



# Appetitive and aversive classical conditioning: Self-reports and physiological responses

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## ABSTRACT

Understanding the neural mechanisms underlying appetitive and aversive conditioning has important clinical implications because maladaptive associative learning processes are thought to contribute to various mental disorders, including anxiety, mood and eating disorders, as well as addiction and chronic pain. Since brain areas related to appetitive and aversive conditioning overlap with one another, but are probably also distinct, it is of interest to directly compare appetitive and aversive conditioning in behavioral and imaging studies. To what extent do behavioral outcome recordings in appetitive and aversive conditioning tasks match? We compared self-reports and physiological responses (skin conductance responses and pupil size) using commonly applied appetitive and aversive unconditioned stimuli (US) in 40 young and healthy participants (20 women). Different to animal studies, secondary reinforcers, particularly monetary rewards, are most commonly used as appetitive US in humans. Therefore, the first study compared self-reports and physiological assessments that were elicited by electric shock and three monetary rewards (one Euro, two Euros and five Euros). In the second study, differential aversive and appetitive conditioning were performed on two consecutive days with order being randomized between participants. Since outcome measures of electric shock best matched the one Euro reward, one Euro was used as US in the appetitive conditioning paradigm. In both studies, physiological responses were significantly lower towards appetitive conditioned stimuli (CS) and US compared to aversive CS and US (all *p*-values < 0.001). Self-reports, on the other hand, showed much fewer differences in response magnitude and differential CS responding comparing appetitive and aversive CS and US. Overall, self-reports of valence were higher towards monetary rewards compared to the electrical stimulus considering both responses to the US in study 1 and CS in study 2 (*p*-values < 0.001). Our findings show that full comparability between behavioral outcomes can probably not be achieved in appetitive and aversive conditioning paradigms since outcomes might easily diverge. Future studies comparing the neural responses in processing of aversive and appetitive stimuli using brain imaging, electroencephalography or other neurobiological methods need to control for possible differences in response magnitudes and learning rates.

## 1. Introduction

Associative learning, that is, learning about relationships between different events, comprises a key element of shaping our behavior [1,2]. Classical, or Pavlovian, conditioning is frequently used to study the neural mechanisms underlying associative learning [3–5]. In Pavlovian

conditioning, an initially neutral stimulus is reliably followed by a biologically salient event (unconditioned stimulus, US), which may either be appetitive or aversive [6], and then becomes a conditioned stimulus (CS). Primary US such as food and electric shock are inherently rewarding and aversive, respectively. In contrast, secondary US like money derive their reinforcing properties through association with

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primary reinforcers (for example, money can be exchanged for food). Learning the CS/US association results in defensive or approaching conditioned responses (CR) towards the CS. To understand the neural mechanisms underlying appetitive and aversive conditioning has important clinical applications because maladaptive associative learning processes are thought to contribute to various mental disorders, including anxiety, mood and eating disorders, as well as addiction and chronic pain [7–9]. Brain areas related to appetitive and aversive conditioning show overlap, but are probably also distinct [10,11]. For example, meta-analyses of human brain imaging studies suggest that fMRI activations due to appetitive and aversive processing are similar in ventral striatum, amygdala, thalamus, insula and cerebellum [11], whereas activations in ventral tegmental area and periaqueductal gray are selectively involved in appetitive and aversive processing, respectively [10]. It is therefore of interest to directly compare appetitive and aversive conditioning in behavioral and imaging studies. Fear conditioning paradigms are frequently employed to study aversive conditioning. An electric shock is often applied as a primary US to elicit fear in both human and animal studies [6]. While in animal studies, primary reinforcers are also utilized as US in appetitive conditioning paradigms, particularly fluids or food in fluid- or food-deprived animals [7], they are much more difficult to use in human studies, both for methodological and ethical reasons [12]. Consequently, secondary reinforcers, such as monetary rewards, are predominantly used in human appetitive conditioning [13,14]. Other secondary reinforcers are social and sensory rewards, such as smiling faces and pleasant touch [15,16]. Monetary rewards, however, have been shown to have stronger behavioral effects than social rewards [17,18]. Furthermore, in contrast to operant (or instrumental) conditioning (that is, using reinforcers coupled to a response), Pavlovian appetitive conditioning studies are performed comparatively rarely in humans [19–22].

We asked to what extent self-reports and physiological responses can be matched in aversive and appetitive conditioning in healthy human participants using commonly applied US, that is using electrical shock and monetary rewards. Two studies were performed. In the first study, self-reported outcome and physiological responses (that is, skin conductance responses and pupil size) were compared between electric shock and three monetary rewards. In the second study, Pavlovian appetitive and aversive conditioning were performed, whereas the appetitive reward was chosen based on findings of the first study.

## 2. Materials and methods

### 2.1. Participants

The sample size for this pilot study was determined using G\*Power software [23]. Based on a repeated measures ANOVA design (within factors design), we assumed a medium effect size ( $f = 0.3$ ) [24], a significance level ( $\alpha$ ) of 0.05, a desired power ( $1 - \beta$ ) of 0.8, and a moderate correlation ( $r = 0.5$ ) among repeated measures (number of measurements – 4). These parameters align with the exploratory nature of the study, where the primary goal is to estimate feasibility and effect sizes for a future, fully powered trial [25]. The analysis indicated that 17 participants would be sufficient for this purpose for each study.

A total of 42 young and healthy participants performed the experiment. Two participants had to be excluded because of technical errors. Thus, 40 participants (20 men, 20 women, mean age: 24.05 (SD = 4.61) years, range: 18–34 years) were included in the final data analysis. Twenty participants performed study 1 (10 men, 10 women, mean age: 21.9 (SD = 3.49) years, range: 18–31 years), and 20 participants performed study 2 (10 men, 10 women, mean age: 26.2 (SD = 4.65) years, range: 20–34 years).

None of the participants presented with neurological or neuropsychiatric disorders based on medical history. None were taking centrally acting drugs. The Depression-Anxiety-Stress-Scale-21 (DASS-21) questionnaire was used to assess participants' depression, anxiety, and stress

[26]. The DASS-21 is a 21-question self-report with seven questions for each of the three subscales. In both studies, stress, anxiety and depression scores were within the normal range in all participants.

To assess socioeconomic status, the amount of (monetary) savings was assessed. Participants were asked to provide the most accurate estimate of their total assets, including bank account balances, savings, real estate, funds, and other assets. In study 1, the mean amount of savings was  $15.157 \pm 28.587$  €, range = 350–130.000 €). In study 2, the mean amount of savings was  $12.337 \pm 20.151$  €, range = 700–80.000 €).

All participants had normal or corrected-to-normal vision. They were informed that they would receive a basic rate of 36 Euros for study 1, and 50 Euros for study 2. In addition, they were informed that any monetary rewards presented during the study they would be paid out in real money (total of 32 Euros in study 1, and 20 Euros in study 2).

Informed consent was obtained from all participants. Studies were approved by the University Hospital Essen ethics committee and conducted in accordance with the Declaration of Helsinki.

### 2.2. Experimental set-up

Participants took part in either study 1 or study 2, during which their pupil size, skin conductance responses (SCRs), heart rate (ECG), pulse and breathing rate were measured (Figs. 1A, 2A). Pupil size was monitored using an EyeLink® 1000 Plus System (SR Research Ltd., Ontario, Canada), positioned 60 cm from participants' eyes. To ensure continuous eye detection throughout the experiment, participants positioned their heads on a headrest device centrally aligned with both the camera and the display screen behind it. Additionally, a calibration of the eye tracking system was conducted prior to each phase. The screen was used to display all visual CS and visual US in both studies using Presentation software (version 16.4, Neurobehavioral Systems Inc., Berkeley, CA). SCRs were recorded via an MP160 Data Acquisition Hardware unit with appropriate filters sampling at 2 kHz (EDA 100C-MRI, BIOPAC Systems Inc., Goleta, CA). The skin conductance electrodes were attached to the participants' hypothenar eminence on the left hand (Fehler! Verweisquelle konnte nicht gefunden werden. Figs. 1A, 2A), positioned approximately 2 cm apart.

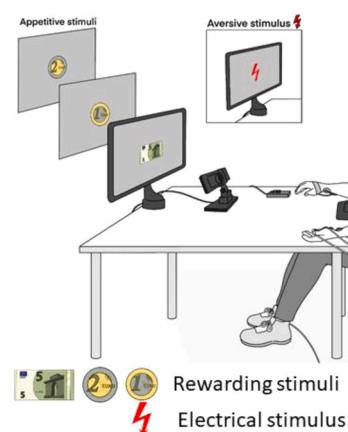
The aversive US consisted of an electrical stimulation produced by a constant current stimulator (DS7A, Digitimer Ltd., London, UK) and delivered to the participants' right hand through a 6.5 mm concentric bipolar electrode (WASP electrode, Specialty Developments, Bexley, UK). It comprised a series of short trains of consecutive 500 µs current pulses with an inter pulse interval of 33 ms and with a maximum output voltage of 400 V. The individual electrical stimulation intensity threshold was established to be perceived as very uncomfortable without being painful. To reach the threshold, the intensity strength was progressively increased and modulated according to each participant's feedback provided through a 1–9 pain perception scale. Similarly to Inoue et al. [27], final US intensity was increased by 20 % and kept the same for each aversive stimulation. The aversive US lasted 100 ms in study 1 and 1000 ms in study 2. The mean current was  $3.65 \pm 1.79$  mA (range: 0.84–7.20 mA) in study 1 and  $1.05 \pm 0.74$  mA (range: 0.48–3.42 mA) in study 2. The set-up was identical for both studies.

### 2.3. Paradigms

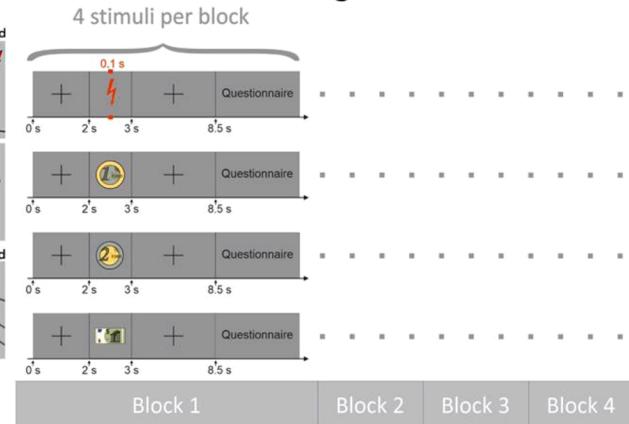
#### 2.3.1. Study 1: Comparison of self-reports and physiological responses to aversive and appetitive (unconditioned) stimuli

Participants were exposed to four different (unconditioned) stimuli: monetary rewards of one, two or five Euros presented on the screen, and an electrical stimulation on the hand, which was simultaneously signaled by a red lightning sign display on the screen (Fig. 1A). Each trial began with a 2 s fixation cross, followed by a 1 s presentation of one of the visual stimuli (either a monetary reward or a red lightning symbol) (Fig. 1B). This was followed by a 5.5 s fixation cross before the questionnaire appeared. In aversive trials, the electrical stimulation started

## A Experimental Set-up

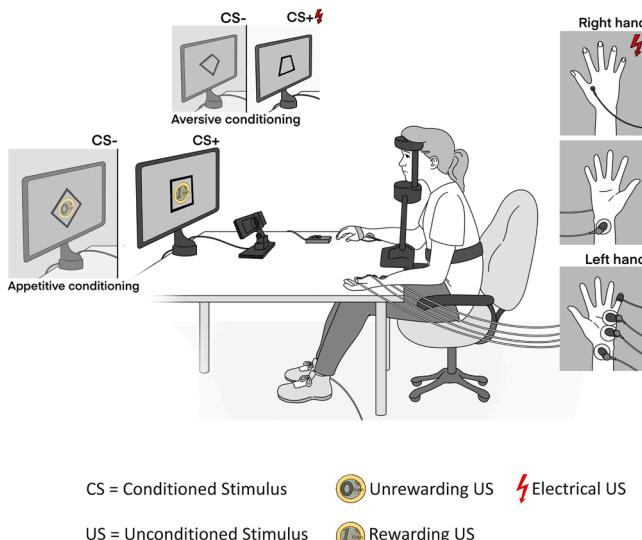


## B Paradigm

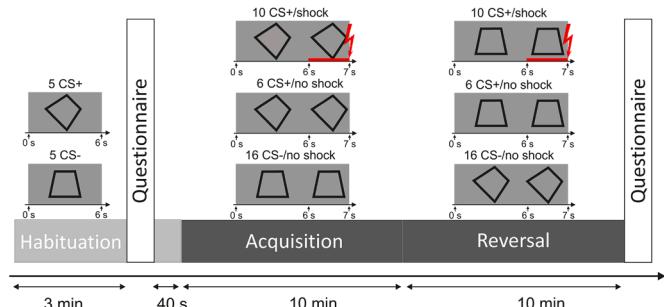


**Fig. 1.** Experimental set-up (A) and paradigm (B) used in study 1. In (A) position of the participant in front of the eyetracking system and screen is shown. Button box to answer questionnaires was positioned near the right hand. Electrode delivering electric shocks was attached to the right hand, electrodes for SCR recording were attached to the hypothenar of the left hand. Additional electrodes were attached for recording of pulse (left pinky), ECG (both wrists, left ankle) and breathing rate (belt) (data not presented in the study). In (B) one of four blocks is shown, containing all of four possible stimuli: one Euro reward, two Euros reward, five Euros reward, and an electric shock (accompanied by a red lightning visual stimulus). Self-reports based on questionnaires were done after each trial. The next three blocks contain the same four stimuli arranged in random order (not shown).

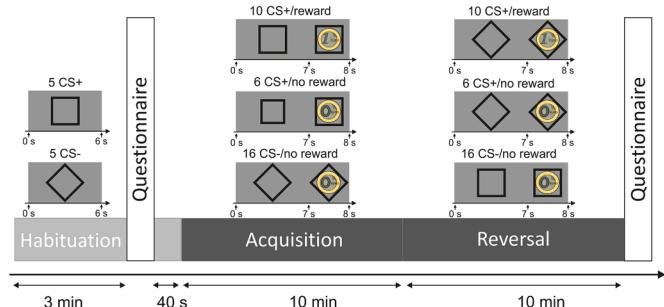
## A Experimental Set-up



## B Aversive conditioning



## Appetitive conditioning



**Fig. 2.** Experimental set-up (A) and paradigm (B) used in study 2. In (A) position of the participant in front of the eyetracking system and screen is shown. Visual CS and US were shown on the screen. Electrode delivering electric shocks was attached to the right hand. Button box to answer questionnaires was positioned near the right hand. Electrodes for SCR recording were attached to the hypothenar of the left hand. Additional electrodes were attached for recording of pulse (left pinky), ECG (both wrists, left ankle) and breathing rate (belt) (data not presented in the study). In (B) aversive and appetitive differential Pavlovian reversal paradigms are shown using one Euro reward or electrical stimulation as appetitive or aversive US, respectively. Habituation phase is followed by initial acquisition training and a reversal phase. Geometric figures represent CS. US overlaps with CS in the last second of presentation (delay paradigm). For acquisition and reversal the number of reinforced and nonreinforced trials is given.

450 ms after the corresponding visual stimulus onset and lasted for 100 ms [39]. Participants rated stimulus perception, fear, valence and arousal based on questionnaires as outlined below. Time interval between stimuli presentations depended on how quickly participants answered the questions. The stimuli were organized into four blocks, each containing all four different stimuli presented in a randomized

order. The randomization of stimulus order was unique for each participant and ensured that no stimulus was presented more than twice consecutively. Participants were informed that the monetary rewards displayed during the experiment would be given to them after the experiment.

### 2.3.2. Study 2: Pavlovian aversive and appetitive conditioning

In study 2, a differential Pavlovian reversal paradigm was used for both appetitive and aversive conditioning (Fig. 2B). All study 2 participants underwent both paradigms on two consecutive days, with the paradigm order being randomized and balanced for sex. For the conditioned stimuli (CS+ and CS-), two pairs of geometrical figures were used: square and diamond (a square tilted 45°) or trapezoid and rhomboid (a trapezoid tilted 45°) (Fig. 2A,B). The pairs of geometrical figures were randomly assigned to either appetitive or aversive conditioning, with assignments varying across participants and balanced for sex. The CS+ and CS- pairs had the same colour intensity and pixel count. In both appetitive and aversive conditioning, delay paradigms were used. The CS were presented for 7 s for appetitive conditioning and 6 s for aversive conditioning, all US for 1 s. CS and US co-terminated. Appetitive US was the “one Euro” sign, identical to the “one Euro” sign used in study 1. Intertrial intervals randomly varied from 7 s to 11 s. A fixation cross was presented during the intertrial intervals. In study 2, participants were exposed to three trial types: a CS followed by a US (paired CS+/US trial), a CS without the US (CS+/noUS trial), and a CS which was never followed by the US (CS-).

Participants were informed about the goal of the study, i.e. to understand the processing of pleasant and unpleasant stimuli. They were also informed that they were not required to do anything except remain attentive and keep looking at the screen the whole time of the experiment, which would display a black cross on a gray background along with various symbols. Normal blinking was allowed, but prolonged eye closure and eye movement were discouraged to ensure accurate measurement of pupil size. Electrodes attached to their left hand measured skin conductance, while additional sensors recorded heart rate and breathing frequency. Participants were advised to minimize movement to prevent the electrodes and sensors from shifting. Experimenters were available to assist if needed. Before aversive conditioning they were informed that the experiment would start by setting the intensity of electric stimuli, beginning with very weak ones that were not perceptible. The intensity would gradually increase based on their feedback until it reached a level that was very unpleasant but not painful. This intensity would remain consistent throughout the experiment. They were also instructed that an electrical shock would be applied only through one electrode, while the other electrodes were for measurements. Before appetitive conditioning, participants were instructed that all monetary symbols they would see would be given to them after the experiment, in addition to the already announced reimbursement. Participants were informed at the beginning of each experiment which kind of stimuli they will receive (that is, monetary rewards or electrical stimuli).

During habituation participants were presented 4 unreinforced CS+ and 4 CS- trials. Habituation started either with a CS+ or a CS- (randomized and balanced for sex). During reward or aversive acquisition training, CS+ and CS- were switched halfway through the corresponding acquisition phase, *unbeknown* to the participants. That is, the CS+ which was reinforced in initial acquisition training was not reinforced anymore, and the CS- was reinforced instead. Following habituation and after reversal phase participants answered the questionnaires.

The full acquisition training consisted of 66 trials and started with unreinforced presentation of one CS+ and CS- trial to refresh habituation (trials were excluded from the analysis). This was followed by 32 initial acquisition trials and 32 reversal trials. During both the acquisition and reversal phases, participants were exposed to 16 CS+ and 16 CS- in a pseudorandomized sequence, ensuring no more than two identical geometrical figures were presented consecutively. In each phase (initial and reversal), 10 out of the 16 CS+ were reinforced (62.5 % reinforcement rate). Each trial type was equally distributed across the first and second halves of both phases. Last trial of acquisition and the first and last trials of reversal were reinforced CS+ trials. CS- was never followed by electric shock in case of aversive conditioning. In the case of appetitive conditioning CS- was always followed with “0

Euro” sign indicating no reward. The order of CS+ and CS- was the same in acquisition and reversal phases, except that during reversal, CS+ was switched to CS- and vice versa.

### 2.4. Self-reports

In study 1, participants were asked to rate US perception, arousal, valence, and fear using a nine-step Likert scale after each trial. Arousal was rated from 1 (very calm) to 9 (very nervous), valence from 1 (very pleasant) to 9 (very unpleasant), and fear from 1 (not afraid) to 9 (very afraid). Stimulus perception ranged from 1 (very gratifying) to 9 (not gratifying at all) for monetary rewards, and from 1 (not unpleasant) to 9 (very unpleasant) for electrical stimulation. To allow for comparison of “absolute” perception and valence, the scales for monetary rewards were reversed for data analysis.

In study 2, participants rated conditioned stimuli (CS+ and CS-) regarding arousal, valence and fear using the same nine-step Likert scale as in study 1. The scale for valence ratings for monetary rewards was reversed for data analysis. In addition, US expectancy (from 1 (US not expected) to 9 (US expected)) was rated. Ratings were done following habituation (except for US expectancy) and at the end of the experiment. At the end of the experiment, participants were asked to rate the initial acquisition training and reversal phase separately. All participants confirmed that they had noticed the switch in the CS/US contingencies.

### 2.5. Physiological data acquisition and analysis

To eliminate high-frequency noise, skin conductance data was low-pass filtered with a 10 Hz cutoff using a hardware filter (EDA100C-MRI module, BIOPAC Systems). Data was downsampled to 1 kHz and semi-automated peak detection was performed using the EDA-Analysis software [28] based on MATLAB (Release 2022b, RRID: SCR\_001622, The MathWorks). SCRs were defined as the maximum trough-to-peak amplitude within a given time interval and with a minimum amplitude of 0.01  $\mu$ S and a minimum rise time of 500 ms [29]. The time interval used for SCR peak detection was from 1 s after stimulus onset to 5 s after stimulus onset [30] in study 1, and from 1 s after CS onset to the end of the CS in study 2. SCR peaks were initially detected by the software algorithm based on the parameters we predefined. These peaks were then manually reviewed to ensure accuracy, and any identified as artifactual, such as those caused by movement or technical issues, were removed. Trials that did not meet the criteria were scored as zero and included in the subsequent data analysis [31]. In all cases, raw SCRs were normalized through a logarithmic [ $\ln(1 + \text{SCR})$ ] transformation [29,32].

### 2.6. Pupilometry analysis

Preprocessing of the raw pupil data was performed in MATLAB (version 9.13 (R2022b), MathWorks, Natick, USA). Blinks and saccades were removed from the data. Furthermore, a simultaneous visual examination of the raw and processed pupil data was carried out to ensure processing coherence and determine whether both eyes were properly recorded and should be kept in the subsequent analysis. By default, means were computed for each phase using data from the right eye. In study 1, the time window starting from 0.5 s to 2.5 s after stimulus offset was analyzed, and the pupil response was calculated as mean value during time window minus mean value during baseline. As baseline we took a 5 s interval starting 20 s before the onset of the stimulus, which was when participants answered the questionnaires. In study 2, pupil size was analyzed in the 2 s interval preceding US onset, which has been shown to show the largest difference between CS+ and CS- during fear acquisition training [33]. For each trial, the baseline was computed as the mean pupil size recorded during the 300 ms prior CS onset and subtracted from the corresponding pupil size during CS [33,34]. To convert raw pupil size data from pixels to mm<sup>2</sup>, a human-sized dummy

eye was used with a known size positioned exactly where a participant's eye would be during the experiment to calculate a conversion factor (0.0016 per pixel).

## 2.7. Statistical analysis

Data analysis was conducted using nonparametric statistical methods. We used nonparametric statistical analysis for repeated measures using rank-based F tests (ANOVA option in the PROC MIXED method in SAS, SAS Studio 3.8, SAS Institute and *nparLD* R package), which has been recommended for dealing with skewed distributions, outliers, or small sample sizes. These methods use ANOVA-type statistic with the denominator degrees of freedom set to infinity [35,36] to enhance the reliability of the ANOVA-type statistic. Using finite denominator degrees of freedom can lead to increased type I errors [37]. Scores on the Likert scale for perception and valence for monetary rewards have been reversed for direct comparability with shock.

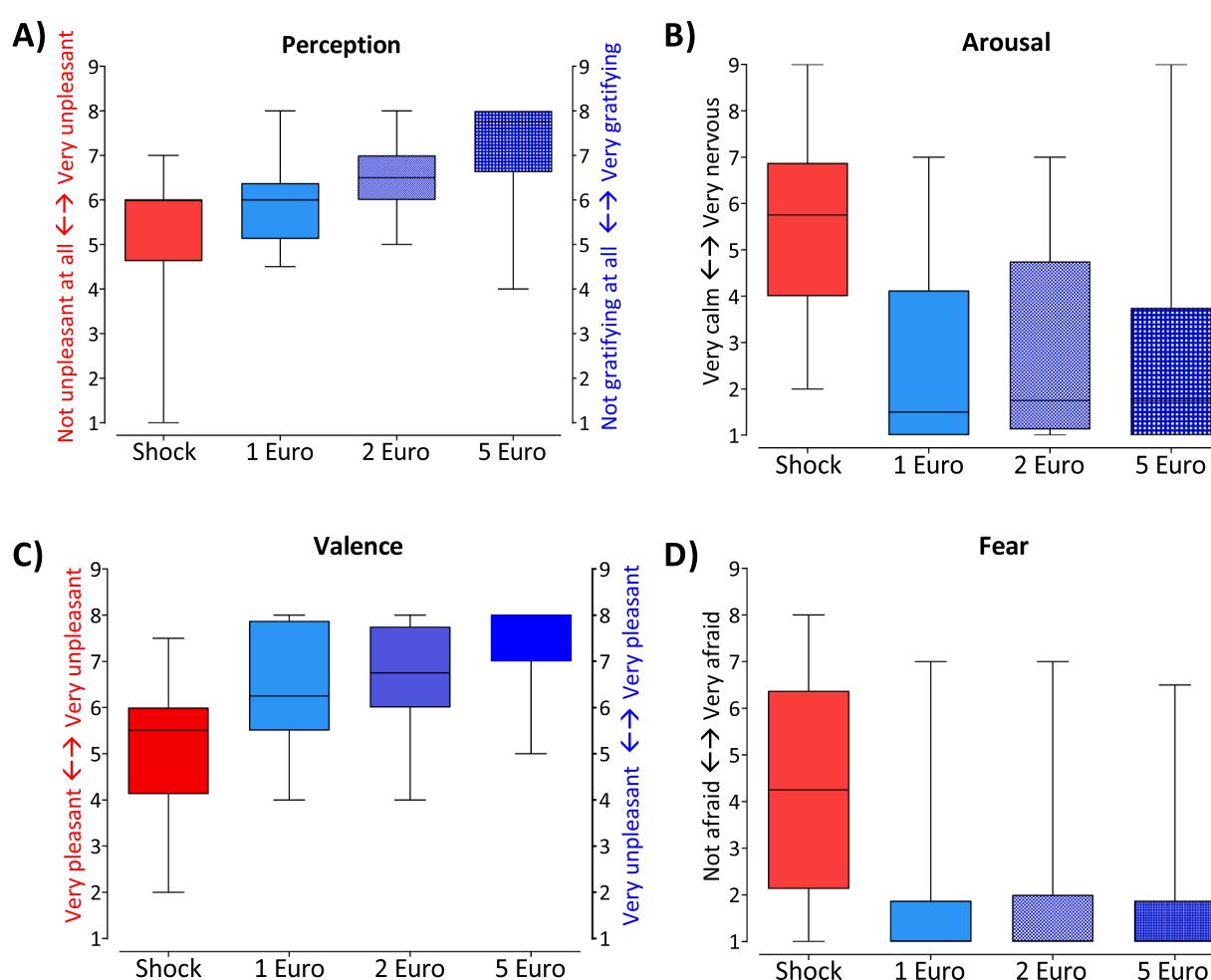
In study 1 measured SCRs and pupil size value was used as dependent variable. Type of Stimulus (one Euro, two Euros, five Euros, electrical stimulation) and Block (1–4) were chosen as within-subjects factors, as well as their interaction. Self-reports were analyzed the same way with the rating value as dependent variable.

In study 2 measured value were taken as dependent variable (self-report value, pupil size change or SCRs), Conditioning Paradigm

(appetitive vs. aversive), Stimulus (CS+ vs. CS-), Phase (initial acquisition vs. reversal) and Block (early vs. late) as within-subjects factors, as well as their interactions. Please note that the initial CS+ and CS- have been recoded to CS- and CS+ in reversal phase.

Throughout the manuscript, in case of significant results of nonparametric ANOVA-type statistic, post hoc comparisons were performed using least square means tests and were adjusted for multiple comparisons using the Tukey-Kramer method. To assess the influence of participants' sex and socioeconomic status on the results, we repeated the statistical analyses for both study 1 and study 2, adding sex and participants' self-reported monetary savings as covariates. For study 2, we included a third covariate: the order of paradigms, that is, whether appetitive conditioning was conducted before or after aversive conditioning. The effects of these covariates were analyzed independently.

To quantify effect sizes, we employed a metric known as relative treatment effect (RTE), also known as the Wilcoxon-Mann-Whitney effect. The RTE, which ranges from 0 to 1, represents the probability that a randomly selected observation from a specific subset of data under a given condition is either larger or smaller than a randomly selected observation from the entire dataset. For example, an RTE value of 0.25 for condition X indicates that there is approximately a 25 % chance that a randomly chosen observation from the entire dataset would score lower than an observation randomly selected from condition X [38].



**Fig. 3.** Self-reports for appetitive and aversive stimuli. Bars show group medians and interquartile ranges. Y-axis represents ratings for (A) stimulus perception for money shown in blue (from 1: 'not gratifying at all' to 9: 'very gratifying'), for shock shown in red (from 1: 'not unpleasant at all' to 9: 'very unpleasant'); (B) arousal (from 1: 'very calm' to 9: 'very nervous'), (C) valence (for money: 1: 'very unpleasant' to 9: 'very pleasant', for shock: 1: 'very pleasant' to 9: 'very unpleasant'), (D) fear (from 1: 'not afraid' to 9: 'very afraid'). The scales for perception and valence regarding money have been reversed for direct comparability. Whiskers range from the first to the third quartile.

### 3. Results

#### 3.1. Study 1: Comparison of self-reports and physiological responses to aversive and appetitive (unconditioned) stimuli

##### 3.1.1. Self-reports

Visual inspection of self-reports in Fig. 3 shows that perception, valence, arousal and fear differed between shock and monetary rewards. Overall, “absolute” ratings were closest between shock and one Euro reward considering perception and valence. Results of nonparametric ANOVA are summarized in Table 1.

##### 3.1.2. Perception

Nonparametric ANOVA-type statistics showed a significant main effect of Stimulus ( $F_{(1,43)} = 48.30, p < 0.001$ ), and Block (1–4) ( $F_{(2,21)} = 3.66, p = 0.022$ ). No interaction effects were found. Post hoc analyses showed significant differences between all monetary rewards ( $p < 0.001$ ) with five Euros being most gratifying and one Euro being least gratifying as could be expected. Shock was significantly different from all monetary rewards ( $p < 0.001$ ), being less “unpleasant” than money “gratifying”. Although there was a significant main effect of Block, post hoc analysis did not reveal significant difference for any of the stimuli across Blocks (one Euro in first block was not different from one Euro in any other block etc.).

##### 3.1.3. Arousal

Nonparametric ANOVA-type statistics showed a significant main effect of Stimulus ( $F_{(1,63)} = 28.61, p < 0.001$ ). No Block or interaction effects were found. Post hoc analysis showed that arousal ratings regarding the shock were significantly higher ( $p < 0.001$ ) than to any of monetary rewards that did not differ significantly from each other.

##### 3.1.4. Valence

Nonparametric ANOVA-type statistics showed a significant main effect of Stimulus ( $F_{(1,76)} = 56.73, p < 0.001$ ), and Block ( $F_{(2,46)} = 4.81, p = 0.005$ ). No interaction effects were found. Post hoc analysis revealed significant differences between all monetary rewards and shock ( $p < 0.001$ ), showing that the value of unpleasantness of the shock was

lower than pleasantness of all monetary rewards. In addition, one Euro was rated as less pleasant than five Euros ( $p = 0.013$ ), and two Euros were rated as less pleasant than five Euros ( $p = 0.009$ ). Ratings of one and two Euros did not differ significantly ( $p = 0.752$ ). Post hoc analysis showed that stimuli were rated higher in the first than in the third block ( $p = 0.030$ ), with no other significant block differences.

##### 3.1.5. Fear

Nonparametric ANOVA-type statistics showed a significant main effect of Stimulus ( $F_{(1,31)} = 22.21, p < 0.001$ ). No Block or interaction effects were found. Post hoc analysis showed that all monetary rewards were rated lower than shock ( $p < 0.001$ ) and did not differ significantly between each other.

### 3.2. Physiological responses

Visual inspection of physiological responses in Fig. 4 shows that SCRs and pupil size responses were larger towards the shock compared to monetary rewards with no difference between one, two and five Euros.

#### 3.2.1. Skin conductance responses

Nonparametric ANOVA-type statistics showed significant main effects of Stimulus ( $F_{(2,3)} = 17.78, p < 0.001$ ) and Block ( $F_{(2,03)} = 3.96, p = 0.018$ ). No significant Stimulus  $\times$  Block ( $p = 0.394$ ) interaction was found. Post hoc analysis showed higher responses to electrical shock than to one Euro, two Euros and five Euros, with no differences between monetary rewards.

#### 3.2.2. Pupil responses

Nonparametric ANOVA-type statistics showed significant main effects of Stimulus ( $F_{(2,95)} = 69.34, p < 0.001$ ), Block ( $F_{(1,99)} = 29.12, p < 0.001$ ) and Stimulus  $\times$  Block ( $F_{(7,79)} = 4.60, p < 0.001$ ) interaction. Post hoc analysis showed that pupil response to electrical shock was higher than response to any of monetary rewards ( $p < 0.001$ ). Response to one Euro was lower than response to two Euros ( $p < 0.001$ ) and lower than response to five Euros ( $p < 0.001$ ). Two Euros and five Euros did not differ ( $p = 0.727$ ). Responses in the first block were higher than responses in all other blocks ( $p < 0.001$ ). This block effect was most prominent for the aversive stimulus (Fig. 4D), likely reflecting habituation processes.

Overall, self-reports of arousal and fear as well as physiological responses were significantly higher for the aversive stimulus compared to appetitive stimuli. Ratings of perception and valence were significantly higher for appetitive stimuli compared to the aversive stimulus. Because one Euro showed the smallest differences to the shock based on self-reported valence and perception, we decided to use the one Euro reward in study 2 (Pavlovian appetitive conditioning).

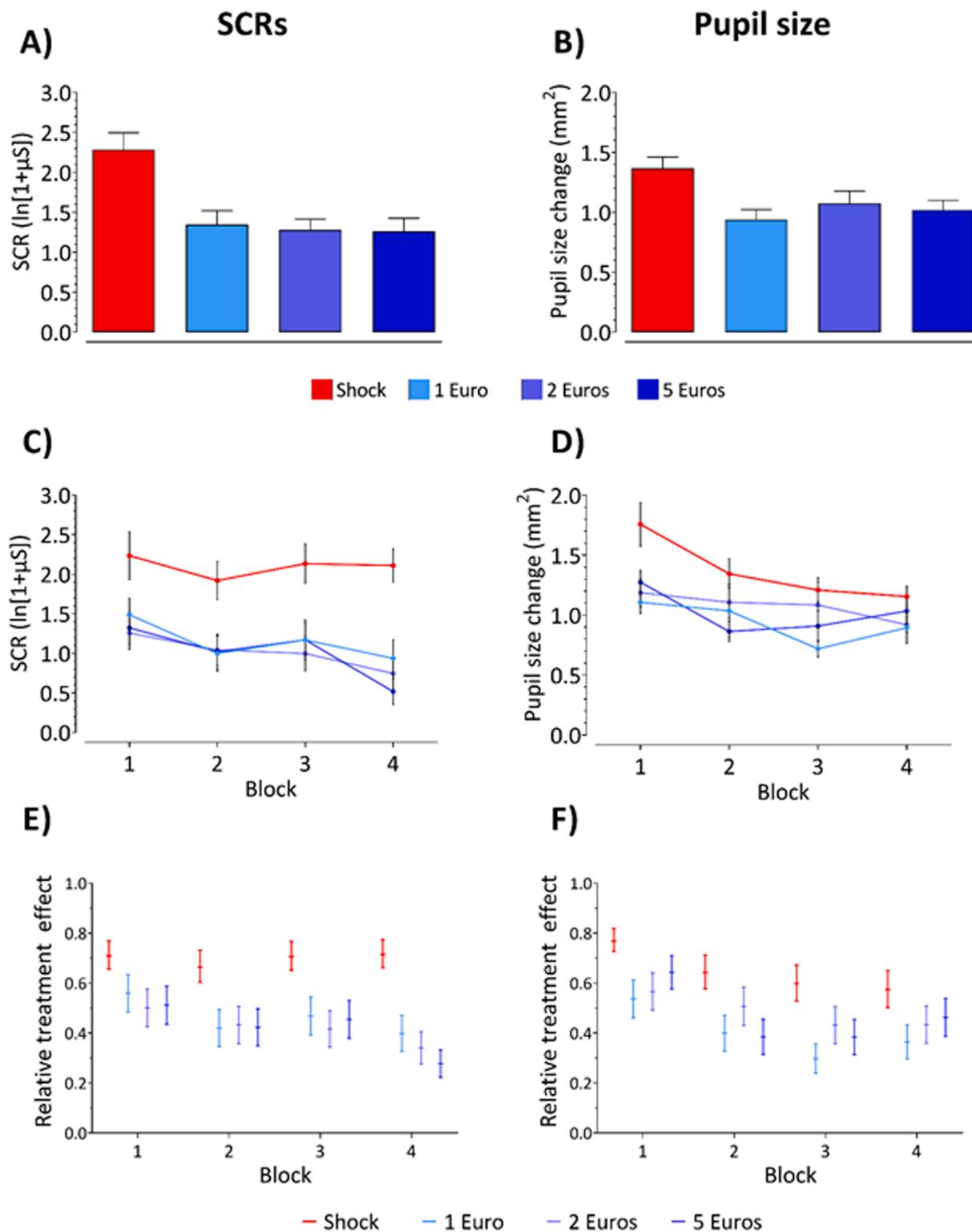
#### 3.3. Sex and (monetary) savings effects

Sex of participants had a significant impact on ratings of Valence ( $p < 0.001$ ) and Fear ( $p = 0.006$ ), which was rated higher by women for both, with no significance for Perception ( $p = 0.562$ ), and Arousal ( $p = 0.150$ ). SCRs and pupil size responses were not significantly different between men and women (both  $p$ -values = 0.351). The amount of savings had a significant impact on ratings of Arousal ( $p < 0.001$ ), Valence ( $p < 0.001$ ), and a trend like significance on Perception ( $p = 0.065$ ). Participants who had less money rated those values higher. The amount of savings had no significant impact on fear ratings ( $p = 0.729$ ). Savings had a significant impact on pupil size responses ( $p = 0.025$ ). Participants with more savings had higher pupil size responses. There was a trend-like effect on SCRs ( $p = 0.095$ ): participant with more savings tended to have lower SCRs. Note that the main findings of the results (comparing appetitive and aversive stimuli) remained unchanged when including sex and savings as a covariate (see Tables S1-S2 and Figures S1-S12 in supplementary materials).

**Table 1**  
Summary of nonparametric ANOVA in study 1 considering self-reports and physiological parameters.

Actor	Numerator Df	F	P
<b>Perception</b>			
Block	2.21	3.66	<b>0.022</b>
Stimulus	1.43	22.21	<b>&lt; .001</b>
Block $\times$ Stimulus	4.64	1.36	0.863
<b>Arousal</b>			
Block	1.86	2.10	0.126
Stimulus	1.63	28.61	<b>&lt; .001</b>
Block $\times$ Stimulus	5.23	0.81	0.546
<b>Valence</b>			
Block	2.46	4.81	<b>0.005</b>
Stimulus	1.76	56.73	<b>&lt; .001</b>
Block $\times$ Stimulus	5.23	0.39	0.864
<b>Fear</b>			
Block	2.18	1.75	0.171
Stimulus	1.31	22.21	<b>&lt; .001</b>
Block $\times$ Stimulus	5.43	1.36	0.232
<b>SCRs</b>			
Block	2.03	3.96	<b>0.018</b>
Stimulus	2.30	17.78	<b>&lt; .001</b>
Block $\times$ Stimulus	5.04	1.04	0.394
<b>Pupil size</b>			
Block	1.99	29.12	<b>&lt; .001</b>
Stimulus	2.95	69.34	<b>&lt; .001</b>
Block $\times$ Stimulus	7.79	4.60	<b>&lt; .001</b>

Bold font indicates statistical significance at  $p < 0.05$



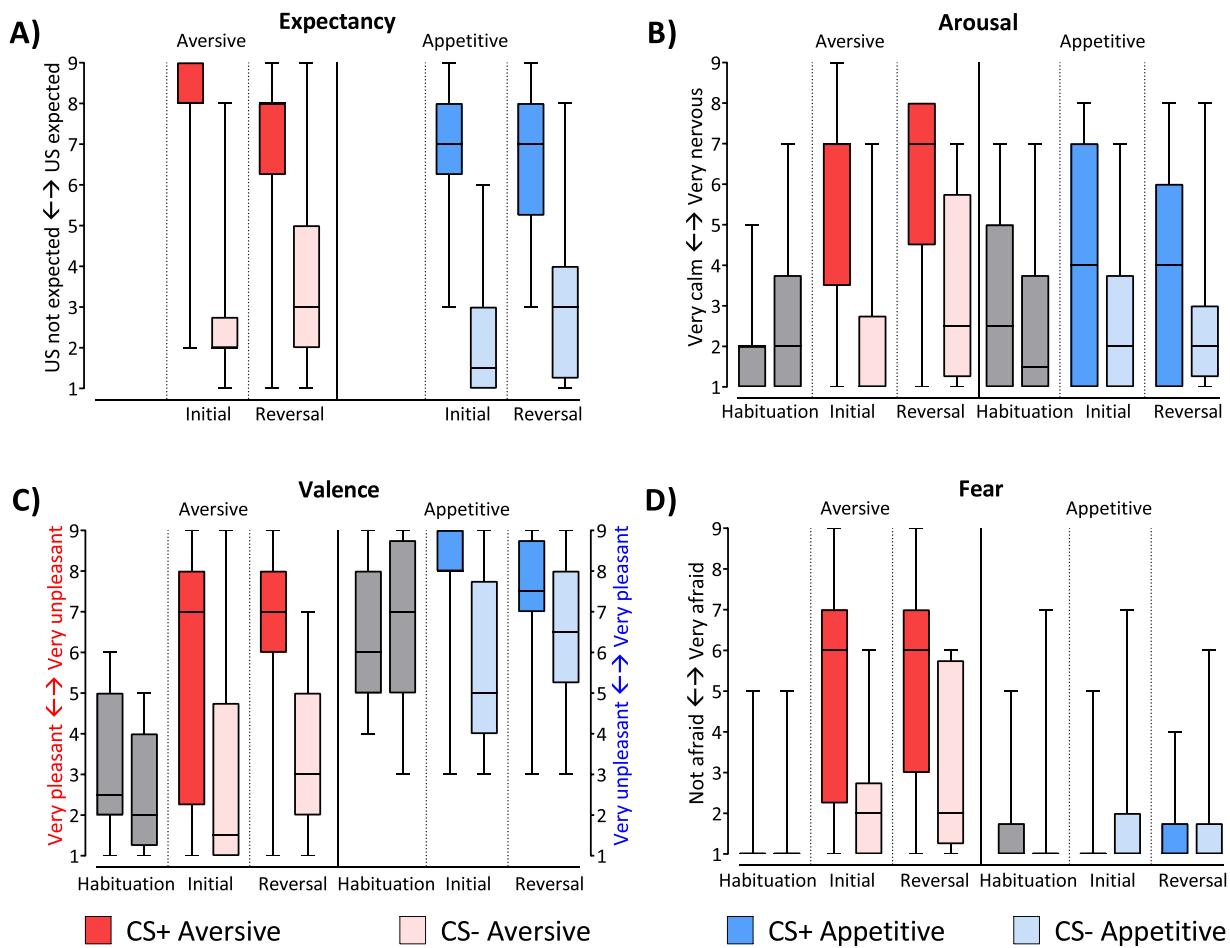
**Fig. 4.** SCRs (A,C) and pupil responses (B,D) to the shock, one Euro reward, two Euros reward, five Euros rewards averaged across blocks (A,B) and for individual blocks (C,D). Data are presented as means and standard errors of the mean (SEM). Relative treatment effect (RTE) estimates for individual blocks of (E) SCRs and (F) pupil responses. Horizontal lines represent median RTEs, and whiskers indicate 95 % confidence intervals.

### 3.4. Study 2: Pavlovian aversive and appetitive conditioning

#### 3.4.1. Self-reports

Visual inspection of self-reports in Fig. 5 shows participants learned to differentiate between the CS+ and the CS- both in the initial acquisition training and the reversal phase in the aversive and the appetitive

conditioning paradigm. Expectancy, arousal and fear ratings were higher in aversive conditioning, whereas absolute valence ratings were higher in appetitive conditioning. Results of nonparametric ANOVA are summarized in Table 2.



**Fig. 5.** Self-reports of appetitive and aversive CS pre-/post-conditioning. Median ratings, regarding expectancy (A), arousal (B), valence (C) and fear (D) on a Likert scale. Data shown are medians and interquartile ranges. Appetitive paradigm is represented by dark blue (CS+) and light blue (CS-), aversive paradigm is represented by dark red (CS+) and light red (CS-), and ratings in habituation in grey. Please note that during reversal, the initial CS+ is no longer followed by the US and the initial CS- is followed by the US (the initial CS+ and CS- have been recoded to CS- and CS+ in reversal phase). Initial = initial acquisition training, reversal = reversal training. Horizontal lines denote median values. Whiskers range from the first to the third quartile.

### 3.4.2. US Expectancy

Nonparametric ANOVA-type statistics showed a significant main effect of Stimulus (CS+ vs CS-:  $F_{(1)} = 7856$ ,  $p < 0.001$ ), and Conditioning Paradigm (appetitive vs aversive:  $F_{(1)} = 15.12$ ,  $p < 0.001$ ). The Stimulus x Phase interaction effect was significant ( $F_{(1)} = 6.14$ ,  $p = 0.013$ ). Inspection of Fig. 5A shows that expectancy of the US was higher following the CS+ in aversive compared to appetitive conditioning, although the differences were small. The difference between the CS+ and CS- was less pronounced in the reversal phase compared to initial acquisition training. Post hoc tests showed that US expectancy after initial acquisition training was not significantly different from after the reversal phase both considering the CS+ ( $p = 0.150$ ) and CS- ( $p = 0.141$ ). Likewise, no significant US expectancy differences were found between CS+ appetitive and CS+ aversive ( $p = 0.130$ ) as well as between CS- appetitive and CS- aversive ( $p = 0.602$ ).

### 3.4.3. Arousal

Nonparametric ANOVA-type statistics showed significant main effects of Stimulus (CS+ vs CS-) ( $F_{(1)} = 54.74$ ,  $p < 0.001$ ), Conditioning Paradigm (appetitive vs aversive) ( $F_{(1)} = 9.97$ ,  $p = 0.002$ ) and an interaction effects of Conditioning Paradigm x Stimulus ( $F_{(1)} = 10.77$ ,  $p = 0.001$ ). Overall, arousal ratings were higher in aversive compared to appetitive conditioning, but differences were small (Fig. 5B). The Stimulus x Phase x Conditioning Paradigm interaction was significant ( $F_{(1)} = 4.07$ ,  $p = 0.044$ ). Post hoc tests showed that arousal ratings

regarding the CS+ were higher than CS- in the reversal phase of aversive conditioning ( $p = 0.003$ ) but not in the reversal phase of appetitive conditioning ( $p = 0.995$ ).

### 3.4.4. Valence

Nonparametric ANOVA-type statistics showed significant main effects of Stimulus (CS+ vs CS-:  $F_{(1)} = 41.84$ ,  $p < 0.001$ ), and Conditioning Paradigm (appetitive vs aversive:  $F_{(1)} = 20.30$ ,  $p < 0.001$ ). The Stimulus x Conditioning Paradigm interaction did not reach significance. Post hoc analysis showed that (absolute) valence of the CS+ appetitive was significantly higher than CS+ aversive ( $p = 0.023$ ). Self-reports in absolute values were higher for appetitive CS+ than for aversive CS+, showing that appetitive CS+ was more rated as pleasant, than the aversive CS+ as unpleasant.

### 3.4.5. Fear

There were significant main effects of Conditioning Paradigm (appetitive vs aversive:  $F_{(1)} = 50.53$ ,  $p < 0.001$ ), and Stimulus (CS+ vs CS-:  $F_{(1)} = 8.52$ ,  $p < 0.006$ ). The Conditioning Paradigm (appetitive vs aversive) x Phase (initial vs reversal:  $F_{(1)} = 6.55$ ,  $p = 0.011$ ) and the Conditioning Paradigm x Stimulus interactions ( $F_{(1)} = 27.06$ ,  $p < 0.001$ ) were significant. Post hoc test showed, as expected, no difference in fear ratings for the appetitive CS+ and CS- ( $p = 0.291$ ), but higher fear ratings for the CS+ compared to the CS- in aversive conditioning ( $p < 0.001$ ). Ratings of fear for the reversal phase were significantly

**Table 2**  
Summary of nonparametric ANOVA in study 2 considering self-reports.

Factor	Numerator Df	F	p
<b>US Expectancy</b>			
Stimulus	1	78.56	< .001
Conditioning Paradigm	1	15.12	< .001
Phase	1	0.01	0.938
Stimulus × Conditioning	1	0.31	0.575
Stimulus × Phase	1	6.14	<b>0.013</b>
Conditioning × Phase	1	0.25	0.621
Stimulus × Conditioning × Phase	1	0.01	0.925
<b>Arousal</b>			
Stimulus	1	54.74	< .001
Conditioning Paradigm	1	9.97	<b>0.002</b>
Phase	1	1.93	0.165
Stimulus × Conditioning	1	10.77	<b>0.001</b>
Stimulus × Phase	1	1.42	0.233
Conditioning × Phase	1	1.24	0.266
Stimulus × Conditioning × Phase	1	4.07	<b>0.044</b>
<b>Valence</b>			
Stimulus	1	41.84	< .001
Conditioning Paradigm	1	20.30	< .001
Phase	1	1.44	0.229
Stimulus × Conditioning	1	3.71	0.054
Stimulus × Phase	1	1.38	0.241
Conditioning × Phase	1	1.44	0.230
Stimulus × Conditioning × Phase	1	1.46	0.227
<b>Fear</b>			
Stimulus	1	8.52	0.004
Conditioning Paradigm	1	50.53	< .001
Phase	1	1.80	0.180
Stimulus × Conditioning	1	27.06	< .001
Stimulus × Phase	1	0.35	0.556
Conditioning × Phase	1	6.55	<b>0.011</b>
Stimulus × Conditioning × Phase	1	3.27	0.71

Bold font indicates statistical significance at  $p < 0.05$

higher than of initial acquisition training for aversive conditioning ( $p = 0.003$ ).

### 3.5. Physiological responses

Fig. 6 shows data averaged for the early (first half of the trials) and late (second half of the trials) block of each learning phase (initial acquisition training and reversal), and Fig. 7 shows data considering individual trials. SCR and pupil responses showed differential responses to the CS+ and CS- in aversive conditioning, during both initial acquisition training and the reversal phase. For appetitive conditioning, physiological responses were lower, and differential responses were mainly seen in SCR data. Results of nonparametric ANOVA are summarized in Table 3.

#### 3.5.1. Skin conductance responses

Nonparametric ANOVA-type statistics revealed significant main effects of Conditioning Paradigm (appetitive vs aversive:  $F_{(1)} = 96.40$ ,  $p < 0.001$ ), Stimulus (CS+ vs CS-:  $F_{(1)} = 64.48$ ,  $p < 0.001$ ), Phase (initial vs reversal:  $F_{(1)} = 24.73$ ,  $p < 0.001$ ) and Block (early vs late:  $F_{(1)} = 29.12$ ,  $p < 0.001$ ). The Stimulus × Conditioning Paradigm interaction was significant ( $F_{(1)} = 24.97$ ,  $p < 0.001$ ). Post hoc tests showed that the aversive CS+ were accompanied by significantly higher SCRs than appetitive CS+ ( $p < 0.001$ ), aversive CS+ by higher SCRs than aversive CS- ( $p < 0.001$ ), and appetitive CS+ by higher SCRs than appetitive CS- ( $p = 0.04$ ). There was also a significant Conditioning × Phase interaction effect ( $F_{(1)} = 18.05$ ,  $p < 0.001$ ). Post hoc tests showed that responses during initial acquisition training of aversive conditioning were significantly higher than during the reversal phase of aversive conditioning ( $p < 0.001$ ). There was no significant difference between initial acquisition training and the reversal phase for appetitive conditioning. ( $p = 0.001$ ). In addition, there was a significant Conditioning × Block interaction effect ( $F_{(1)} = 27.43$ ,  $p < 0.001$ ). Visual inspection of Fig. 6

shows that SCRs decreased across blocks in aversive but not appetitive conditioning. In fear conditioning, decreasing SCRs is a common finding which reflects habituation processes [39,40]. Post hoc tests revealed that responses in the early block of aversive conditioning were significantly higher than responses during the late block of aversive conditioning ( $p < 0.001$ ). For appetitive conditioning, this difference was not significant ( $p = 0.903$ ).

Finally there was a significant Stimulus × Conditioning Paradigm × Block interaction ( $F_{(1)} = 15.21$ ,  $p < 0.001$ ). Post hoc tests showed that responses to appetitive CS+ were higher compared to CS- in late ( $p < 0.004$ ) but not early Block ( $p = 0.348$ ). For aversive conditioning, CS+ caused higher responses compared to CS- both in early and late blocks ( $p < 0.001$ ).

#### 3.5.2. Pupil responses

Nonparametric ANOVA-type statistics revealed significant main effects of Conditioning Paradigm (appetitive vs aversive:  $F_{(1)} = 17.91$ ,  $p < 0.001$ ), Stimulus (CS+ vs CS-:  $F_{(1)} = 13.40$ ,  $p < 0.001$ ) and a trend significance of Stimulus × Conditioning Paradigm interaction effect ( $F_{(1)} = 3.96$ ,  $p = 0.063$ ). Post hoc tests showed a significant difference between CS+ and CS- during aversive conditioning ( $p = 0.004$ ), but not during appetitive conditioning ( $p = 0.58$ ). In addition, pupil size towards the CS+ during appetitive conditioning was significantly lower compared to the CS+ during aversive conditioning ( $p = 0.002$ ). Visual inspection of trial-by-trial mean pupil size responses in Fig. 7B shows increased responses to the appetitive CS+ compared to the appetitive CS- in the reversal phase. Exploratory post hoc analysis considering only the reversal phase in appetitive conditioning revealed a significant Stimulus effect (CS+ vs CS-:  $F_{(1)} = 4.27$ ,  $p = 0.039$ ).

#### 3.6. Sex, (monetary) savings and order effects

Women showed higher SCRs than men ( $p < 0.001$ ), but pupil responses did not significantly differ ( $p = 0.859$ ). Sex had no significant impact on self-reports.

The amount of savings did not have a significant impact on self-reports (Arousal ( $p = 0.723$ ), Valence ( $p = 0.102$ ), Fear ( $p = 0.232$ ), Expectation ( $p = 0.168$ )) or pupil size responses ( $p = 0.495$ ). There was a significant effect on SCRs ( $p = 0.043$ ) with more savings leading to lower responses.

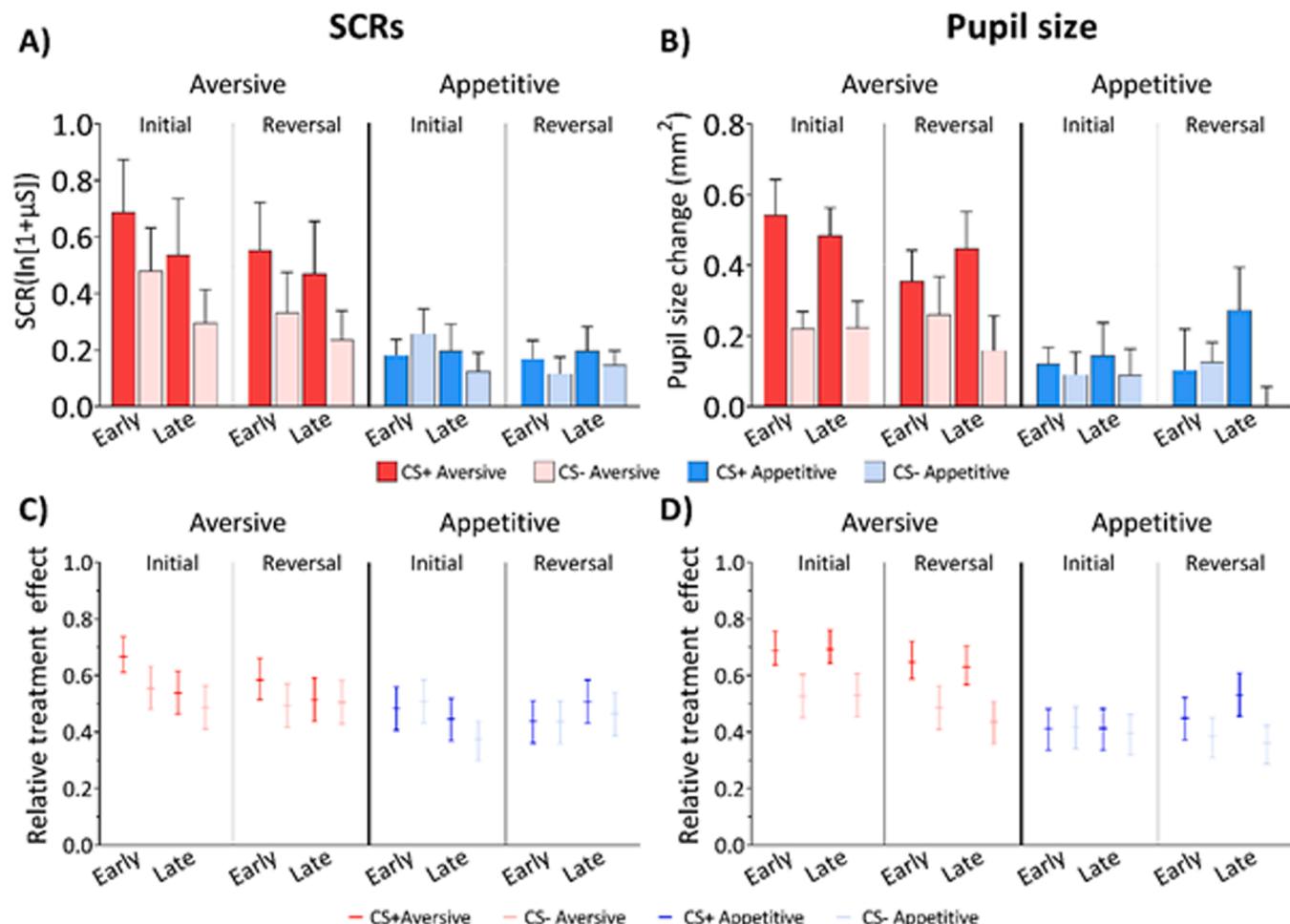
Participants who started Study 2 with appetitive conditioning had significantly higher ratings for Arousal ( $p = 0.054$ ), Fear ( $p = 0.042$ ) and lower ratings of Expectation ( $p = 0.010$ ). Ratings of Valence ( $p = 0.424$ ) did not differ significantly. Order of paradigm did not have a significant effect on pupil size ( $p = 0.348$ ) and SCRs ( $p = 0.182$ ).

Note that the main findings of the results (comparing appetitive and aversive conditioning) did not change when sex, monetary savings or order were included as covariates (see Tables S3-S8 and Figures S13-S30, in supplementary materials).

## 4. Discussion

In two studies, we compared self-reports and physiological responses (SCRs and pupil size) using commonly applied appetitive and aversive US in healthy human participants. In the first study, self-reports and physiological assessments were compared towards electric shock and three monetary rewards. In the second study, Pavlovian aversive and appetitive conditioning were performed. In both studies, physiological responses were significantly less pronounced towards appetitive CS and US compared to aversive CS and US, whereas magnitudes of self-reports were much more similar.

SCRs can be considered a physiological measure of stimulus salience or arousal [41]. Stimulus salience affects the magnitude of associative learning [5,42]. The more salient aversive US therefore explains stronger magnitudes of differential learning compared to the appetitive US based on physiological responses and stronger expectancy of the US



**Fig. 6.** SCRs (A) and pupil (B) responses and respective relative treatment effects (C,D) during appetitive (blue colors) and aversive (red colors) conditioning, grouped by phase: initial acquisition training, reversal, and block: early and late. Dark red: aversive CS+, light red: aversive CS-, dark blue: appetitive CS+, light blue: appetitive CS-. Please note that during reversal, the initial CS+ is no longer followed by the US and the initial CS- is followed by the US (the initial CS+ and CS- have been recoded to CS- and CS+ in reversal phase). A,B) Columns represent means and error bars represent SEM C,D) Horizontal lines represent median RTEs, and whiskers indicate 95 % confidence intervals. See Methods section for details.

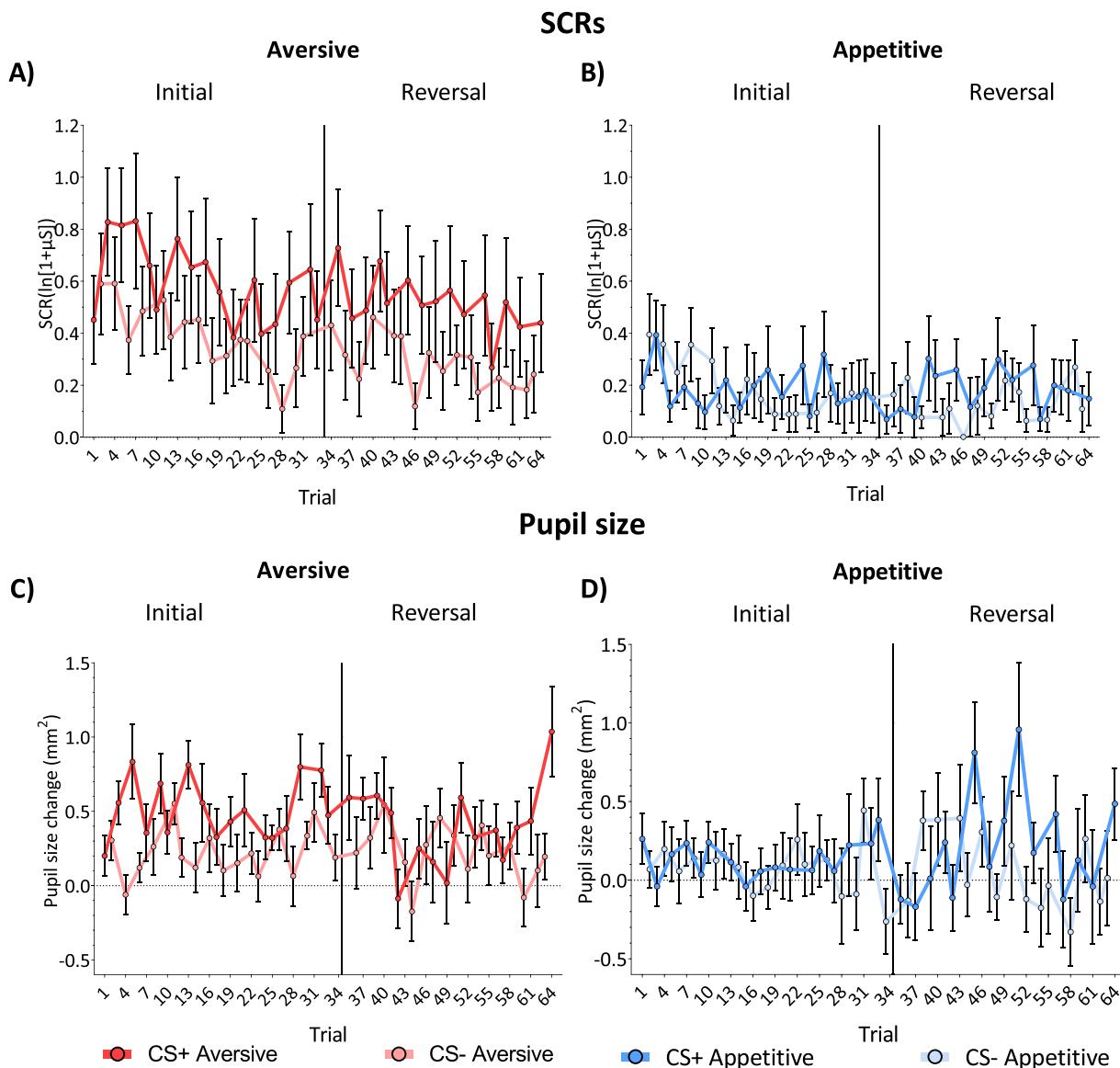
based on self-reports.

Our finding that conditioned and unconditioned physiological responses to monetary rewards are generally small and significantly less compared to aversive electrical stimulations are in line with the literature [43]. One possible cause is that physiological responses may be stronger to primary compared to secondary reinforcers. For example, Andreatta and Pauli [44] found comparable conditioning effects based on SCRs using food as appetitive US and electrical stimulation as aversive US. Another possible cause is that physiological conditioned and unconditioned responses to aversive stimuli may be generally higher towards aversive compared to appetitive stimuli because of their biological significance and potential harm. For example, Hermann et al. [45] and Exner et al. [46] found conditioned SCRs towards unpleasant (aversive) but not pleasant (appetitive) olfactory US, despite odors being primary reinforcers. Furthermore, van der Schaaf et al. [47] observed significant conditioning effects based on SCRs using pain as US for aversive conditioning, but not using pain relief as US for appetitive conditioning. Likewise, using aversive and appetitive pictures as US, aversive conditioning occurred in SCRs towards the aversive but not the appetitive images [48]. However, SCR changes have also been reported to be higher to monetary losses compared to wins [21]. Thus, a second reason may be that unconditioned and conditioned physiological responses are generally more pronounced towards aversive compared to appetitive stimuli. A third reason may be individual variability in

physiological responses. For example, some participants show an increase of pupil size in expectation of a monetary reward ("sign-trackers"), whereas other show an increase only during the presentation ("goal-trackers") [49]. This adds to variability, and differential conditioned responding may be missed.

Self-reports, on the other hand, showed much fewer differences in response magnitude and differential CS responding when comparing appetitive and aversive CS and US. In the present study, (absolute) valence ratings were even higher towards monetary rewards compared to the electrical stimulus, considering both responses to the US in study 1 and CS in study 2. Both self-reports of stimulus perception and valence scaled with increasing amount of money. Findings agree with findings in the literature that also found robust responses in self-reports but much lower or absent physiological responses in appetitive conditioning [45, 46]. Furthermore, higher absolute valence ratings in appetitive conditioning have also been reported by others. Andreatta and Pauli [44] found that appetitive food CS received higher absolute valence ratings than aversive CS (painful electrical shock). Higher valence (and arousal) ratings, however, have been reported by others towards the CS associated with aversive compared to the appetitive olfactory US [45,46].

Valence is a verbal report of the pleasantness or unpleasantness of a stimulus. In the present study, participants rated the electric shock as less unpleasant in absolute terms than they rated the monetary reward as pleasant. Although an electric shock is potentially harmful, the



**Fig. 7.** Average SCRs and pupil responses during appetitive and aversive conditioning. Trial-by-trial aversive (A, C) and appetitive (B, D), SCRs (A, B) and pupil responses (C, D). Circles represent average values and error bars represent standard error of the mean. CS responses are in red for aversive conditioning and in blue for appetitive conditioning. Please note that during reversal, the initial CS+ is no longer followed by the US and the initial CS- is followed by the US (the initial CS+ and CS- have been recoded to CS- and CS+ in reversal phase). Vertical line between trial 34 and 35 indicates start of reversal phase.

stimulation strength was individually calibrated to be unpleasant but not painful, as frequently done in fear conditioning studies [39,40]. Money, on the other hand, is a powerful secondary reinforcer, which can be exchanged to any kind of primary reinforcers. The amount of money, which can be reasonably used in an experimental setting, may be, however, not salient enough to engage the autonomic system, as for example, a one-million-dollar reward may do. Valence of electrical stimulation on the other hand may have been limited, because it was below the pain threshold, but, because it is a primary reinforcer, engaged the autonomic system.

Whereas classical appetitive and aversive Pavlovian conditioning was used in the present study (and also by, for example, van der Schaaf et al. [49]), the majority of other studies applied operant (or instrumental) conditioning [19–22]. Similar differences between physiological and self-reports to unconditioned and conditioned stimuli were reported for operant conditioning, which demonstrates their generality.

Aversive and appetitive conditioning processes are altered in mental disorders such as anxiety and addiction, which are characterized by

excessive avoidance and approach behavior, respectively [50–53]. Neuroimaging studies in healthy participants and patients are one way to better understand the underlying neural mechanisms of the diseases and possible treatments (for example, exposure therapy in anxiety disorders). Our data show that it is difficult to fully match outcome parameters in aversive and appetitive conditioning studies.

Of note, it is not uncommon that multimodal outcome recordings in associative learning paradigms do not converge [6]. Furthermore, it is not uncommon that multimodal outcomes partly differ comparing appetitive and aversive conditioning paradigms even when US from the same modalities are used (for example, pleasant and unpleasant odors; pain and pain relief). That is, studies comparing the neural responses in processing of aversive and appetitive stimuli using brain imaging, electroencephalography or other neurobiological methods, need to control for possible differences in magnitude and rate of outcomes. Another possible out-read are neural responses, for example, electroencephalography or fMRI responses. For the interpretation of findings of these neural responses, it is important to consider that different brain

**Table 3**

Summary of nonparametric ANOVA-type statistics for study 2 considering physiological data (SCRs and pupil size).

Factor	Numerator Df	F	p
<b>SCRs</b>			
Stimulus	1	63.48	
Conditioning Paradigm	1	96.40	<.001<.001
Phase	1	24.73	<.001
Block	1	29.12	<.001
Stimulus × Conditioning	1	24.97	<.001<.001
Stimulus × Phase	1	0.08	0.774
Conditioning × Phase	1	18.05	<.001
Stimulus × Conditioning × Phase	1	1.51	0.219
Stimulus × Block	1	1.52	0.218
Conditioning × Block	1	27.43	<.001
Phase × Block	1	37.89	<.001
Stimulus × Conditioning × Block	1	15.21	<.001
Stimulus × Conditioning × Phase	1	7.63	0.006
Stimulus × Conditioning × Block × Phase	1	0.35	0.554
Stimulus × Phase × Block	1	0.02	0.876
<b>Pupil size</b>			
Stimulus	1	13.40	<.001
Conditioning Paradigm	1	17.91	<.001
Phase	1	0.78	0.377
Block	1	0.10	0.754
Stimulus × Conditioning	1	3.45	0.063
Stimulus × Phase	1	0.18	0.672
Conditioning × Phase	1	0.98	0.323
Stimulus × Conditioning × Phase	1	0.30	0.585
Stimulus × Block	1	0.21	0.648
Conditioning × Block	1	0.02	0.898
Phase × Block	1	0.68	0.410
Stimulus × Conditioning × Block	1	0.14	0.711
Stimulus × Conditioning × Block × Phase	1	0.00	0.987
Stimulus × Phase × Block	1	0.22	0.639

Bold font indicates statistical significance at  $p < 0.05$

regions may be involved in different aspects of the associative learning tasks, which are reflected by differences in outcome measures. Likewise, depending on the question one has, the type of CS and US should be chosen. Furthermore, one needs to know that direct comparisons between neural network responses are limited comparing aversive and appetitive conditioning tasks in particular when the primary out-read are autonomic measures.

Physiological responses in conditioning tasks and self-reports are known to differ in male and female participants and depend on sex hormone levels [6,54]. Furthermore, the relative value of monetary rewards likely varies across socioeconomic or cultural contexts, potentially impacting the generalizability of the results. In fact, we did observe sex- and socioeconomic status related differences, in particular in self-reports towards the unconditioned stimuli in study 1. Nevertheless the observed differences in self-reports and physiological responses between appetitive and aversive conditioning remained even when considering sex and socioeconomic status as covariates in statistical analysis. The present study, however, was not powered to assess sex and socioeconomic status effects in more detail.

Another potential limitation is the order of paradigms in study 2. Participants who started with the appetitive conditioning paradigm rated CS higher in terms of arousal, fear, and lower in expectation. There were no order effects regarding the physiological parameters (SCRs and pupil size responses). Thus, there may be less differences between aversive and appetitive conditioning in between-group study designs compared to within-group study designs, at least considering self-reports.

In conclusion, full comparability between multimodal outcomes can

probably not be achieved in appetitive and aversive conditioning paradigms. Still, with our approach we could show that self-reports lead to a better match than physiological parameters using commonly applied aversive and appetitive US.

### CRediT authorship contribution statement

**Ernst Thomas M.:** Validation, Software, Resources, Project administration, Methodology, Data curation. **Nio Enzo:** Software, Data curation. **Coenen Lena:** Writing – review & editing, Investigation, Data curation. **Doubliez Alice:** Writing – original draft, Visualization. **Batsikadze Giorgi:** Writing – review & editing, Visualization, Validation, Software, Project administration, Formal analysis, Data curation. **Petrenko Mykola:** Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Merz Christian J.:** Writing – review & editing, Conceptualization. **Timmann Dagmar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Uengoer Metin:** Writing – review & editing, Conceptualization. **Cheng Sen:** Writing – review & editing, Conceptualization. **Diekmann Nicolas:** Writing – review & editing, Conceptualization.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bbr.2025.115509](https://doi.org/10.1016/j.bbr.2025.115509).

### Data Availability

Data will be made available on request.

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