iMotions - The World's Leading Biosensor Platform

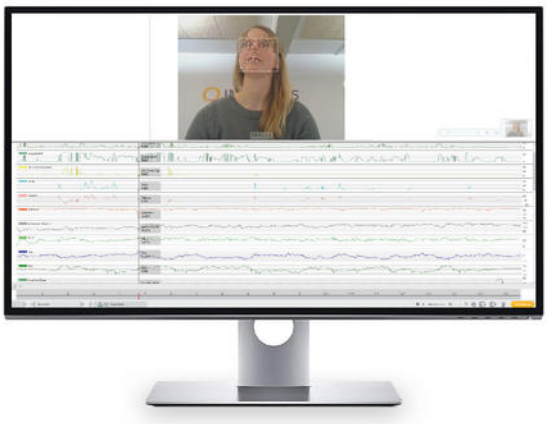
iMotions is a software platform that seamlessly integrates and synchronizes multiple biosensors, such as eye trackers, EEG, EDA, facial expression analysis, ECG, and EMG, in one place.

- Hardware agnostic software with 50+ biosensor devices
- Full and continuous support, 24/7
- Modify as you need with the API and/or LSL connections
- Full scalability when you need it

Execute research faster - Increase validity and reliability - Spend more time concluding

Examples of what clients are currently using iMotions for include:

- Autism biomarker exploration with EEG, eye tracking, and EDA
- Using EEG to study the effect of VR on learning
- Exploring psychophysiological responses in bipolar patients
- The physiological effects of transdermal neuromodulation



iMotions is used at 41 of the top ranking universities in the world

Harvard University · Mayo Clinic · McGill · University of Oxford · UCL · Stanford University · Australian National University · Massachusetts Institute of Technology

sales@imotions.com www.imotions.com Tel +1 617-520-4958

Received: 9 April 2019 | Revised: 20 July 2019 | Accepted: 22 July 2019

DOI: 10.1111/psyp.13463

ORIGINAL ARTICLE

PSYCHOPHYSIOLOGY SPR WILEY

Pupil dilation as an implicit measure of appetitive Pavlovian learning

Charlotte Pietrock[1] | **Claudia Ebrahimi**[1] | **Teresa M. Katthagen**[1] | **Stefan P. Koch**[1] | **Andreas Heinz**[1,2,3] | **Marcus Rothkirch**[1] | **Florian Schlagenhauf**[1,3,4]

[1]Department of Psychiatry and Psychotherapy, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[2]Cluster of Excellence NeuroCure, Charité–Universitätsmedizin Berlin, Berlin, Germany

[3]Bernstein Center for Computational Neuroscience Berlin, Humboldt-Universität zu Berlin, Berlin, Germany

[4]Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

Correspondence
Charlotte Pietrock, Department of Psychiatry and Psychotherapy, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charitéplatz 1, 10117 Berlin, Germany.
Email: charlotte.pietrock@charite.de

Funding information
Deutsche Forschungsgemeinschaft (DFG) grant (SCHL1969/2-2, as part of FOR1617, SCHL1969/4-1) (to F.S.); Elsa-Neumann scholarship, Charité scholarship (to C.E.)

Abstract

Appetitive Pavlovian conditioning is a learning mechanism of fundamental biological and pathophysiological significance. Nonetheless, its exploration in humans remains sparse, which is partly attributed to the lack of an established psychophysiological parameter that aptly represents conditioned responding. This study evaluated pupil diameter and other ocular response measures (gaze dwelling time, blink duration and count) as indices of conditioning. Additionally, a learning model was used to infer participants' learning progress on the basis of their pupil dilation. Twenty-nine healthy volunteers completed an appetitive differential delay conditioning paradigm with a primary reward, while the ocular response measures along with other psychophysiological (heart rate, electrodermal activity, postauricular and eyeblink reflex) and behavioral (ratings, contingency awareness) parameters were obtained to examine the relation among different measures. A significantly stronger increase in pupil diameter, longer gaze duration and shorter eyeblink duration was observed in response to the reward-predicting cue compared to the control cue. The Pearce-Hall attention model best predicted the trial-by-trial pupil diameter. This conditioned response was corroborated by a pronounced heart rate deceleration to the reward-predicting cue, while no conditioning effect was observed in the electrodermal activity or startle responses. There was no discernible correlation between the psychophysiological response measures. These results highlight the potential value of ocular response measures as sensitive indices for representing appetitive conditioning.

KEYWORDS
associative learning, attention, eye-tracking, pupil dilation, reward

1 | INTRODUCTION

Appetitive Pavlovian conditioning is the learning process by which an initially neutral stimulus (CS, conditioned stimulus), after repeated pairings with a salient pleasant experience (US, unconditioned stimulus), is able to elicit the innate physiological response that was originally confined to the US (Pavlov, 1927; Rescorla, 1988). This constitutes a central learning mechanism that enables organisms to survive and thrive in dynamic environments; however, if maladaptive, it can also contribute to pathological states including addiction, depression, and eating disorders (Grosshans, Loeber,

& Kiefer, 2011; Kiefer & Dinter, 2013; Martin-Soelch, Linthicum, & Ernst, 2007; Robinson & Berridge, 2000; van den Akker, Jansen, Frentz, & Havermans, 2013).

In contrast to its aversive counterpart (Delgado, Jou, & Phelps, 2011; Fullana et al., 2016; Li & McNally, 2014), appetitive conditioning is only rarely explored in humans (Andreatta & Pauli, 2015; Konova & Goldstein, 2018). This is predominantly ascribed to two challenges: the identification of suitable reinforcement as well as clear criteria for established conditioning. Regarding the first, it is difficult to determine a US whose rewarding properties or subjective pleasantness is inter-individually equivalent. So far, a variety of both primary and secondary stimuli have been used for appetitive reinforcement, for example, food (Andreatta & Pauli, 2015; Blechert, Testa, Georgii, Klimesch, & Wilhelm, 2016; van den Akker et al., 2017a; Wardle, Lopez-Gamundi, & Flagel, 2018), drink (Ebrahimi et al., 2019; O'Doherty, Buchanan, Seymour, & Dolan, 2006; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003; Pauli et al., 2015; Prévost, McNamee, Jessup, Bossaerts, & O'Doherty, 2013), odor (Gottfried, O'Doherty, & Dolan, 2002; Hermann, Ziegler, Birbaumer, & Flor, 2000; Stussi, Delplanque, Corai, Pourtois, & Sander, 2018), attractive faces (Bray & O'Doherty, 2007), erotic images (Klucken et al., 2009, 2013, 2015; Klucken, Wehrum-Osinsky, Schweckendiek, Kruse, & Stark, 2016), and money (Austin & Duka, 2010; Delgado, Gillis, & Phelps, 2008; Ebrahimi et al., 2017; Tapia León, Kruse, Stalder, Stark, & Klucken, 2018). Although there exists a certain overlap, primary and secondary rewards are processed in distinct neural systems (Sescousse, Caldú, Segura, & Dreher, 2013). Both primary and secondary appetitive reinforcers rarely result in physiological responses comparable to those evoked by reinforcers in aversive conditioning research (e.g., pain and noise), and the appetitive value of the US is difficult to standardize (Martin-Soelch et al., 2007; Stussi et al., 2018). Furthermore, the physiological responses toward secondary reinforcers may be weaker compared to those elicited by primary reinforcers (Andreatta & Pauli, 2015; Ebrahimi et al., 2017).

Associated with this is the second challenge facing human appetitive conditioning research, namely, the lack of an established gold standard measurement to assess conditioned responding. A frequently implemented method to confirm successful conditioning are ratings, for example, CS valence (Andreatta & Pauli, 2015; Ebrahimi et al., 2017, 2019; Klucken et al., 2015, 2009, 2013, 2016; Prévost et al., 2013), CS dichotomous preference (Bray & O'Doherty, 2007; Kahnt, Heinzle, Park, & Haynes, 2011; Metereau & Dreher, 2013; Prévost et al., 2013), US expectancy (van den Akker, Havermans, & Jansen, 2015), and contingency awareness (Bray & O'Doherty, 2007; Ebrahimi et al., 2017, 2019; Klucken et al., 2015, 2009, 2013, 2016; Stussi et al., 2018; Tapia León et al., 2018). A shortcoming of ratings is that they only reflect the explicit component of learning and are prone to influences of social desirability when the learning task is simple. Therefore, a thorough investigation of appetitive associative learning should incorporate both explicit and implicit conditioning indices. Unfortunately, due to the scarcity of multi-methodological studies that compare implicit learning parameters, along with nonstandardized approaches of analysis, it is still unclear which measure is most suited in appetitive conditioning experiments (Stussi et al., 2018; Wardle et al., 2018).

Implicit behavioral indices of appetitive conditioning, like reaction time, are hitherto inconclusive, with results showing both conditioned increases (O'Doherty et al., 2006), decreases (Ebrahimi et al., 2017; Gottfried et al., 2002), or no differentiation (Ebrahimi et al., 2019; Metereau & Dreher, 2013) in response times. Psychophysiological measures similarly often present inconsistent results. Electrodermal activity, which is a common learning index used in aversive conditioning paradigms (Lonsdorf et al., 2017; Ney et al., 2018), has shown both an enhanced skin conductance response (SCR; Andreatta & Pauli, 2015; Ebrahimi et al., 2019; Klucken et al., 2013, 2015, 2016; Tapia León et al., 2018), as well as no differential response (Klucken et al., 2009; Stussi et al., 2018; van den Akker et al., 2017a) to the reward-associated stimulus and appears to be dependent on task context (van den Akker et al., 2017b). Heart period response (HPR) has seldom been examined in an appetitive context and has not yielded a conclusive differential effect (Hermann, Ziegler, Birbaumer, & Flor, 2000; Wardle et al., 2018). Interestingly, fear-conditioned cardiac deceleration (bradycardia) has been observed in experiments using aversive US (Castagnetti et al., 2016; Prévost et al., 2013). Both SCR and HPR are characterized by long response latencies and durations, which unfortunately prolong the experiment's duration (Lonsdorf et al., 2017; Sjouwerman & Lonsdorf, 2018). In contrast, acoustic startle responses (eyeblink reflex, EBR; Andreatta & Pauli, 2015; Ebrahimi et al., 2019; Hermann et al., 2000; Stussi et al., 2018; Wardle et al., 2018) and the vestigial postauricular microreflex (PAR; Aaron & Benning, 2016; Ebrahimi et al., 2019; Sandt, Sloan, & Johnson, 2009; Stussi et al., 2018) have short reaction latencies; however, their inherent aversive quality limits their utility in the appetitive conditioning domain, where they are confined to being post-hoc measures.

In the current study, we decided to explore the ocular response as a potential measure of appetitive conditioning. Eye-tracking is an accurate, non-invasive tool and specifically pupil diameter constitutes a powerful implicit measure in cognitive tasks with short response latency (van der Wel & van Steenbergen, 2018). Non-luminance-mediated pupil dilation is generally associated with a broad range of cognitive processes causing sympathetic nervous activation (Sirois & Brisson, 2014; van der Wel & van Steenbergen, 2018), including, but not limited to, mental processing load (Just, Carpenter, & Miyake, 2003; Kahneman & Beatty, 1966), emotional processing (Granholm & Steinhauer, 2004;

Kinner et al., 2017), arousal (Bradley, Miccoli, Escrig, & Lang, 2008; Leuchs, Schneider, Czisch, & Spoormaker, 2017; Prévost et al., 2013; Seymour, Daw, Dayan, Singer, & Dolan, 2007), attention (Eldar, Cohen, & Niv, 2013; Laeng, Sirois, & Gredebäck, 2012; Lasaponara et al., 2019), surprise (Kloosterman et al., 2015), exerted effort (Varazzani, San-Galli, Gilardeau, & Bouret, 2015), learning and memory (Aston-Jones & Cohen, 2005; Brocher & Graf, 2016; Eldar et al., 2013; Goldinger & Papesh, 2012; Nassar et al., 2012; Silvetti, Vassena, Abrahamse, & Verguts, 2018; Tzovara, Korn, & Bach, 2018). Prior research in the context of appetitive conditioning is scarce and has focused only peripherally on pupil diameter (Bray, Rangel, Shimojo, Balleine, & O'Doherty, 2008; O'Doherty et al., 2003, 2006; Seymour et al., 2007; Pauli et al., 2015; Prévost et al., 2013), showing pupil dilation toward both primary (Pauli et al., 2015; Prévost et al., 2013; O'Doherty et al., 2003, 2006) and secondary (Seymour et al., 2007) conditioned stimuli. Moreover, we decided to explore gaze dwelling time, that is, the amount of time gaze lingers on a stimulus, as a measure of visual attention (Isaac, Vrijsen, Rinck, Speckens, & Becker, 2014) in conditioned learning. We further investigated blink responding (blink frequency and duration). Analyses of blink frequency have thus far been isolated to the aversive conditioning domain, where a greater frequency to the aversive conditioned stimulus has been observed (Pauli et al., 2015; Prévost et al., 2013). Blink duration is commonly used as an indicator of alertness, as long blinks are found to signal drowsiness and fatigue (Caffier, Erdmann, & Ullsperger, 2003; Stern, Boyer, & Schroeder, 1994). Both gaze dwelling time and blink responses have, to our knowledge, never been systematically examined in an appetitive conditioning paradigm. With the purpose of contributing to the quest for a sensitive psychophysiological parameter, we tested whether the ocular response measures (pupil diameter, gaze dwelling time, blink duration, blink count) are suitable measures for representing appetitive conditioning.

To address the elaborated challenges in appetitive conditioning, we designed a conditioning paradigm using a primary reinforcer to test the hypothesis that pupil dilation is a sensitive marker for appetitive conditioning. Furthermore, we, to our knowledge, for the first time assess additional ocular response measures such as gaze dwelling time and blink responding in the appetitive conditioning context.

In line with budding research (Koenig, Uengoer, & Lachnit, 2018; Leuchs et al., 2017), we investigated whether latent and dynamic learning mechanisms could be inferred from the trial-by-trial pupil response by means of computational modeling techniques. Using learning models based on a Rescorla-Wagner framework, we explored whether this trial-by-trial measure depicted the expected stimulus value or its associated Pearce-Hall attention weight. To corroborate our data and search for possible relations between ocular and other psychophysiological measures (Wardle et al., 2018), we assessed additional psychophysiological parameters (SCR, HPR, EBR, and PAR) previously used in appetitive conditioning research.

2 | METHOD

2.1 | Participants

A total of 32 right-handed, healthy volunteers participated in the present study. Participants were recruited via the student mailing lists of the Humboldt-University of Berlin and Charité–Universitätsmedizin Berlin. All participants were free of current or past neurological, psychiatric, and metabolic disorders, had normal or corrected-to-normal vision, intact color vision, and consumed no therapeutic or recreational drugs. Inclusion criteria were regular daily food intake and no allergies or dietary limitations. Students of psychology were not permitted to take part in the experiment. Three participants were excluded from the analysis (two as a cause of technical difficulties during data acquisition and one due to an average negative US rating (≤50 %), see Section 2.3.1., Ratings, for further details). This left 29 participants (16 female) ranging in age from 18–30 years, $M(SD)_{age} = 24.49(3.45)$ years and ranging in body mass index (BMI) from 18–27 kg/m^2, $M(SD)_{BMI} = 22.12(2.26)$ kg/m^2. All participants provided written informed consent and received 20€ for their participation. The study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the Charité.

2.2 | Experimental procedure

Participants completed an appetitive Pavlovian learning task, where they learned to associate sequentially presented audiovisual stimuli (two female faces coupled with a distinct bell chime) with a rewarding outcome (juice delivery) or no reward, respectively. Throughout the task, we acquired a variety of psychophysiological measures (ocular response measures, heart period, electrodermal activity). Directly before and after the learning task, participants rated the CS and US and indicated their awareness for CS-US contingency. As a further parameter of conditioning, an auditory startle task was performed following the conditioning task.

To enforce the craving of the US, participants were asked to abstain from eating and drinking in the respective 6 and 4 hrs preceding the experiment (Ebrahimi et al., 2019; Metereau & Dreher, 2013). The mean reported fasting time was 9.6 hr for food and 4.4 hr for drink. Participants selected and rated their preferred US from four fruit juices (apple, orange, mango-passion fruit, berry) and, after viewing a 4-min priming presentation showing various appetizing dishes and drinks, rated their current state of hunger and thirst on a visual analogue scale (VAS) ranging from 0–100%.

2.2.1 | Design

The differential delay conditioning procedure consisted of 96 trials (48 trials with CS+ condition, 48 with CS− condition). An additional habituation phase of 8 CS presentations (4 per condition) with no reinforcement, but analogous timing, preceded the experiment. During the conditioning phase, each trial began with the presentation of the CS for 6 s to the left or right side of a central fixation cross. In half of the CS+ trials (24 trials), the CS+ was followed by the US 5 s after CS onset (50% reinforcement schedule). In reinforced trials, the phrase "Please swallow!" appeared on screen during the intertrial interval (ITI) with a jittered interval of 3–6 s after US delivery in order to mitigate swallowing artifacts (Pauli et al., 2015). The CS− was never reinforced. Trials were separated by a variable ITI starting at CS offset with a mean duration of 11 s (min. 9 s, max. 16 s; see Figure 1a).

2.2.2 | Trial order

The experiment was divided into two halves. Before the start of the first and second half of the experiment, a standardized 9-point eye-tracking calibration was carried out. Stimuli were presented in a pseudorandomized order: The first and second half consisted of quasi-identical trial sequences, where the first two appearances of the CS+ in the experiment were always reinforced. The three possible pairings of CS and outcome (CS+ reinforced, CS+ unreinforced, and CS− trials) appeared equally often in the first and second half of the experiment (Klucken et al., 2016). Additionally, the following criteria were applied to the trial sequences: There were never more than three consecutive trials of the same condition, cues were never displayed for more than three successive trials on the same side of the fixation cross, and there was a balanced succession of CS+ and CS− trials following a trial with US delivery (Ebrahimi et al., 2017, 2019).

2.2.3 | Stimuli

Visual stimuli were presented on a 36.5 cm × 27.4 cm computer monitor with a spatial resolution of 1,280 × 960 pixels. The monitor was placed 60 cm in front of the participant, whose head was stabilized on a chin rest. Two high-resolution images of young, female faces with a neutral facial expression from the FACES database (Max Planck Institute for Human Development, Berlin; image ID 132, 182; Ebner, Riediger, & Lindenberger, 2010) served as CS. The images presented resembled each other regarding relevant perceptual and social parameters comprising perceived attractiveness, competence, dominance, familiarity, trustworthiness, and distinctiveness, based on empirical ratings provided in an aesthetic preference study (Kiiski, Cullen, Clavin, & Newell, 2016). Both stimuli had equal mean luminance and were presented on a gray background. The stimuli were cropped onto an 82.1 mm × 70.0 mm ellipsoid template and covered 5.65% of the whole screen each. The CS+ and CS− were each coupled with a distinct bell sound (50 dB, duration: 100 ms, 2,349.32 Hz = D_7 and 2,637.02 Hz = E_7) that coincided with CS onset and was presented binaurally via headphones. We employed compound CS as this permits two different sensory modalities to be associated with the US and therefore facilitates the conditioning procedure (Talmi, Seymour, Dayan, & Dolan, 2009). The assignment of the visual-auditory stimuli to the CS+ and CS− condition was counterbalanced across participants. The experiment was coded in MATLAB R2016a (The Mathworks, Natick, MA) using Psychtoolbox-3 (http://psychtoolbox.org; Brainard, 1997; Kleiner et al., 2007). The individually selected appetitive liquid was delivered by a programmable syringe pump (World Precision Instruments, Inc., Sarasota, FL). The pump administered 3 ml of the juice through a 3-m long polyvinyl tube (Oldoplast GmbH, Marl, Germany; outside diameter: 6 mm, inside diameter: 4 mm) with an attached exchangeable straw continuously held between the individual's lips.

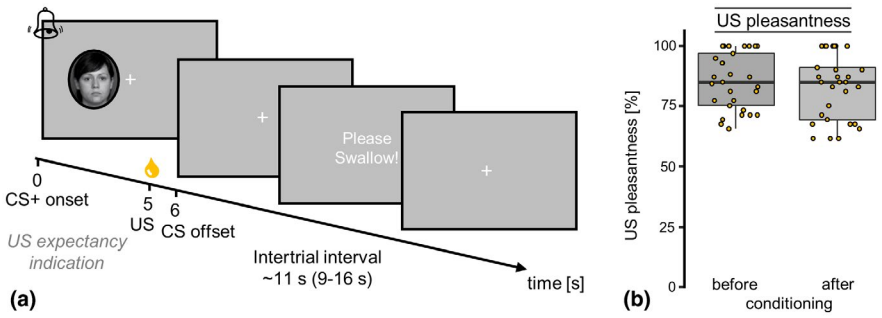


FIGURE 1 Appetitive conditioning procedure. (a) Sequence and timing of an example reinforced Pavlovian learning trial. Participants learned to associate two neutral audiovisual stimuli with a reward or no reward. At the beginning of a trial, one of two female faces was displayed to the left or right of a central fixation cross for 6 s. Upon display of cue, participants had to indicate their binary juice expectancy via button press. In reinforced CS+ trials, the US was delivered 5 s after CS onset. The intertrial interval ranged from 9–16 s (mean: 11 s) after cue offset. In reinforced CS+ trials, the signal to swallow appeared 2–5 s following cue offset. (b) US pleasantness rating before and after conditioning. All error bars represent *SEM*. *$p \leq .05$

2.2.4 | US expectancy

In each trial, participants were instructed to indicate their binary expectancy of US delivery as quickly as possible via button press using their dominant hand. The button indicating a positive or negative expectation was counterbalanced across participants. Due to a technical error, no responses exceeding a reaction latency of 1.5 s after trial onset were recorded causing a loss of 55.3% of the data. Analyses of US expectancy and reaction time are therefore restricted to the online supporting information, Appendix S1, and to be treated with caution.

2.3 | Ratings

2.3.1 | Dimensional ratings

Immediately before and after the conditioning experiment, participants rated the CS attractiveness, pleasantness, and arousal each on a dimensional 100-point VAS ranging from *very unattractive* to *very attractive* for attractiveness and correspondingly, *very unpleasant* to *very pleasant* for pleasantness, and *not at all arousing* to *very arousing* for arousal rating. US pleasantness was also rated on a 100-point VAS ranging from *very unpleasant* to *very pleasant* before and after conditioning. To ensure that the US fulfilled its appetitive potency, participants with an average negative rating (<50%) were excluded from the analysis ($n = 1$).

2.3.2 | Dichotomous preference rating

In addition, participants performed a dichotomous preference rating: Both cues and four further images of young female faces from the FACES database (image ID 63, 22, 150, 171) most similar in rated attractiveness were used (Kiiski et al., 2016). During each choice, two stimuli were presented simultaneously, and participants were asked to promptly indicate via button press which image they preferred based on their current judgment (Bray & O'Doherty, 2007; Kahnt et al., 2011; Metereau & Dreher, 2013; Prévost et al., 2013). Each image was paired with every other image exactly once, resulting in 15 choices.

2.3.3 | Contingency awareness

After the learning session, participants' explicit contingency awareness regarding the pairing of visual stimuli with reward outcomes was assessed on a categorical four-level Likert-type scale. To this end, each CS was presented individually and participants had to indicate how often they received the juice after the respective image was presented. The response options were *always*, *sometimes*, *never*, and *I am unsure*. Participants were considered contingency aware when they chose the always or sometimes options for the CS+ and the never option for the CS−. Using this awareness criterion, 28 participants reached awareness and 1 participant was unsure. An additional dimensional awareness measure on a VAS from 0–100% also confirmed CS+ versus CS− differentiation ($t = 8.71$, $p < .001$; paired t test; Tapia León et al., 2018).

2.4 | Ocular response measures: data acquisition and pre-processing

We tracked participants' eye movement and pupil diameter using a high-speed video-based eye-tracker (Cambridge Research Systems Ltd., UK; sampling rate: 250 Hz, spatial accuracy: 0.05°). For each participant, the activity of the right eye was measured. Preprocessing of eye-tracking data comprised a visual inspection of the raw data. Untracked data points were treated as missing data points. Subsequently, data were smoothed using a second-order Savitzky-Golay filter over seven consecutive data points. The data were segmented from CS onset until potential US onset (0–5 s after CS onset within each trial).

2.4.1 | Pupil diameter

The pupil diameter data were baseline corrected using the mean pupil diameter in a time window of 2 s prior to CS onset for correction. Due to the temporal proximity between the calibration of the eye-tracker and the start of the second half of the conditioning experiment, baseline correction was not possible for the first trial of the second half, resulting in the elimination of this trial from all further eye-tracker analyses. We performed statistical analyses on the pre-outcome pupil size (4–5 s after CS onset) as this is considered the interval of strongest CS differentiation (Koenig et al., 2018; Leuchs et al., 2017). All participants with ≥35% missing data were excluded from analyses (Korn, Staib, Tzovara, Castegnetti, & Bach, 2017). In addition to the latter analysis, we also performed a model-based approach (Korn et al., 2017; Korn & Bach, 2016) on pupil size response (PSR) using the PsPM toolbox (version 4.0, http://pspm.sourceforge.net/, details of analysis below).

2.4.2 | Dwelling time

Dwelling time was computed by averaging the percentage of time participants' gaze fell on the displayed stimulus in the segmented time window of 0–5 s after CS onset (Rothkirch, Stein, Sekutowicz, & Sterzer, 2012). Within this window of analysis, participants had the opportunity of gazing at the displayed CS, the fixation cross, or anywhere else on the gray background. Presenting the CS on the left or right side of the central fixation cross allowed us to assess the relative gaze proportion of the participants on the stimulus. All trials with ≥25% untracked data points were excluded from further analysis.

2.4.3 | Blink duration and frequency

Blink duration was assessed by calculating the mean blink length per condition in the segmented time window. A blink was defined as a series of continuous missing data points with a duration of 50–750 ms (Holmqvist et al., 2011; Stern, Walrath, & Goldstein, 1984). All blinks that coincided with the start or end of the designated time window were removed from the analysis. For the eyeblink rate, the number of eyeblinks in the identical time frame was counted.

2.5 | Further psychophysiological measures: data acquisition and pre-processing

Heart period, electrodermal activity, breathing, and startle responses were recorded using a BrainAmp MR amplifier (Brain Products GmbH, Munich, Germany; sampling frequency: 250 Hz). Due to a technical malfunction, data from one participant in these parameters were lost. All data were preprocessed using MATLAB R2016a. For HPR, SCR, and PSR, we used psychophysiological modeling techniques by means of the PsPM toolbox (Bach et al., 2018).

2.5.1 | HPR

Heart rate was measured using electrocardiography (ECG) with bipolar leads. Pre-gelled adhesive electrodes (45 mm) were placed in the right parasternal second intercostal space and fifth intercostal space in the left midclavicular line. All raw data underwent a visual inspection. Two participants were removed from further analysis due to data loss. The data were band-pass filtered using the PsPM default second-order Butterworth filter with desired cutoff frequencies of 5–15 Hz. QRS detection was performed semiautomatically using PsPM's modified version of the Pan & Tompkins algorithm (Pan & Tompkins, 1985). All deviating detected or undetected QRS complexes were manually corrected if necessary. The ECG signal was linearly interpolated at a 10 Hz sampling rate, converted to heart period, and normalized (Castegnetti et al., 2016; Paulus, Castegnetti, & Bach, 2016).

2.5.2 | SCR

A pair of 11-mm Ag/AgCl-electrodes placed on the medial phalanx of the second and third digit of the nondominant hand and secured with eudermic tape was used to detect SCR. An initial visual inspection was performed on the raw SCR data, resulting in the exclusion of 8 data sets due to poor signal quality (i.e., flatline due to disconnection of electrodes). The remaining data were filtered using PsPM's default 0.05–5 Hz unidirectional first-order Butterworth filter and downsampled to 10 Hz (Bach et al., 2013).

2.5.3 | PsPM first-level general linear model for HPR, SCR, and PSR

For HPR, SCR, and PSR separately, we executed a first-level analysis using PsPM's general linear convolution model (Bach, Flandin, Friston, & Dolan, 2010; Bach, Friston, & Dolan, 2013; Castegnetti et al., 2016; Korn et al., 2017). Psychophysiological modeling of HPR, SCR, and PSR has been shown to discriminate conditioned CS+ from CS− responses more precisely than corresponding model-free alternatives (Bach, 2014; Castegnetti et al., 2016; Korn et al., 2017). Each general linear model (GLM) included six regressors of interest, modeling cue onsets for CS+ unreinforced, CS+ reinforced, and CS−, for both halves of the experiment separately. Cue onsets of the habituation phase and US onsets were included as regressors of no interest. Regressors were convolved with the modality-specific (i.e., canonical HPR, SCR, and PSR) response function, yielding a beta estimate of each regressor. For primary group analysis, CS+ (mean of unreinforced and reinforced) and CS− estimates for each phase entered the second level. To assess the influence of conditioning on the responses uncontaminated by US, these analyses were complemented by an analysis of only unreinforced CS+ versus CS− responses.

2.5.4 | Startle task

Auditory startle reflexes were assessed subsequently to the learning session as a further index of appetitive conditioning. The startle session consisted of eight trials (four per condition, with no reinforcement) in which the cues were presented individually at the center of the screen. Participants did not have to indicate US expectancy. At asynchronous onset latencies (0.3, 0.6, 0.9, 1.2 s after stimulus onset), a white noise startle probe (90 dB, duration: 50 ms) was presented binaurally via headphones. Additionally, four startle probes occurred 0.1 s after ITI onset, in order to prevent a CS-startle association. Four initial habituation startle probes with analogous timing, but no cue display preceded the startle session. The ITI had a mean duration of 3.5 s after CS offset (min. 1.4 s, max. 5.8 s). Trial order and timing were randomized within and counterbalanced across participants.

2.5.5 | Startle response

The startle-induced EBR was measured using electromyography (EMG) of the left musculus orbicularis oculi. Two 5-mm Ag/AgCl electrodes were used and, adhering to human EMG eyeblink startle guidelines (Blumenthal et al., 2005), placed 1 cm below the eye's central vertical axis and 1 cm temporal of the lateral canthus. The PAR was measured using EMG of the left musculus auricularis posterior by positioning two 5-mm Ag/AgCl electrodes 1 cm posterior of the auricular auris directly above and below

the height of the meatus acusticus externus. Due to high electrical impedance noise detected in the primary visual examination of the data, only $n = 13$ and $n = 17$ data sets remained in the EBR and PAR analysis, respectively. The remaining data were fourth-order high-pass Butterworth filtered with a cutoff frequency of 40 Hz (EBR) and 28 Hz (PAR). Mains hum was removed using a 50 Hz notch filter. The EMG signal was rectified, and the orbicularis oculi data were further smoothed with a fourth-order low-pass Butterworth filter using a time constant of 3 ms (equivalent to 53.05 Hz; Khemka, Tzovara, Gerster, Quednow, & Bach, 2017). The peak startle magnitude was defined as the maximum value in the time interval of 20–120 ms for EBR (Blumenthal et al., 2005; Schumacher et al., 2018) and 5–35 ms for PAR (Aaron & Benning, 2016; Gable & Harmon-Jones, 2009; Sandt et al., 2009; Stussi et al., 2018) after startle onset subtracted by the mean EMG amplitude in a time window of 10 ms before startle onset for baseline correction. All negative peak values were transformed to zero. We applied the following quality criteria: (a) all trials with a baseline shift ≥5 μV were rejected from further analysis (EBR, PAR); (b) peak startle magnitudes ≤5 μV in the window of analysis were converted to zero (EBR; Genheimer, Andreatta, Asan, & Pauli, 2017; Glotzbach-Schoon, Andreatta, Mühlberger, & Pauli, 2015). Lastly, all data were *t* scored (*z* scored × 10 + 50).

2.6 | Self-report questionnaires

Prior to the learning task, participants completed the following self-report questionnaires: NEO-FFI (Neo Five-Factor Inventory; Costa & McCrae, 1992; German version: Borkenau & Ostendorf, 1993), BIS/BAS (Behavioral Inhibition System/ Behavioral Activation System Scale; Carver & White, 1994), and STAI (State-Trait Anxiety Inventory; Laux, Glanzmann, Schaffner, & Spielberger, 1981). For sample characteristics, see supporting information, Table S1.

2.7 | Statistical analysis

We conducted all statistical analyses using the R software environment (version 3.4.3, R Core Team, 2017) with an alpha level set at 0.05. Partial eta squared (η_p^2) or Cohen's *d* were used to estimate effect size. Ratings were analyzed using separate 2 × 2 repeated measures analyses of variance (rmANOVAs) with within-subject factors condition (CS+ vs. CS−) and time (pre- vs. postconditioning). Ocular response measures, HPR, SCR, reaction time, and US expectancy, were analyzed analogously, with time referring to the first versus second half of the experiment. Habituation trials were excluded from analyses to reduce orienting response confounds (Kruse, Tapia León, Stark, & Klucken, 2017). In addition, for the physiological measures, only unreinforced CS+ responses were initially contrasted with CS− responses. As these analyses did not change our results substantially (see Appendix S1), the differentiation between both CS+ types (reinforced/unreinforced) was henceforth discontinued. The reported results contrast all CS+ with all CS− responses. The startle data were analyzed using a paired *t* test, contrasting CS+ versus CS− conditions. To investigate intraindividual associations between conditioning indices, bivariate correlations between measures showing a significant differential conditioning effect were computed (Pearson's product-moment correlation or Spearman's rank correlation for associations with CS preference rating scores). As the personality traits neuroticism and extraversion potentially modulate the responsiveness to appetitive conditioning (Depue & Fu, 2013; Hooker, Verosky, Miyakawa, Knight, & D'Esposito, 2008; Schweckendiek, Stark, & Klucken, 2016), we correlated these subscales from the NEO-FFI with CS-related pupil size, HPR, and dichotomous CS ranking.

2.8 | Computational modeling of pupil data

As pupil diameter was strongly affected by appetitive conditioning, we investigated whether latent and dynamic learning mechanisms could be inferred from trial-by-trial pupil responses (individual trial-by-trial means, determined for the pre-outcome pupil size time window). By using computational modeling techniques, individual pupil responses were predicted by either (a) expected values of the displayed CS, or (b) the dynamic attention weight associated with the displayed CS. All learning models were based on a Rescorla-Wagner framework, where trial-wise prediction errors (reflecting the discrepancy between the received reward and the expected value; see Equation 1: *k* denotes trial number; $\delta_{v'}^{(k)}$ denotes the prediction error on trial *k*; *r* is the received reward, and $v'^{(k)}$ is the expected value) are used to update the expected value of the displayed CS (Equation 2).

$$\delta_{v'}^{(k)} = r^{(k)} - v'^{(k)} \tag{1}$$

$$v'^{(k+1)} = v'^{(k)} + \alpha^{(k)}\delta_{v'}^{(k)} \tag{2}$$

In our model space, the influence of the prediction errors on the value update was varied via (a) fixed learning rates (one free parameter α for both stimuli vs. two separate parameters per outcome), or (b) dynamic attention weights. The latter was determined via a Pearce-Hall update rule (as used by Diederen et al., 2016) that takes into account a general decay across accumulating trials, as well as the absolute prediction error from the previous trial (Equation 3: γ denotes the decay constant; $\left|\delta_{v'}^{(k-1)}\right|$ denotes the absolute prediction error from the preceding trial). Transferred to our paradigm,

the latter operationalization would model relatively steady attention weights for the values attributed to the CS+ because prediction errors remain high due to the 0.5 reinforcement rate. In contrast, the attention weights for the CS− would slowly decrease when participants have learned that this stimulus is not followed by the reward, reflected by expected values and prediction errors approximating 0 (figure 4b; Pearce & Hall, 1980).

$$\alpha_{v'}^{(k)} = \gamma \left| \delta_{v'}^{(k-1)} \right| + (1-\gamma)\, \alpha_{v'}^{(k-1)} \tag{3}$$

In a trial where the respective CS was not shown, the attention weight as well as the expected value remained constant. Further, the prediction error of the last trial where the respective CS was shown was used in the Pearce-Hall update rule. Learning trajectories ($learning^k$; reflecting either expected values or dynamic attention weights) were defined to linearly predict individual trial-by-trial pupil responses (Equation 4: ζ = Gaussian noise).

$$pupil\ diameter = \beta_0 + \beta_1 learning^k + \zeta \tag{4}$$

In sum, there were six Rescorla-Wagner learning models: (1) one fixed learning rate and expected value as predictor (RW-1α), (2) two fixed learning rates and expected value as predictor (RW-2α), (3) Rescorla-Wagner Pearce-Hall hybrid model with value as predictor with the same parameters for both conditioned stimuli (RW-PH-value-same), and (4) with distinct parameters (RW-PH-value-distinct), as well as these hybrid models with the attention weights predicting the pupil response—(5) RW-PH-attention-same, (6) RW-PH-attention-distinct. In order to compare whether pupil responses reflected such dynamic learning effects or stationary reactions to two cues, a null model was added that only predicted pupil responses via the displayed CS. Models were fitted using the HGF toolbox 4.15 (http://www.translationalneuromodeling.org/tapas/; Mathys, Daunizeau, Friston, & Stephan, 2011; Mathys et al., 2014) applying a quasi-Newton algorithm for optimization. For prior means and variances of parameters, see supporting information, Table S2.

2.8.1 | Model selection

A random-effects Bayesian model selection (Stephan, Penny, Daunizeau, Moran, & Friston, 2009) was used to compare the negative variational free energy of the following model families: null model, Rescorla-Wagner (RW-1α, RW-2α; value predicting pupil responses), Pearce-Hall models with values predicting responses (RW-PH-value-same, RW-PH-value-distinct), and Pearce-Hall attention weight (RW-PH-attention-same, RW-PH-attention-distinct). The exceedance probability (XP) of each model family, which reflects the certainty about the probability that the data from a randomly chosen participant are best explained by this respective model (i.e., this model family is more likely than any of the others considered) was reported. In addition to the family-wise comparison, all models were compared directly, which was quantified using the protected exceedance probability (PXP) that is protected against the null hypothesis that there are no differences across models (Rigoux, Stephan, Friston, & Daunizeau, 2014).

2.8.2 | Recovery of raw data effects

As a sanity check of our modeling data, the same analyses performed on the raw pupil data were repeated on the simulated data based on the best fitting model (rmANOVA with condition and time as within-subject factors (see Section 2.7, Statistical analysis).

2.8.3 | Confusion matrix

We calculated a confusion matrix in order to probe the specificity of our models (Tzovara et al., 2018; Wilson & Collins, 2019; Wilson & Niv, 2012). We simulated 200 data sets for each of the seven models from our model space, for which we drew parameter values from distributions based on our empirical data. We then fitted the seven models to these simulated data sets. For the confusion matrix, we compared the Bayes information criterion (BIC) scores from these 7 × 7 model fits within every individual subject. For every simulated model (columns), we summed up in how many subjects (percentage) the fitted model (rows) explained the data best. Thus, the diagonal in the created matrix shows how often the true model explained these simulated data best compared to the other candidate models in the model space.

3 | RESULTS

3.1 | Motivational state and perceived US valence

Ratings confirmed that participants were in a hungry and thirsty state before conditioning took place (hunger: $M(SD)$ = 64.5% (22.0); thirst: $M(SD)$ = 63.3% (20.5). The final study population evaluated the US as very pleasant, $M(SD)$ = 84.2% (12.5); this was also consistent over the course of the experiment (before conditioning: $M(SD)$ = 85.4% (11.6); after conditioning: $M(SD)$ = 83.0% (13.4); Figure 1b).

3.2 | CS ratings and US expectancy

Ratings of CS+ and CS− face stimuli before and after the experiment showed no significant conditioning effects regarding pleasantness, arousal, and attractiveness (all Fs(1, 28) ≤ 2.88, $p \geq .101$, $\eta_p^2 \leq .09$), except for a trend-wise time

effect for CS attractiveness, $F(1, 28) = 3.47$, $p = .073$, $\eta_p^2 = .11$.

However, in a dichotomous preference rating, participants chose the CS+ more often after conditioning when selecting between two out of six face stimuli including the CS+ and CS−. We observed a significant Condition × Time interaction, $F(1, 28) = 7.34$, $p = .011$, $\eta_p^2 = .21$, and main effect of time, $F(1, 28) = 5.40$, $p = .028$, $\eta_p^2 = .16$ (Figure S1a). Post-hoc analyses (with Bonferroni correction) showed that the CS+ was preferred more often after conditioning had taken place ($t = -4.54$, $p < .001$; paired t test) and also became the most preferred stimulus from all six stimuli (Figure S1b). CS preference did not change significantly over time ($t = 0.53$, $p = .602$; paired t test) and the difference between CS+ and CS− postconditioning did not reach statistical significance ($t = 1.81$, $p = .081$; paired t test).

Trial-by-trial US expectancy ratings indicated that learning was successful; due to the amount of missing data, these results are to be interpreted with caution (Appendix S1).

3.3 | Ocular response measures

3.3.1 | Pupil diameter

A significant main effect of condition, $F(1, 24) = 9.64$, $p = .005$, $\eta_p^2 = .29$, along with a trend in Condition × Time interaction, $F(1, 24) = 3.23$, $p = .085$, $\eta_p^2 = .12$, was found for the pupil diameter response (Figure 2a,b). The rmANOVA of the model-based PSR showed a significant main effect of condition, $F(1, 24) = 15.15$, $p = .001$, $\eta_p^2 = .39$, and time, $F(1, 24) = 4.71$, $p = .04$, $\eta_p^2 = .16$, along with a trend in Condition × Time interaction, $F(1, 24) = 4.23$, $p = .051$, $\eta_p^2 = .15$. In both analysis approaches, the CS+ elicited a stronger pupil dilation in comparison to the CS−, and this difference was more pronounced in the second half of the experiment.

3.3.2 | Dwelling time

The rmANOVA showed a significant main effect of condition, $F(1, 26) = 7.74$, $p = .010$, $\eta_p^2 = .23$ (Figure 2c) with a longer gaze-dwelling time on CS+ stimuli than on CS− stimuli, yet no main effect of time or Condition × Time interaction (all Fs$(1, 26) \leq 0.62$, $p \geq .437$, $\eta_p^2 \leq .02$).

3.3.3 | Blink duration

A significant main effect of condition, $F(1, 28) = 10.99$, $p = .003$, $\eta_p^2 = .28$, and time, $F(1, 28) = 9.69$, $p = .004$, $\eta_p^2 = .26$, but no Condition × Time interaction between these two variables, $F(1, 28) = 0.39$, $p = .537$, $\eta_p^2 = .01$, was found in the rmANOVA examining mean blink duration. The blink duration was generally increased in CS− trials and in the latter part of the experiment (Figure 2d).

3.3.4 | Blink count

The mean amount of blinks quantified after CS onset showed a trend for the condition, $F(1, 28) = 3.17$, $p = .086$, $\eta_p^2 = .10$, with a higher frequency of blinks in CS− trials. There was no main effect of time or Condition × Time interaction (all Fs$(1, 28) \leq 0.34$, $p \geq .567$, $\eta_p^2 \leq .01$).

3.4 | Modeling results

To further elucidate the mechanism of the observed conditioning effect on pupil dilation, we used different computational models to explain the individual trial-by-trial pupil response in combination with Bayesian model comparison. This revealed that the Pearce-Hall models that predicted the pupil response via the dynamic attention weights associated with the displayed stimulus explained the data best ($XP_{PearceHallAttention} = .6433$, $XP_{RescorlaWagnerValue} = .2361$, $XP_{NullModel} = .0881$, $XP_{PearceHallValue} = .0325$; Figure 3a). The pattern was more ambiguous in the direct comparison of all single models, but in line with the family comparison, the Pearce-Hall attention model with distinct learning parameters per stimulus (RW-PH-attention-distinct) displayed the best model fit (PXP = .1529).

Next, we simulated pupil response data using the best fitting model (RW-PH-attention-distinct). When performing the same analyses as for the raw data, we were able to recover the raw pupil data effects from the simulated pupil responses. The Condition × Time ANOVA across the two halves of the experiment revealed a significant Condition × Time interaction, $F(1, 24) = 30.58$, $p < .001$, $\eta_p^2 = .56$, as well as a significant main effect of condition, $F(1, 24) = 35.25$, $p < .001$, $\eta_p^2 = .59$, and time, $F(1, 24) = 28.09$, $p < .001$, $\eta_p^2 = .54$ (Figure 4a).

Our confusion matrix discerning the specificity of the candidate models showed that, apart from the null model that does not use any dynamic learning trajectories (23%), the other true models clearly predominate the model fits (≥70% of subjects' data are best explained by their true model) with our best fitting model also showing the highest specificity (82%; Figure 3b).

3.5 | Additional psychophysiological measures

The rmANOVA of the HPR showed a significant main effect of condition, $F(1, 25) = 98.85$, $p < .001$, $\eta_p^2 = .80$, with no main effect of time or Condition × Time interaction (all Fs(1,

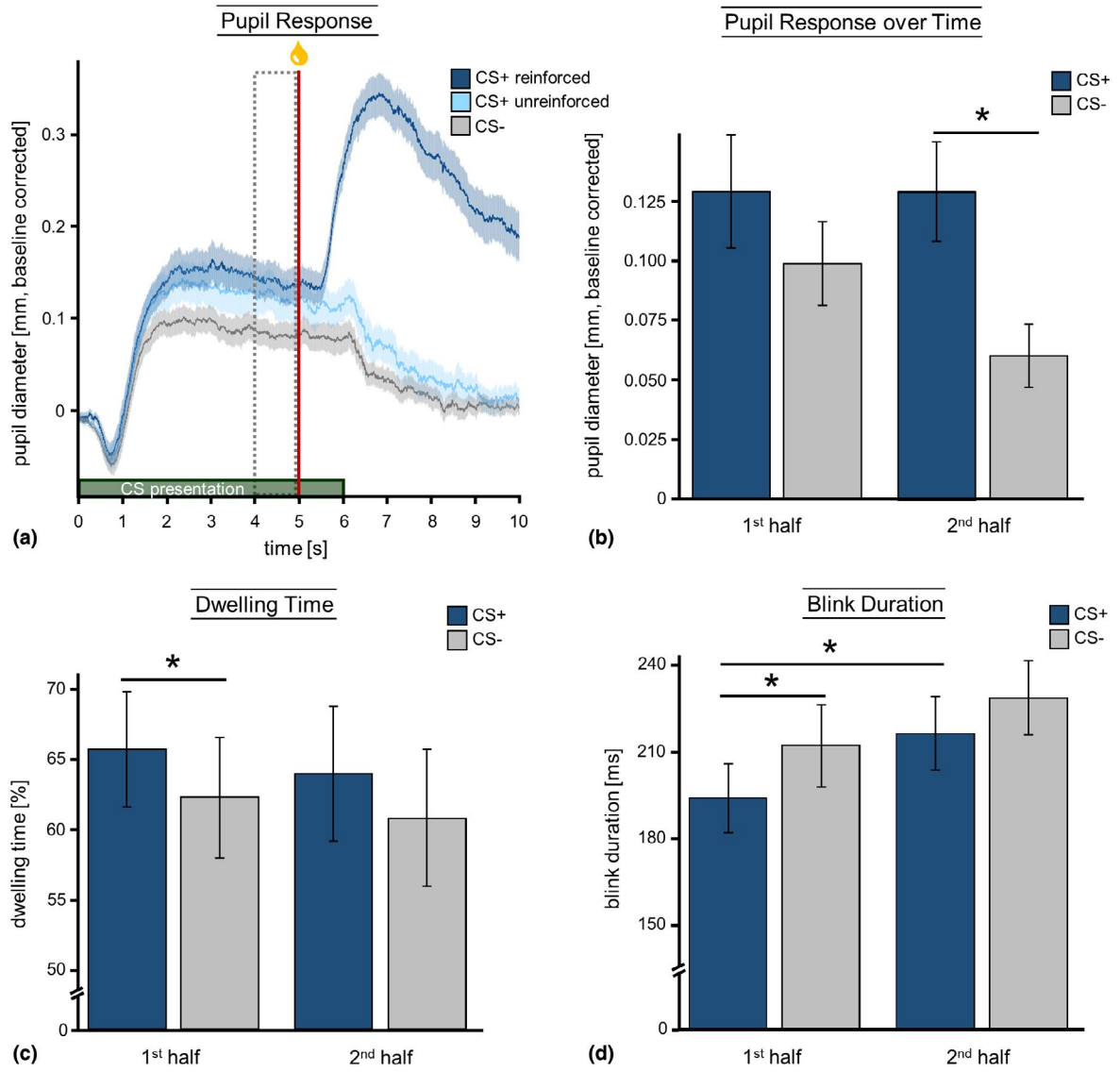


FIGURE 2 Ocular response measures. (a) Mean pupil diameter (baseline corrected) in reinforced/unreinforced CS+ and CS− trials over all participants. The CS+ elicited a stronger pupil dilation compared to the CS− in the predetermined time window (Second 4–5 after CS onset, dotted area). (b) Mean pupil diameter per condition in the first and second half of the experiment. The stronger pupil dilation to the reward-predicting stimulus is especially prominent in the second half of the experiment. (c) Average time participants' gaze fell on the displayed cue in the first and second half of the experiment. There was a longer gaze-dwelling time on the reward-predicting cue than on the control cue. (d) Blink duration contrasted by condition in the first and second half of the experiment. Blink duration was significantly shorter in CS+ trials and the first half of the experiment. All error bars represent *SEM*. *$p \leq .05$

25) ≤ 1.33, $p \geq .261$, $\eta_p^2 \leq .05$), indicating a heart period increase (heart rate deceleration) following CS+ compared to CS− presentations (Figure S2a,b; for computational results of the HPR, see Appendix S1, Figure S3). No conditioning effect was found in the SCR: The rmANOVA showed no significant main effects or Condition × Time interaction when contrasting CS+ with CS− (all Fs(1, 19) ≤ 2.80, $p \geq .111$, $\eta_p^2 \leq .13$). No significant startle potentiation difference was found between CS+ versus CS− in the EBR ($t = 1.30$, $p = .217$; paired t test) and PAR ($t = -0.61$, $p = .551$; paired t test).

3.6 | Correlations

Significant correlations were found neither between the ocular response measures showing significant conditioning effects (i.e., pupil diameter, gaze dwelling time, blink duration), nor between these ocular response measures, HPR, and dichotomous preference rating (all rs $\leq .28$, ps $\geq .117$). As expected, we found a significant positive correlation between the conditioning effect in pupil diameter and model-based PSR ($r = .82$, $p < .001$). We further explored associations between personality traits (extraversion and neuroticism) and

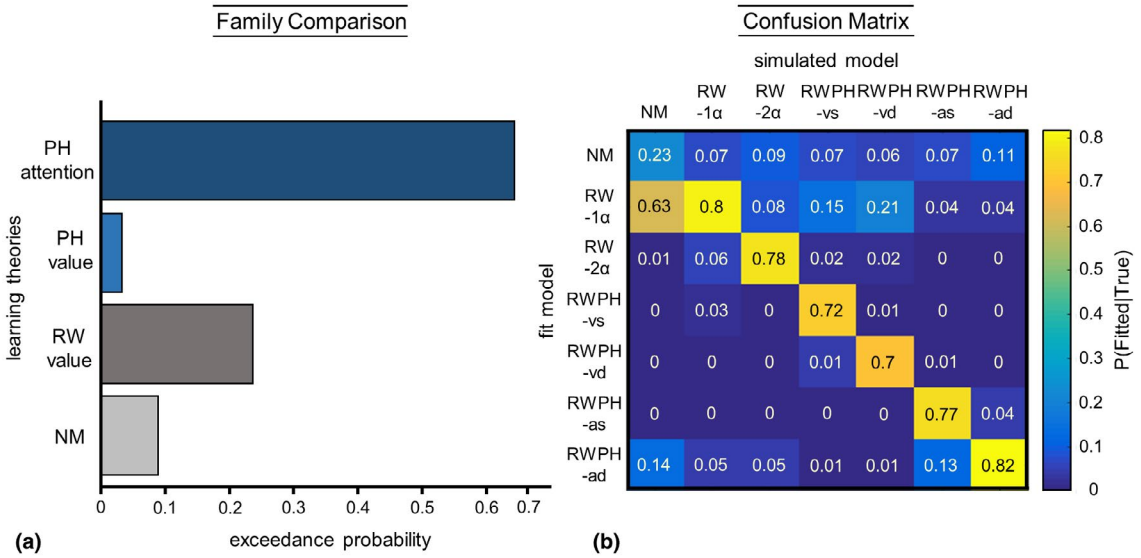


FIGURE 3 Modeling results. (a) Comparison of model families according to their exceedance probabilities. Pearce-Hall models that inferred the pupil response using the dynamic attention weights explained the data best. PH attention = Pearce-Hall models with attention weight (RW-PH-attention same, RW-PH-attention distinct); PH value = Pearce-Hall models with value weight (RW-PH-value-same, RW-PH-value distinct); RW value = Rescorla-Wagner value predicting pupil responses (RW-1α, RW-2α); NM = null model. (b) Confusion matrix: Recovery rates of models. NM = null model; RW-1α = Rescorla-Wagner with one fixed learning rate and expected value as predictor; RW-2α = Rescorla-Wagner with two fixed learning rates and expected value as predictor; RWPH-vs (value same) = Rescorla-Wagner Pearce-Hall hybrid with value as predictor and same parameters for both conditioned stimuli; RWPH-vd (value distinct) = Rescorla-Wagner Pearce-Hall hybrid with value as predictor and distinct parameters for the conditioned stimuli; RWPH-as (attention same) = Rescorla-Wagner Pearce-Hall hybrid with attention as predictor and same parameters for both conditioned stimuli; RWPH-ad (attention distinct) = Rescorla-Wagner Pearce-Hall hybrid with attention as predictor and distinct parameters for both conditions

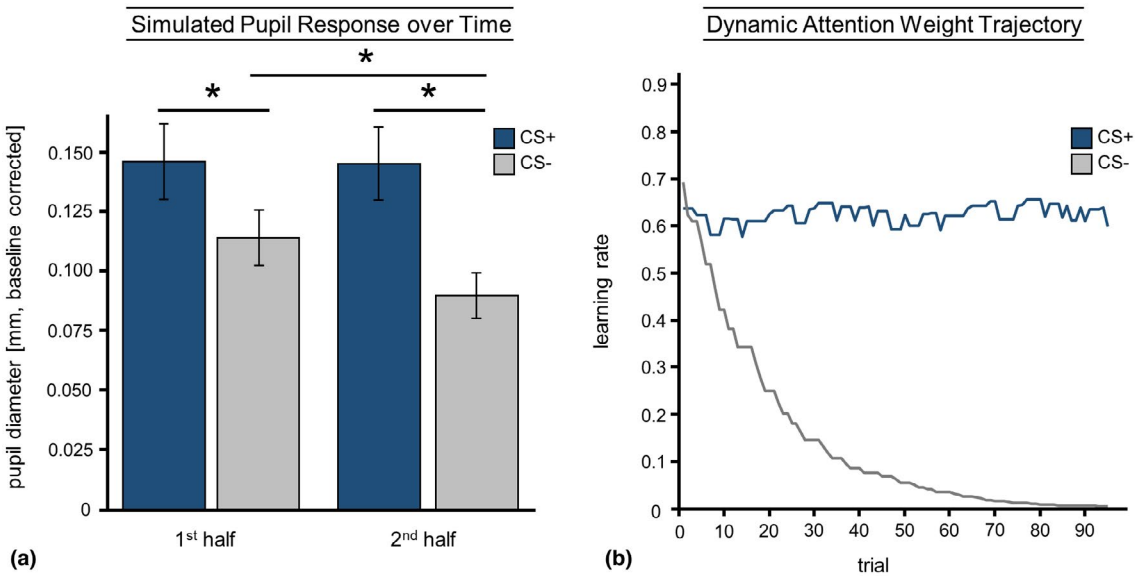


FIGURE 4 Modeling results. (a) Mean simulated pupil response per condition in the first and second half of the experiment. Results are comparable to the raw pupil data (Figure 2b). (b) Example attention weight trajectory of participant #26. The attention weight considers a general decay across accumulating trials along with the absolute prediction error of the previous trial. CS+ achieves relatively steady attention weights due to the high prediction error caused by a 0.5 reinforcement rate. Conversely, the attention weights for CS− slowly decrease as participants learn to not expect a reward following this stimulus. All error bars represent *SEM*. *$p \leq .05$

pupil diameter, HPR, and dichotomous preference applying Bonferroni correction (0.05/6 = 0.0083). We found that the dichotomous preference ratings correlated significantly with extraversion ($r = .48$, $p = .008$; Figure S4), while no other conditioned response was correlated with either extraversion or neuroticism (all rs $\leq .18$, ps $\geq .377$).

4 | DISCUSSION

The present study evaluated pupil diameter and other ocular response measures (gaze dwelling time, blink duration, and count) as psychophysiological indices of appetitive conditioned responding in humans. To this purpose, we designed a differential delay conditioning experiment, where two audiovisual stimuli were systematically paired with either a liquid primary reinforcer or no reward, while ocular response measures, as well as other psychophysiological (SCR, HPR, EBR, PAR) and behavioral (ratings, contingency awareness) parameters were acquired. We found that pupil diameter not only constitutes a sensitive index for representing appetitive conditioning, but also precisely reflects individual trial-by-trial learning mechanisms. Using different computational models and Bayesian model comparison to further elucidate the observed conditioned pupil response, we found that a Pearce-Hall attention-weighted learning model best explains the individual pupil responses. Moreover, we provide initial evidence that gaze dwelling time and blink duration are additional valuable psychophysiological indices of conditioning.

4.1 | Increased pupil dilation towards appetitive conditioned stimuli

We were able to initiate and extend evidence that the ocular response measures represent appetitive conditioning on a psychophysiological level. We specifically examined pupil dilation, which is associated with a variety of cognitive processes causing sympathetic nervous activation (Sirois & Brisson, 2014; van der Wel & van Steenbergen, 2018;). In the current study, participants showed a stronger pupil dilation in response to the conditioned reward-predicting cue (CS+) compared to the control CS−, and this differentiation trend-wise increased over time. Within a trial, we found an initial pupil constriction following CS onset that has been observed previously in paradigms using visual cues as CS (Reinhard, Lachnit, & Koenig, 2006). The differentiation between CS+ and CS− occurred approximately 2 s after CS onset and remained stable until US presentation (Figure 2a). In line with our findings, pupil dilation to appetitive conditioned stimuli has been described previously: Imaging studies using liquid primary reinforcers during Pavlovian conditioning reported pupil dilation to the appetitive conditioned CS in early trials of the experiment in a time window of 0–3 s after CS onset, which was not stable over time and consequently ascribed to potential habituation effects (O'Doherty et al., 2003). Another fMRI study using five different liquid primary reinforcers revealed increased pupil dilation to both the most and least preferred US in a time window of 0–5 s after CS onset (O'Doherty et al., 2006). Pupil dilation was also observed for an earlier time window after CS onset (0.5–1.5/2 s) during a Pavlovian task with a reversal component (Prévost et al., 2013) and for the proximal cue during a higher-order conditioning task (Pauli et al., 2015) using juice as US. In a mixed appetitive-aversive learning task with monetary reinforcement, differential pupil diameter responding was observed toward stimuli associated with rewards and losses (Seymour et al., 2007) using the peak light reflex after cue presentation in each trial (Bitsios, Szabadi, & Bradshaw, 2004). We found that learning about CS-US associations was expressed in stronger pupil dilation toward the CS+ relative to the CS− throughout the experiment. The assumption that change in pupil diameter is prone to early habituation was not observed in the present study (see also Leuchs, Schneider, & Spoormaker, 2018). Our finding showing increased pupil dilation to appetitive conditioned stimuli is therefore in accordance with previous findings, but the first to affirm pupil dilation as a conditioned response throughout the experiment in a design focused on appetitive classical conditioning using primary reinforcement in the established pre-outcome time window. This finding was substantiated by the conditioning effect observed in the PSR using psychophysiological modeling. This supports and complements previous evidence in that pupillary responding represents a promising measure for appetitive conditioning research.

4.2 | Gaze and blink duration as novel appetitive conditioned response measures

Besides pupil dilation, we observed a conditioning effect as participants' gaze remained on the CS+ longer and blink duration was shorter during CS+ compared to CS− presentations. Longer gaze dwelling time is likely explained in part by the attentional capture of reward-associated cues (Anderson, Laurent, & Yantis, 2011; Le Pelley, Pearson, Griffiths, & Beesley, 2015). Blink responding showed a trend of a greater blink rate for the CS− compared to the CS+. Previous studies have described a greater eyeblink rate to aversively conditioned stimuli compared to neutral stimuli (Pauli et al., 2015; Prévost et al., 2013), which may indicate that our CS− was perceived as qualitatively aversive in comparison with the appetitive cue as it was never associated with reward. As a novel measure, we found a significantly shorter blink duration on reward-associated stimuli and in the earlier phase of the experiment. Blink duration is commonly deemed an indicator of drowsiness and fatigue (Caffier et al., 2003; Stern et al., 1994), which would be compatible with the temporal component of our result. The differential responding toward both cues possibly indicates increased alertness or arousal to the reward-associated cue.

4.3 | Pearce and Hall's attention model predicts trial-by-trial pupil diameter change

We used computational models of trial-by-trial pupil diameter change to elucidate the cognitive process in more detail.

We tested whether the pupil responses were predicted more accurately by either the dynamic expected value or dynamic attention weight of the displayed stimuli. We observed that the Pearce-Hall learning model with distinct attention weights per CS type best predicted the pupil response. While this is an interesting result, it is important to recognize that XP only expresses the relative model fit within the considered model space. Pearce-Hall's learning theory describes the circumstances in which the attention given to a CS evolve in reaction to the experienced consequences, remaining high when the CS outcome is unpredictable and contrastingly decreasing when the CS outcome is highly predictable (Pearce & Hall, 1980). This is in accordance with our finding where we see steady attention weights to the CS+ where the outcome is uncertain in contrast to a declining attention weight in the CS− where the outcome (i.e., lack of reward) is certain. When examining appetitive and aversive higher-order learning, pupil diameter has shown to be modulated by an interaction of both CS value and prediction error (Pauli et al., 2015). An aversive learning experiment found that the trial-by-trial PSR predominantly reflects expected CS outcome (Tzovara et al., 2018). Interestingly, earlier studies have also found evidence in support of the Pearce-Hall learning theory in gaze-dwelling time, showing longer gaze durations on stimuli associated with appetitive and aversive uncertain outcome (Hogarth, Dickinson, Austin, Brown, & Duka, 2008; Koenig, Kadel, Uengoer, Schubö, & Lachnit, 2017), which is consistent with our result. Taken together, while pupil dilation was a sensitive measure of appetitive conditioning in our study, it seems to be more related to attentional processes rather than appetitive value.

In line with our modeling finding, pupil dilation has shown to be a robust measure for orienting attention toward cues that reliably predict an outcome (Lasaponara et al., 2019). Trial-by-trial pupil metrics have further been related to more complex learning processes such as change-point probability and relative uncertainty, which were associated with pupil change and pupil average, respectively (Nassar et al., 2012). Change in pupil diameter also distinctly reflects perceptual content and level of surprise (Kloosterman et al., 2015). Imaging (Murphy, O'Connell, O'Sullivan, Robertson, & Balsters, 2014) and translational animal model studies (Joshi, Li, Kalwani, & Gold, 2016; Rajkowski, Kubiak, & Aston-Jones, 1993; Varazzani et al., 2015) have associated pupil dilation with locus coeruleus activation and increased noradrenaline release (Aston-Jones & Cohen, 2005). Theories propose that noradrenaline is relevant for signaling unexpected uncertainty in a volatile environment (Yu & Dayan, 2005). Therefore, pupil diameter, as a proxy of locus coeruleus activation and noradrenaline release, presents a valuable outcome measure in the multi-dimensional learning and decision-making framework (Silvetti et al., 2018).

4.4 | Confirming successful conditioning through additional psychophysiological measures

The present study was able to corroborate the conditioning effect as differential CS responding was also observed in other independent parameters. Although the explicit valence rating was not a sensitive measure of conditioning, the more implicit dichotomous preference rating showed a clear conditioned preference increase to the reward-predicting stimulus. A possible explanation for the lack of a prominent valence differentiation is that we used neutral faces as CS, which are already afflicted with many social characteristics and contain a strong preference bias (Todorov, Olivola, Dotsch, & Mende-Siedlecki, 2015). As already established in fear-conditioning experiments (Castegnetti et al., 2016; Prévost et al., 2013), we observed conditioned bradycardia to the reward-associated stimulus, which is a novel finding in the appetitive conditioning domain. No conditioning effect was observed in the SCR or acoustic startle responses (EBR, PAR). Previous studies showed both significant SCR effects during appetitive conditioning (Andreatta & Pauli, 2015; Ebrahimi et al., 2019; Klucken et al., 2013, 2015, 2016; Tapia León et al., 2018), as well as no significant differential response to the conditioned CS (Ebrahimi et al., 2017; Klucken et al., 2009; Stussi et al., 2018; van den Akker et al., 2017b). Our non-significant finding may result from insufficient statistical power (especially due to the exclusion of eight participants from the SCR analysis) or habituation effects (i.e., a decrement in response amplitude with repeated CS presentation), which particularly afflicts experiments with a longer duration as used in our study (Leuchs et al., 2018; Lonsdorf et al., 2017). Although the acoustically evoked PAR has been suggested as a sensitive index of appetitive responding (Ebrahimi et al., 2019; Sandt et al., 2009; Stussi et al., 2018), the present study could not replicate this. We attribute the lack of a conditioned effect to the low sample size due to low data quality in this measure as well as the low sampling rate. Furthermore, the startle stimulus occurred comparatively early after CS onset, possibly conglomerating response effects.

Psychophysiological response measures with disparate results are common in conditioning research (Hermann et al., 2000; Stussi et al., 2018; Wardle et al., 2018). Interestingly, there was no discernible correlation between the different measures showing a conditioning effect. This is in accordance with prior findings theorizing that there are interindividual differences in the preferred response system or that the various measures are influenced by distinct psychological components of reward (Berridge, Robinson, & Aldridge, 2009; Wardle et al., 2018). The weak relationships among measures emphasize the importance of a multi-methodological

approach when investigating appetitive Pavlovian conditioning. Furthermore, it is would be desirable to standardize approaches of analysis, for instance, by using psychophysiological modeling techniques (Bach et al., 2018).

4.5 | Outlook

In conclusion, the present study highlights the potential value of ocular response measures when examining appetitive conditioning in humans. Although appetitive Pavlovian conditioning is a central learning mechanism and fundamental for understanding various pathological states, it remains vastly underexplored, largely due to the lack of a sensitive psychophysiological measure to represent conditioned responding. We propose the incorporation of eye-tracking measures when examining appetitive conditioning, as they provide multiple accurate, noninvasive measures with short reaction latencies that show clear conditioned differentiation. A further advantage is that ocular response measures have a high signal-to-noise ratio and are not susceptible to magnetic field artifacts, making them ideal measures in an fMRI environment. This could help expedite appetitive conditioning research and assist the exploration of neural correlates of appetitive learning derivatives like extinction and reinstatement (Konova & Goldstein, 2018) or reward prediction (Bach, Symmonds, Barnes, & Dolan, 2017). To conclude, our findings contribute evidence toward the establishment of a much-needed gold standard learning criterion in the human appetitive conditioning domain.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (DFG) grant to F.S. SCHL1969/2-2 (as part of FOR1617), SCHL1969/4-1. C.E. was supported by an Elsa-Neumann scholarship and a Charité scholarship. Raw data are available and can be uploaded into a public repository upon request.

ORCID

Charlotte Pietrock https://orcid.org/0000-0001-9358-5973

REFERENCES

Aaron, R. V., & Benning, S. D. (2016). Postauricular reflexes elicited by soft acoustic clicks and loud noise probes: Reliability, prepulse facilitation, and sensitivity to picture contents. *Psychophysiology*, *53*(12), 1900–1908. https://doi.org/10.1111/psyp.12757

Anderson, B. A., Laurent, P. A., & Yantis, S. (2011). Value-driven attentional capture. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(25), 10367–10371. https://doi.org/10.1073/pnas.1104047108

Andreatta, M., & Pauli, P. (2015). Appetitive vs. aversive conditioning in humans. *Frontiers in Behavioral Neuroscience*, *9*, 128. https://doi.org/10.3389/fnbeh.2015.00128

Aston-Jones, G., & Cohen, J. D. (2005). An integrative theory of locus coeruleus-norepinephrine function: Adaptive gain and optimal performance. *Annual Review of Neuroscience*, *28*, 403–450. https://doi.org/10.1146/annurev.neuro.28.061604.135709

Austin, A. J., & Duka, T. (2010). Mechanisms of attention for appetitive and aversive outcomes in Pavlovian conditioning. *Behavioural Brain Research*, *213*(1), 19–26. https://doi.org/10.1016/j.bbr.2010.04.019

Bach, D. R. (2014). A head-to-head comparison of SCRalyze and Ledalab, two model-based methods for skin conductance analysis. *Biological Psychology*, *103*, 63–68. https://doi.org/10.1016/j.biopsycho.2014.08.006

Bach, D. R., Castegnetti, G., Korn, C. W., Gerster, S., Melinscak, F., & Moser, T. (2018). Psychophysiological modeling: Current state and future directions. *Psychophysiology*, *55*(11), e13214. https://doi.org/10.1111/psyp.13209

Bach, D. R., Flandin, G., Friston, K. J., & Dolan, R. J. (2010). Modelling event-related skin conductance responses. *International Journal of Psychophysiology*, *75*(3), 349–356. https://doi.org/10.1016/j.ijpsycho.2010.01.005

Bach, D. R., Friston, K. J., & Dolan, R. J. (2013). An improved algorithm for model-based analysis of evoked skin conductance responses. *Biological Psychology*, *94*(3), 490–497. https://doi.org/10.1016/j.biopsycho.2013.09.010

Bach, D. R., Symmonds, M., Barnes, G., & Dolan, R. J. (2017). Whole-brain neural dynamics of probabilistic reward prediction. *Journal of Neuroscience*, *37*(14), 3789–3798. https://doi.org/10.1523/JNEUROSCI.2943-16.2017

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'Liking', 'wanting', and learning. *Current Opinion in Pharmacology*, *9*(1), 65–73. https://doi.org/10.1016/j.coph.2008.12.014

Bitsios, P., Szabadi, E., & Bradshaw, C. M. (2004). The fear-inhibited light reflex: Importance of the anticipation of an aversive event. *International Journal of Psychophysiology*, *52*(1), 87–95. https://doi.org/10.1016/j.ijpsycho.2003.12.006

Blechert, J., Testa, G., Georgii, C., Klimesch, W., & Wilhelm, F. H. (2016). The Pavlovian craver: Neural and experiential correlates of single trial naturalistic food conditioning in humans. *Physiology & Behaviour*, *158*, 18–25. https://doi.org/10.1016/j.physbeh.2016.02.028

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology*, *42*(1), 1–15. https://doi.org/10.1111/j.1469-8986.2005.00271.x

Borkenau, P., & Ostendorf, F. (1993). *NEO-Fünf-Faktoren Inventar (NEO-FFI) nach Costa und McCrae*. Handanweisung. Göttingen, Bern, Toronto, Seattle: Hogrefe.

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*(4), 602–607. https://doi.org/10.1111/j.1469-8986.2008.00654.x

Brainard, D. H. (1997). The psychophysics toolbox. *Spatial Vision*, *10*(4), 433–436. https://doi.org/10.1163/156856897X00357

Bray, S., & O'Doherty, J. P. (2007). Neural coding of reward-prediction error signals during classical conditioning with attractive faces. *Journal of Neurophysiology*, *97*(4), 3036–3045. https://doi.org/10.1152/jn.01211.2006

Bray, S., Rangel, A., Shimojo, S., Balleine, B., & O'Doherty, J. P. (2008). The neural mechanisms underlying the influence of Pavlovian cues on human decision making. *Journal of Neuroscience*, *28*(22), 5861–5868. https://doi.org/10.1523/JNEUROSCI.0897-08.2008

Brocher, A., & Graf, T. (2016). Pupil old/new effects reflect stimulus encoding and decoding in short-term memory. *Psychophysiology*, *53*(12), 1823–1835. https://doi.org/10.1111/psyp.12770

Caffier, P. P., Erdmann, U., & Ullsperger, P. (2003). Experimental evaluation of eye-blink parameters as a drowsiness measure. *European Journal of Applies Physiology*, *89*(3–4), 319–325. https://doi.org/10.1007/s00421-003-0807-5

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology*, *67*(2), 319–333.

Castegnetti, G., Tzovara, A., Staib, M., Paulus, P. C., Hofer, N., & Bach, D. R. (2016). Modeling fear-conditioned bradycardia in humans. *Psychophysiology*, *53*(6), 930–939. https://doi.org/10.1111/psyp.12637

Costa, P. T., & McCrae, R. R. (1992). *Revised NEO Personality inventory and NEO five-factor inventory (Professional Manual)*. Odessa: Psychological Assessment Resources.

Delgado, M. R., Gillis, M. M., & Phelps, E. A. (2008). Regulating the expectation of reward via cognitive strategies. *Nature Neuroscience*, *11*(8), 880–881. https://doi.org/10.1038/nn.2141

Delgado, M. R., Jou, R. L., & Phelps, E. A. (2011). Neural systems underlying aversive conditioning in humans with primary and secondary reinforcers. *Frontiers in Neuroscience*, *5*, 71. https://doi.org/10.3389/fnins.2011.00071

Depue, R. A., & Fu, Y. (2013). On the nature of extraversion: Variation in conditioned contextual activation of dopamine-facilitated affective, cognitive, and motor processes. *Frontiers in Human Neuroscience*, *7*, 288. https://doi.org/10.3389/fnhum.2013.00288

Diederen, K. M., Spencer, T., Vestergaard, M. D., Fletcher, P. C., & Schultz, W. (2016). Adaptive prediction error coding in the human midbrain and striatum facilitates behavioral adaptation and learning efficiency. *Neuron*, *90*(5), 1127–1138. https://doi.org/10.1016/j.neuron.2016.04.019

Ebner, N., Riediger, M., & Lindenberger, U. (2010). FACES—A database of facial expressions in young, middle-aged, and older women and men: Development and validation. *Behavior Research Methods*, *42*(1), 351–362. https://doi.org/10.3758/BRM.42.1.351

Ebrahimi, C., Koch, S. P., Friedel, E., Crespo, I., Fydrich, T., Ströhle, A., … Schlagenhauf, F. (2017). Combining D-cycloserine with appetitive extinction learning modulates amygdala activity during recall. *Neurobiology of Learning and Memory*, *142*(Pt B), 209–217. https://doi.org/10.1016/j.nlm.2017.05.008

Ebrahimi, C., Koch, S. P., Pietrock, C., Fydrich, T., Heinz, A., & Schlagenhauf, F. (2019). Opposing roles for amygdala and vmPFC in the return of appetitive conditioned responses in humans. *Translational Psychiatry*, *9*(1), 148. https://doi.org/10.1038/s41398-019-0482-x

Eldar, E., Cohen, J. D., & Niv, Y. (2013). The effects of neural gain on attention and learning. *Nature Neuroscience*, *16*(8), 1146–1153. https://doi.org/10.1038/nn.3428

Fullana, M. A., Harrison, B. J., Soriano-Mas, C., Vervliet, B., Cardoner, N., Àvila-Parcet, A., & Radua, J. (2016). Neural signatures of human fear conditioning: An updated and extended meta-analysis of fMRI studies. *Molecular Psychiatry*, *21*(4), 500–508. https://doi.org/10.1038/mp.2015.88

Gable, P. A., & Harmon-Jones, E. (2009). Postauricular reflex responses to pictures varying in valence and arousal. *Psychophysiology*, *46*(3), 487–490. https://doi.org/10.1111/j.1469-8986.2009.00794.x

Genheimer, H., Andreatta, M., Asan, E., & Pauli, P. (2017). Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. *Scientific Reports*, *7*(1), 17786. https://doi.org/10.1038/s41598-017-18183-3

Glotzbach-Schoon, E., Andreatta, M., Mühlberger, A., & Pauli, P. (2015). Reinstatement of contextual anxiety in humans: Effects of state anxiety. *International Journal of Psychophysiology*, *98*(3 Pt 2), 557–566. https://doi.org/10.1016/j.ijpsycho.2015.07.013

Goldinger, S. D., & Papesh, M. H. (2012). Pupil dilation reflects the creation and retrieval of memories. *Current Directions in Psychological Science*, *21*(2), 90–95. https://doi.org/10.1177/0963721412436811

Gottfried, J. A., O'Doherty, J. P., & Dolan, R. J. (2002). Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *Journal of Neuroscience*, *22*(24), 10829–10837. https://doi.org/10.1523/JNEUROSCI.22-24-10829.2002

Granholm, E., & Steinhauer, S. R. (2004). Pupillometric measures of cognitive and emotional processes. *International Journal of Psychophysiology*, *52*(1), 1–6. https://doi.org/10.1016/j.ijpsycho.2003.12.001

Grosshans, M., Loeber, S., & Kiefer, F. (2011). Implications from addiction research towards the understanding and treatment of obesity. *Addiction Biology*, *16*(2), 189–198. https://doi.org/10.1111/j.1369-1600.2010.00300.x

Hermann, C., Ziegler, S., Birbaumer, N., & Flor, H. (2000). Pavlovian aversive and appetitive odor conditioning in humans: Subjective, peripheral, and electrocortical changes. *Experimental Brain Research*, *132*(2), 203–215. https://doi.org/10.1007/s002210000343

Hogarth, L., Dickinson, A., Austin, A., Brown, C., & Duka, T. (2008). Attention and expectation in human predictive learning: The role of uncertainty. *Quarterly Journal of Experimental Psychology*, *61*(11), 1658–1668. https://doi.org/10.1080/17470210701643439

Holmqvist, K., Nyström, M., Andersson, R., Dewhurst, R., Jarodzka, H., & van der Weijer, J. (2011). *Eye tracking: A comprehensive guide to methods and measures*. Oxford, UK: Oxford University Press.

Hooker, C. I., Verosky, S. C., Miyakawa, A., Knight, R. T., & D'Esposito, M. (2008). The influence of personality on neural mechanisms of observational fear and reward learning. *Neuropsychologia*, *46*(11), 2709–2794. https://doi.org/10.1016/j.neuropsychologia.2008.05.005

Isaac, L., Vrijsen, J. N., Rinck, M., Speckens, A., & Becker, E. S. (2014). Shorter gaze duration for happy faces in current but not remitted depression: Evidence from eye movements. *Psychiatry Research*, *218*(1–2), 79–86. https://doi.org/10.1016/j.psychres.2014.04.002

Joshi, S., Li, Y., Kalwani, R., & Gold, J. I. (2016). Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. *Neuron*, *89*(1), 221–234. https://doi.org/10.1016/j.neuron.2015.11.028

Just, M. A., Carpenter, P. A., & Miyake, A. (2003). Neuroindices of cognitive workload: Neuroimaging, pupillometric and event-related potential studies of brain work. *Theoretical Issues in Ergonomics Science*, *4*(1), 56–88. https://doi.org/10.1080/14639220210159735

Kahneman, D., & Beatty, J. (1966). Pupil diameter and load on memory. *Science*, *154*(3756), 1583–1585. https://doi.org/10.1126/science.154.3756.1583

Kahnt, T., Heinzle, J., Park, S. Q., & Haynes, J. D. (2011). Decoding the formation of reward predictions across learning. *Journal of Neuroscience*, *31*(41), 14624–14630. https://doi.org/10.1523/JNEUROSCI.3412-11.2011

Khemka, S., Tzovara, A., Gerster, S., Quednow, B. B., & Bach, D. R. (2017). Modeling startle eyeblink electromyogram to assess fear learning. *Psychophysiology*, *54*(2), 204–214. https://doi.org/10.1111/psyp.12775

Kiefer, F., & Dinter, C. (2013). New approaches to addiction treatment based on learning and memory. *Current Topics in Behavioral Neurosciences*, *13*, 671–684. https://doi.org/10.1007/7854_2011_147

Kiiski, H. S., Cullen, B., Clavin, S. L., & Newell, F. N. (2016). Perceptual and social attributes underlining age-related preferences for faces. *Frontiers in Human Neuroscience*, *10*, 437. https://doi.org/10.3389/fnhum.2016.00437

Kinner, V. L., Kuchinke, L., Dierolf, A. M., Merz, C. J., Otto, T., & Wolf, O. T. (2017). What our eyes tell us about feelings: Tracking pupillary responses during emotion regulation processes. *Psychophysiology*, *54*(4), 508–518. https://doi.org/10.1111/psyp.12816

Kleiner, M., Brainard, D., Pelli, D., Ingling, A., Murray, R., & Broussard, C. (2007). What's new in Psychtoolbox-3. *Perception*, *36*(14), 1–16.

Kloosterman, N. A., Meindertsma, T., van Loon, A. M., Lamme, V. A., Bonneh, Y. S., & Donner, T. H. (2015). Pupil size tracks perceptual content and surprise. *European Journal of Neuroscience*, *41*(8), 1068–1078. https://doi.org/10.1111/ejn.12859

Klucken, T., Kruse, O., Wehrum-Osinsky, S., Hennig, J., Schweckendiek, J., & Stark, R. (2015). Impact of COMT Val158Met-polymorphism on appetitive conditioning and amygdala/prefrontal effective connectivity. *Human Brain Mapping*, *36*(3), 1093–1101. https://doi.org/10.1002/hbm.22688

Klucken, T., Schweckendiek, J., Merz, C. J., Tabbert, K., Walter, B., Kagerer, S., … Stark, R. (2009). Neural activations of the acquisition of conditioned sexual arousal: Effects of contingency awareness and sex. *Journal of Sexual Medicine*, *6*(11), 3071–3085. https://doi.org/10.1111/j.1743-6109.2009.01405.x

Klucken, T., Wehrum, S., Schweckendiek, J., Merz, C. J., Hennig, J., Vaitl, D., & Stark, R. (2013). The 5-HTTLPR polymorphism is associated with altered hemodynamic responses during appetitive conditioning. *Human Brain Mapping*, *34*(10), 2549–2560. https://doi.org/10.1002/hbm.22085

Klucken, T., Wehrum-Osinsky, S., Schweckendiek, J., Kruse, O., & Stark, R. (2016). Altered appetitive conditioning and neural connectivity in participants with compulsive sexual behavior. *Journal of Sexual Medicine*, *13*(4), 627–636. https://doi.org/10.1016/j.jsxm.2016.01.013

Koenig, S., Kadel, H., Uengoer, M., Schubö, A., & Lachnit, H. (2017). Reward draws the eye, uncertainty holds the eye: Associative learning modulates distractor interference in visual search. *Frontiers in Behavioral Neuroscience*, *11*, 128. https://doi.org/10.3389/fnbeh.2017.00128

Koenig, S., Uengoer, M., & Lachnit, H. (2018). Pupil dilation indicates the coding of past prediction errors: Evidence for attentional learning theory. *Psychophysiology*, *55*(4), e13020. https://doi.org/10.1111/psyp.13020

Konova, A. B., & Goldstein, R. Z. (2018). The emerging neuroscience of appetitive and drug cue extinction in humans. *Psychopharmacology*, *236*(1), 407–414. https://doi.org/10.1007/s00213-018-5098-y

Korn, C. W., & Bach, D. R. (2016). A solid frame for the window on cognition: Modelling event-related pupil responses. *Journal of Vision*, *16*(3), 28. https://doi.org/10.1167/16.3.28

Korn, C. W., Staib, M., Tzovara, A., Castegnetti, G., & Bach, D. R. (2017). A pupil size response model to assess fear learning. *Psychophysiology*, *54*(3), 303–343. https://doi.org/10.1111/psyp.12801

Kruse, O., Tapia León, I., Stark, R., & Klucken, T. (2017). Neural correlates of appetitive extinction in humans. *Social Cognitive and Affective Neuroscience*, *12*(1), 106–115. https://doi.org/10.1093/scan/nsw157

Laeng, B., Sirois, S., & Gredebäck, G. (2012). Pupillometry: A window to the preconscious? *Perspectives on Psychological Science*, *7*(1), 18–27. https://doi.org/10.1177/1745691611427305

Lasaponara, S., Fortunato, G., Dragone, A., Pellegrino, M., Marson, F., Silvetti, M., … Doricchi, F. (2019). Expectancy modulates pupil size both during endogenous orienting and during re-orienting of spatial attention: A study with isoluminant stimuli. *European Journal of Neuroscience*. Advance online publication. https://doi.org/10.1111/ejn.14391

Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. (1981). *Das State-Trait-Angstinventar. Theoretische Grundlagen und Handanweisung*. Weinheim: Beltz Test GmbH.

Le Pelley, M. E., Pearson, D., Griffiths, O., & Beesley, T. (2015). When goals conflict with values: Counterproductive attentional and oculomotor capture by reward-related stimuli. *Journal of Experimental Psychology*, *144*(1), 158–171. https://doi.org/10.1037/xge0000037

Leuchs, L., Schneider, M., Czisch, M., & Spoormaker, V. I. (2017). Neural correlates of pupil dilation during human fear learning. *NeuroImage*, *147*, 186–197. https://doi.org/10.1016/j.neuroimage.2016.11.072

Leuchs, L., Schneider, M., & Spoormaker, V. I. (2018). Measuring the conditioned response: A comparison of pupillometry, skin conductance, and startle electromyography. *Psychophysiology*, *56*(1), e13283. https://doi.org/10.1111/psyp.13283

Li, S. S., & McNally, G. P. (2014). The conditions that promote fear learning: Prediction error and Pavlovian fear conditioning. *Neurobiology of Learning and Memory*, *108*, 14–21. https://doi.org/10.1016/j.nlm.2013.05.002

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., … Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience and Biobehavioral Reviews*, *77*, 247–285. https://doi.org/10.1016/j.neubiorev.2017.02.026

Martin-Soelch, C., Linthicum, J., & Ernst, M. (2007). Appetitive conditioning: Neural bases and implications for psychopathology. *Neuroscience and Biobehavioral Reviews*, *31*(3), 426–440. https://doi.org/10.1016/j.neubiorev.2006.11.002

Mathys, C. D., Daunizeau, J., Friston, K. J., & Stephan, K. E. (2011). A Bayesian foundation for individual learning under uncertainty. *Frontiers in Human Neuroscience*, *5*, 39. https://doi.org/10.3389/fnhum.2011.00039

Mathys, C. D., Lomakina, E. I., Daunizeau, J., Iglesias, S., Brodersen, K. H., Friston, K. J., & Stephan, K. E. (2014). Uncertainty in perception and the hierarchical Gaussian filter. *Frontiers in Human Neuroscience*, *8*, 825. https://doi.org/10.3389/fnhum.2014.00825

Metereau, E., & Dreher, J. C. (2013). Cerebral correlates of salient prediction error for different rewards and punishments. *Cerebal Cortex*, *23*(2), 477–487. https://doi.org/10.1093/cercor/bhs037

Murphy, P. R., O'Connell, R. G., O'Sullivan, M., Robertson, I. H., & Balsters, J. H. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Human Brain Mapping*, *35*(8), 4140–4154. https://doi.org/10.1002/hbm.22466

Nassar, M. R., Rumsey, K. M., Wilson, R. C., Parikh, K., Heasly, B., & Gold, J. I. (2012). Rational regulation of learning dynamics by pupil-linked arousal systems. *Nature Neuroscience*, *15*(7), 1040–1046. https://doi.org/10.1038/nn.3130

Ney, L. J., Wade, M., Reynolds, A., Zuj, D. V., Dymond, S., Matthews, A., & Felmingham, K. L. (2018). Critical evaluation of current data analysis strategies for psychophysiological measures of fear conditioning and extinction in humans. *International Journal of Psychophysiology*, *134*, 95–107. https://doi.org/10.1016/j.ijpsycho.2018.10.010

O'Doherty, J. P., Buchanan, T. W., Seymour, B., & Dolan, R. J. (2006). Predictive neural coding of reward preference involves dissociable responses in human ventral midbrain and ventral striatum. *Neuron*, *49*(1), 157–166. https://doi.org/10.1016/j.neuron.2005.11.014

O'Doherty, J. P., Dayan, P., Friston, K., Critchley, H., & Dolan, R. J. (2003). Temporal difference models and reward-related learning in the human brain. *Neuron*, *38*(2), 329–337. https://doi.org/10.1016/S0896-6273(03)00169-7

Pan, J., & Tompkins, W. J. (1985). A real-time QRS detection algorithm. *IEEE Transaction on Bio-Medical Engineering*, *32*(3), 230–236. https://doi.org/10.1109/TBME.1985.325532

Pauli, W. M., Larsen, T., Collette, S., Tyszka, J. M., Seymour, B., & O'Doherty, J. P. (2015). Distinct contributions of ventromedial and dorsolateral subregions of the human substantia nigra to appetitive and aversive learning. *Journal of Neuroscience*, *35*(42), 14220–14233. https://doi.org/10.1523/JNEUROSCI.2277-15.2015

Paulus, P. C., Castegnetti, G., & Bach, D. R. (2016). Modeling event-related heart period responses. *Psychophysiology*, *53*(6), 837–846. https://doi.org/10.1111/psyp.12622

Pavlov, I. P. (1927). *Conditioned reflexes*. Oxford, UK: Oxford University Press.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review*, *87*(6), 532–552. https://doi.org/10.1037/0033-295X.87.6.532

Prévost, C., McNamee, D., Jessup, R. K., Bossaerts, P., & O'Doherty, J. P. (2013). Evidence for model-based computations in the human amygdala during Pavlovian conditioning. *PLOS Computational Biology*, *9*(2), e1002918. https://doi.org/10.1371/journal.pcbi.1002918

Rajkowski, J., Kubiak, P., & Aston-Jones, G. (1993). Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Society for Neuroscience*, Abstract, Washington, DC.

Reinhard, G., Lachnit, H., & Koenig, S. (2006). Tracking stimulus processing in Pavlovian pupillary conditioning. *Psychophysiology*, *43*(1), 73–83. https://doi.org/10.1111/j.1469-8986.2006.00374.x

Rescorla, R. A. (1988). Pavlovian conditioning. It's not what you think it is. *American Psychologist*, *43*(3), 151–160. https://doi.org/10.1037/0003-066X.43.3.151

Rigoux, L., Stephan, K. E., Friston, K. J., & Daunizeau, J. (2014). Bayesian model selection for group studies—Revisited. *NeuroImage*, *84*, 971–985. https://doi.org/10.1016/j.neuroimage.2013.08.065

Robinson, T. E., & Berridge, K. C. (2000). The psychology and neurobiology of addiction: An incentive-sensitization view. *Addiction*, *95*(Supp 2), S91–S117. https://doi.org/10.1046/j.1360-0443.95.8s2.19.x

Rothkirch, M., Stein, T., Sekutowicz, M., & Sterzer, P. (2012). A direct oculomotor correlate of unconscious visual processing. *Current Biology*, *22*(13), 514–515. https://doi.org/10.1016/j.cub.2012.04.046

Sandt, A. R., Sloan, D. M., & Johnson, K. J. (2009). Measuring appetitive responding with the postauricular reflex. *Psychophysiology*, *46*(3), 491–497. https://doi.org/10.1111/j.1469-8986.2009.00797.x

Schumacher, S., Oe, M., Wilhelm, F. H., Rufer, M., Heinrichs, M., Weidt, S., … Martin-Soelch, C. (2018). Does trait anxiety influence effects of oxytocin on eye-blink startle reactivity? A randomized, double-blind, placebo-controlled crossover study. *PLOS One*, *13*(1), e0190809. https://doi.org/10.1371/journal.pone.0190809

Schweckendiek, J., Stark, R., & Klucken, T. (2016). Neuroticism and extraversion moderate neural responses and effective connectivity during appetitive conditioning. *Human Brain Mapping*, *37*(8), 2992–3002. https://doi.org/10.1002/hbm.23221

Sescousse, G., Caldú, X., Segura, B., & Dreher, J. C. (2013). Processing of primary and secondary rewards: A quantitative meta-analysis and review of human functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews*, *37*(4), 681–696. https://doi.org/10.1016/j.neubiorev.2013.02.002

Seymour, B., Daw, N., Dayan, P., Singer, T., & Dolan, R. (2007). Differential encoding of losses and gains in the human striatum. *Journal of Neuroscience*, *27*(18), 4826–4831. https://doi.org/10.1523/JNEUROSCI.0400-07.2007

Silvetti, M., Vassena, E., Abrahamse, E., & Verguts, T. (2018). Dorsal anterior cingulate-brainstem ensemble as a reinforcement meta-learner. *PLOS Computational Biology*, *14*(8), e1006370. https://doi.org/10.1371/journal.pcbi.1006370

Sirois, S., & Brisson, J. (2014). Pupillometry. *Wiley Interdisciplinary Reviews-Cognitive Science*, *5*(6), 679–692. https://doi.org/10.1002/wcs.1323

Sjouwerman, R., & Lonsdorf, T. B. (2018). Latency of skin conductance responses across stimulus modalities. *Psychophysiology*, *56*, e13307. https://doi.org/10.1111/psyp.13307

Stephan, K. E., Penny, W. D., Daunizeau, J., Moran, R. J., & Friston, K. J. (2009). Bayesian model selection for group studies. *NeuroImage*, *46*(4), 1004–1017. https://doi.org/10.1016/j.neuroimage.2009.03.025

Stern, J. A., Boyer, D., & Schroeder, D. (1994). Blink rate: A possible measure of fatigue. *Human Factors*, *36*(2), 285–297. https://doi.org/10.1177/001872089403600209

Stern, J. A., Walrath, L. C., & Goldstein, R. (1984). The endogenous eyeblink. *Psychophysiology*, *21*(1), 22–33. https://doi.org/10.1111/j.1469-8986.1984.tb02312.x

Stussi, Y., Delplanque, S., Corai, S., Pourtois, G., & Sander, D. (2018). Measuring Pavlovian appetitive conditioning in humans with the postauricular reflex. *Psychophysiology*, *55*(8), e13073. https://doi.org/10.1111/psyp.13073

Talmi, D., Seymour, B., Dayan, P., & Dolan, R. J. (2009). Human Pavlovian-instrumental transfer. *Journal of Neuroscience*, *28*(2), 360–368. https://doi.org/10.1523/JNEUROSCI.4028-07.2008

Tapia León, I., Kruse, O., Stalder, T., Stark, R., & Klucken, T. (2018). Neural correlates of participantive CS/UCS association in appetitive conditioning. *Human Brain Mapping*, *39*(4), 1637–1646. https://doi.org/10.1002/hbm.23940

Todorov, A., Olivola, C. Y., Dotsch, R., & Mende-Siedlecki, P. (2015). Social attributions from faces: Determinants, consequences, accuracy, and functional significance. *Annual Review of Psychology*, *66*, 519–545. https://doi.org/10.1146/annurev-psych-113011-143831

Tzovara, A., Korn, C. W., & Bach, D. R. (2018). Human Pavlovian fear conditioning conforms to probabilistic learning. *PLOS Computational Biology*, *14*(8), e1006243. https://doi.org/10.1371/journal.pcbi.1006243

van den Akker, K., Havermans, R. C., & Jansen, A. (2015). Effects of occasional reinforced trials during extinction on the reacquisition of conditioned responses to food cues. *Journal of Behavior Therapy and Experimental Psychiatry*, *48*, 50–58. https://doi.org/10.1016/j.jbtep.2015.02.001

van den Akker, K., Jansen, A., Frentz, F., & Havermans, R. C. (2013). Impulsivity makes more susceptible to overeating after contextual appetitive conditioning. *Appetite*, *70*, 73–80. https://doi.org/10.1016/j.appet.2013.06.092

van den Akker, K., Nederkoorn, C., & Jansen, A. (2017a). Electrodermal responses during appetitive conditioning are sensitive to contingency instruction ambiguity. *International Journal of Psychophysiology*, *118*, 40–47. https://doi.org/10.1016/j.ijpsycho.2017.06.004

van den Akker, K., Schyns, G., & Jansen, A. (2017b). Altered appetitive conditioning in overweight and obese women. *Behavior Research and Therapy*, *99*, 78–88. https://doi.org/10.1016/j.brat.2017.09.006

van der Wel, P., & van Steenbergen, H. (2018). Pupil dilation as an index of effort in cognitive control tasks: A review. *Psychonomic Bulletin and Review*, *25*(6), 2005–2015. https://doi.org/10.3758/s13423-018-1432-y

Varazzani, C., San-Galli, A., Gilardeau, S., & Bouret, S. (2015). Noradrenaline and dopamine neurons in the reward/effort trade-off: A direct electrophysiological comparison in behaving monkeys. *Journal of Neuroscience*, *35*(20), 7866–7877. https://doi.org/10.1523/JNEUROSCI.0454-15.2015

Wardle, M. C., Lopez-Gamundi, P., & Flagel, S. B. (2018). Measuring appetitive conditioned responses in humans. *Physiology and Behavior*, *188*, 140–150. https://doi.org/10.1016/j.physbeh.2018.02.004

Wilson, R. C., & Collins, A. G. E. (2019). Ten simple rules for the computational modeling of behavioral data. *PsyArXiv*. https://doi.org/10.31234/osf.io/46mbn

Wilson, R. C., & Niv, Y. (2012). Inferring relevance in a changing world. *Frontiers in Human Neuroscience*, *5*, 189. https://doi.org/10.3389/fnhum.2011.00189

Yu, A. J., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron*, *46*(4), 681–692. https://doi.org/10.1016/j.neuron.2005.04.026

SUPPORTING INFORMATION

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

Appendix 1
Tables S1, S2
Figures S1–S4

How to cite this article: Pietrock C, Ebrahimi C, Katthagen TM, et al. Pupil dilation as an implicit measure of appetitive Pavlovian learning. *Psychophysiology*. 2019;00:e13463. https://doi.org/10.1111/psyp.13463