participants liking of the milkshake. This change was not persistent as milkshake liking normalized again once rewards and punishments were dropped.

3.3.7. EMG data

No differential learning was found in the EMG data. Therefore, these outcome measures are not discussed in the result section. Instead, a detailed description of the findings can be found in Supplementary material 1.

4. Discussion

The present study investigated whether rewarding food avoidance and punishing food consumption would result in a frequent and decisive avoidance of high-calorie foods, as well as reduced appetitive and increased aversive conditioned responses upon seeing stimuli predictive of food intake. The present study also investigated the function of avoidance behaviours, by comparing conditioned responses during phases where avoidance behaviours were possible and those where avoidance behaviours were not possible. In addition, we looked at the persistence of changes in avoidance behaviours and conditioned responses after rewards and punishments were set on extinction. In line with our expectations, participants in the experimental condition showed cue-elicited food avoidance, increased fear responses, and reduced eating desires after the introduction of reward and punishment. This behaviour became stronger during trials without the possibility to avoid and proved to be persistent when rewards and punishments were not delivered anymore.

In the acquisition phase both conditions successfully learned differential appetitive conditioned responses as indicated by increased US-expectancy, desire to drink the milkshake and valence ratings for CS + vs. CS- trials. Thereby, participants displayed a typical response pattern for healthy individuals (van den Akker et al., 2019; Wardle et al., 2018). However, after the introduction of reward and punishment, the experimental condition avoided the milkshake more often and executed this behaviour with more direct mouse movements than the control condition. Additionally, participants in the experimental condition showed increased fear, reduced eating desires and less liking on CS + trials compared to controls. Thereby, our findings support the idea that food avoidance behaviours and reactions to predictors of food intake, can be changed by rewarding food avoidance and punishing food intake. Comparable classical and operant learning processes may play a crucial role in the development and maintenance of dysfunctional eating behaviours in anorexia nervosa. Accordingly, patients learn to associate food intake with aversive outcomes, such as (uncontrollable) weight gain, loss of control or intra-intestinal malaise (operationalized as the aversive sound in our conditioning task) (Cardi et al., 2019; Hildebrandt et al., 2015; Murray et al., 2018; Schaumberg et al., 2021). Resulting food avoidance behaviours are then maintained via operant conditioning, as they not only remove the previously described aversive outcomes but are also accompanied by rewarding consequences, in the form of weight loss and increased self-esteem (operationalized at the monetary win in our conditioning task) (Melles et al., 2021). Importantly, these learning processes might not only affect individuals' ingestive behaviour but also their conditioned responses to (predictors of) food intake. In the present study, the introduction of reward and punishment for food avoidance/intake, decreased participants' appetitive and increased their aversive conditioned responses to cues that signal eating. These changes mirror the clinical picture often observed in individuals with anorexia nervosa: patients typically report reduced eating desires for high-calorie foods (Steinglass et al., 2015; Stoner et al., 1996); show less appetitive responding to predictors of food intake (for a detailed discussion see Haynos et al., 2020) and experience high levels of fear before and during eating (Buree et al., 1990; Steinglass et al., 2010).

While theoretical models suggest that both aversive and rewarding consequences of food intake/avoidance play a role in the development of patients' dysfunctional eating behaviours (Melles et al., 2021), our results point to a greater role of punishment than reward in the development and maintenance of food avoidance in the current experiment. Firstly, the experimental condition evaluated predictors of food intake as highly unpleasant even though they were coupled with punishment *and* reward. Thus, punishment seemed to have a larger impact on CS evaluations than reward. Secondly, most participants in the experimental condition noted that they avoided the milkshake due to the associated aversive consequences, while only a small number mentioned the reward as a reason for their avoidance behaviours. Individuals with anorexia nervosa associate food intake with a range of aversive consequences, such as gaining weight, losing control or being judged, and report high levels of fear in relation to a variety of eating related situations and stimuli (Levinson & Byrne, 2015; Levinson & Williams, 2020; Melles & Jansen, 2023). The present study provides a first indication that these expected aversive outcomes and fears might be more relevant for the development and maintenance of anorectic food avoidance than potential rewarding consequences (e.g., weight loss and increased self-esteem). As we did not match the intensity of reward and punishment - participants perceived the aversiveness of the scream as more intense than the pleasantness of the monetary reward (see Table 2) – we cannot rule out that differences in the incentive value of these stimuli underlie the present results. To allow for more definite conclusions, future studies should thoroughly control the strength of positive and negative consequences for food avoidance/food intake or present consequences separately from each other (e.g., in a between-subjects design).

Furthermore, our results showed that changes in appetitive and aversive conditioned responses became more extreme when avoidance was not possible (see test trials). While participants already expressed fear upon seeing predictors of food intake when avoidance responses were available, fear further increased when the possibility to avoid was removed. Correspondingly, individuals with anorexia nervosa use eating rituals and restrictive eating as a strategy to cope with eating related fears (Cardi et al., 2019) and situations in which avoidance is not possible, such as social events, evoke heightened levels of anxiety in patients (Levinson & Williams, 2020). Thus, (short-term) anxiety management appears to be an important function of anorectic avoidance, potentially further contributing to the maintenance of the behaviour. Interestingly, participants' conditioned responses did not normalize once avoidance behaviours were possible again; eating desires stayed low and fear stayed high. A possible explanation is that the unannounced switch between the avoidance and the test phase i.e., the

sudden removal of avoidance responses, surprised participants and increased uncertainty in the remaining task. Surprising and uncertain events recruit greater attentional and motivational resources, and thereby, facilitate classical conditioning (Koenig et al., 2017). Presumably, during the test phase participants formed a strong association between the CS+, food intake and the aversive sound resulting in sustained fear and reduced desires. Importantly, uncertainty about the availability of avoidance behaviours might also play a role in the daily experience of individuals with anorexia nervosa; patients cannot always avoid food intake and its associated negative outcomes, which could strengthen eating-disorder related fears and further eradicate eating desires (Kezelman et al., 2016).

On top of that, the current results showed that changed responses towards (predictors of) the milkshake mostly persisted once food avoidance and food intake were not rewarded or punished anymore. While participants' food avoidance can be seen as adaptive when rewards and punishments were in place, persistent avoidance in the absence of these consequences constitutes a dysfunctional behaviour (Krypotos et al., 2015), as participants missed out on the tasty food US for no reason. Similarly, patients with anorexia nervosa continue to rigidly avoid food intake even though initial rewards (such as compliments by others for weight loss) have ceased (Walsh, 2013) and negative outcomes, such as uncontrollable weight gain, do not follow eating. The persistence of food avoidance behaviours and changes in conditioned responses could be due to a relatively short extinction phase: The present conditioning task included more trials with rewards or punishments for (not) drinking the milkshake than trials without these consequences, so that participants possibly lacked time to form strong safety associations. However, as around half of the participants in the experimental group avoided the milkshake on every trial of the extinction phase, their dysfunctional behaviour might be better explained by the so called 'Protection from extinction' effect (Lovibond et al., 2009; Vervliet & Indekeu, 2015). By continuously avoiding the food US, many participants in the present study failed to notice that drinking the milkshake was not punished anymore and therefore, were unable to revise their incorrect threat beliefs. Correspondingly, the rigid and persistent food avoidance seen in anorexia nervosa, might hinder patients from correcting their erroneous expectations. A patient that never eats high-calorie foods (without compensating for it) cannot learn, that food intake is not followed by a feared outcome, such as immediate or uncontrollable weight gain. Thereby, food avoidance behaviours might play an important role in the maintenance of patients' fears and reduced eating desires (Melles et al., 2021).

Finally, the data showed that introducing rewards and punishments for (not) avoiding food intake also affected participants' liking of the food stimulus; participants in the experimental condition rated the milkshake as less tasty than controls. However, the reduction in milkshake liking was relatively small and normalized again once reward and punishment were discontinued and avoidance responses reduced. Thus, the reduction in US liking was more transient than changes in avoidance behaviours or appetitive and aversive conditioned responses. Previous studies comparing patients with anorexia nervosa and healthy individuals, found no differences between both groups in self-reported liking for high-calorie food items, chocolate flavour and sweet sucrose solutions (Cowdrey et al., 2011, 2013; Frank et al., 2012; Monteleone et al., 2017; Stoner et al., 1996). The present findings add to the accumulating evidence that aberrancies in eating desires are more relevant for the development and maintenance of anorexia nervosa than changes in the actual liking of high-calorie foods (Cowdrey et al., 2013).

The present findings also suggest clinical implications. They point to potentially detrimental outcomes if patients' threat beliefs and fears are not addressed during treatment. Removing the possibility to avoid food intake, aggravated participants fear and further reduced their desires to drink the milkshake. Similarly, patients undergoing refeeding therapy report an increasing aversion for food and escalating anxiety about weight gain over the course of their treatment (Kezelman et al., 2016). Thus, psychological interventions that target dysfunctional threat expectancies and fear need to be included already at an early stage of treatment. By showing that classical and operant conditioning may contribute to the development and maintenance of food avoidance behaviours, our findings support the use of learning-based therapies, such as exposure therapy, in the treatment of anorexia nervosa (Cardi et al., 2019; Melles et al., 2021; Steinglass et al., 2012). According to the inhibitory learning approach, exposure therapy helps to violate fear-based associations and introduces a new safety-based meaning of the CS (Craske et al., 2014). A patient with anorexia nervosa might hold the expectation "If I drink a glass of milkshake, I will immediately gain 3 kilos". During exposure therapy, such threat expectations are tested, violated and new safety associations are introduced ("Drinking a glass of milkshake does not make me gain 3 kilos")(Cardi et al., 2019; Melles et al., 2021; Steinglass et al., 2012). Helping patients to recognize that food intake is not followed by aversive consequences, like immediate, extreme, endless or uncontrollable weight gain, should reduce avoidance and increase approach behaviors for food intake (Pittig & Wong, 2021). Laboratory research could constitute a cost-effective method to test whether and under what conditions exposure interventions can help to restore eating desires and to reduce aversive conditioned responses and avoidance behaviours.

We want to highlight that the present study constitutes a first step in understanding the role of classical and operant conditioning for the development of food avoidance, fear of food and eating desires. Our conditioning task was developed based on a theoretical model of food avoidance (Melles et al., 2021; Murray et al., 2018; Strober, 2004) and presents with high face validity. However, more research is needed to be able to speak of a valid experimental model of anorectic food restriction that goes beyond face validity and includes the study of individual differences (for a discussion in the context of anxiety disorder see Krypotos et al., 2018; Vervliet & Raes, 2013). Future studies could test whether dysfunctional learning in conditioning tasks can predict the development of eating disorder symptoms or a reduced treatment response (predictive validity), and whether predictors of restrictive eating outside of the laboratory also increase food avoidance in the laboratory (construct validity).

While our laboratory set-up allows to test a causal mechanism in a controlled environment, this heightened experimental control comes at the price of reduced ecological validity. Additional research is needed to understand how classical and operant conditioning affect food avoidance in a real-life setting (e.g., using ecological momentary assessment (Lavender et al., 2013) and in patients with anorexia nervosa. As we employed a strong manipulation (100% reinforcement rates, highly aversive punishment, contingency instructions), research into individual differences could benefit from turning the conditioning task into a 'weaker situation' (Lissek et al., 2006): individual differences in behavior are more likely to manifest under high ambiguity e.g., when using lower reinforcement rates or eating-disorder related stimuli in the conditioning task.

In sum, the present study shows that a combination of reward and punishment for (not) avoiding food intake can change behavioural and emotional responses to (predictors of) food intake in healthy individuals and thereby, supports the notion that conditioning processes can play a crucial role in the development and maintenance of restrictive eating – a core symptom of anorexia nervosa. Conditioning paradigms may be a useful tool for studying the development, maintenance, and treatment of food restriction or avoidance.

CRediT authorship contribution statement

Michelle Spix: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Franziska Schutzeichel:** Investigation, Writing – review & editing. **Anita Jansen:** Conceptualization, Methodology, Writing – review & editing, All authors approved the final version.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Co-author serves as an editor at Behavioral Research and Therapy - A.J.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brat.2023.104340.

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