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Love at first taste: Activation in reward-related brain regions during single-trial naturalistic appetitive conditioning in humans

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ABSTRACT

Palatable food can trigger appetitive responses, such as salivation and approach tendencies. Though evolutionarily functional, these conditioned responses can encourage overeating and obesity when food is abundant. The current study examines the neural correlates of 'denovo' Pavlovian appetitive conditioning, pairing one class of unknown objects (conditioned stimuli, CS) with their sweet taste (unconditioned stimulus, US) during a single trial. To do so, 23 participants consumed unknown (marzipan) objects of one particular color (CS+) while only interacting with control stimuli of different color and shape (CS-). After this single-trial conditioning procedure, participants viewed and rated images of the marzipan figures and the control objects during functional magnetic resonance imaging (fMRI). Relative to the CS-, the CS+ elicited stronger activation in the dorsal striatum, a brain region associated with cue-reward coupling. Furthermore, conditioning effects in subjective 'craving', defined as increased palatability and desire to eat, were observed, and these were positively related to conditioning effects in the amygdala, a brain region associated with the need-dependent value of a reward. Thus, the study identified reward-related brain regions involved in single-trial appetitive learning, thereby providing a potential mechanism that contributes to the etiology of food craving. These findings might help to understand clinically relevant food cravings in individuals with eating or weight related concerns and might support the development of extinction based treatments.

1. Introduction

Identifying energy-dense foods has historically been of paramount importance to survival. Sweet taste is often indicative of high energy, and it is thus unsurprising that humans are predisposed to develop a preference for sweet foods. However, the kinds of objects are associated with high energy density, is knowledge that needs to be acquired. One such learning mechanism is Pavlovian appetitive conditioning. In Pavlovian appetitive conditioning, an initially neutral stimulus (conditioned stimulus, CS) becomes associated with a biologically salient rewarding stimulus (unconditioned stimulus, US). After a few couplings, the CS alone is able to elicit appetitive responses (conditioned responses, CR), which often resemble the responses elicited by the US (unconditioned responses, UR). In the food context, these appetitive responses include preparatory and consummatory reactions to appetitive food-cues [1]. For example, the mere sight or smell of palatable food (i.e. CS) can initiate cephalic phase responses, such as salivation (i.e. CR), that prepare the gastrointestinal tract for the processing of ingested food (i.e. US) [2, 3]. Thus, after Pavlovian appetitive conditioning, food cues (e.g. sight or smell of the food) elicit appetitive responses (e.g. salivation). However, appetitive responses are not limited to the physiological domain, but extend to the psychological realm, where they influence eating behavior.

One of the psychological reactions that accompany cephalic phase responses is the experience of food craving [2, 4]. Food craving is an intense desire for a specific food [5], which can also be experienced in the absence of hunger [6] According to the conditioning-based incentive sensitization theory, food craving is a state of sensitized incentive salience, i.e. a cue-triggered motivation to consume a food [7]. A meta-analysis by Boswell and Kober [8] showed that cue-induced craving predicts both short term eating behavior and long term weight gain. Furthermore, craving induced by visual food cues had the same predictive effect on these long term outcomes as craving induced by real foods, demonstrating the influence visual food cues on consumption and health.

Visual food cues activate brain regions associated with reward. A meta-analysis of 14 fMRI studies, comparing the impact of visual food cues with the impact of neutral cues, showed reward-related effects of

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food images in the striatum, amygdala, insula, and orbitofrontal cortex (OFC) [9]. Activity in the OFC is thought to relate to the control of appetite, while activity in the mesolimbic regions of the reward system is thought to represent incentive salience of food cues and the inclination towards consumption behavior [10, 11]. Food cue induced activation in the ventral striatum depends on the nutritional composition of the depicted food, with stronger responses to high-calorie foods [12, 13], an effect that predicts subsequent consumption of high-calorie snacks [14, 15]. The dorsal striatum is involved in the formation of eating habits and food cue induced activation of this region predicts future weight gain and obesity [16, 17]. Amygdalar responsiveness to food cues depends on individual needs, with stronger activation during hunger [12, 18]. Finally, the insula (and adjacent operculum) is involved in primary gustatory processing, such as discrimination of experienced taste qualities [19]. Taken together, appetitive food images can be used to induce activation in reward-related brain structures.

However, by using appetitive food images as experimental stimuli, one can only draw indirect conclusions about the etiology of their incentive salience, because most food products known to participants (i.e., CSs) have already been consumed (i.e., US) before and thus have already been coupled multiple times throughout the lifespan. So-called *de-novo* conditioning setups, by contrast, try to reconstruct the original coupling process in a standardized context by pairing an *unknown* and arbitrary CS (e.g., geometric shape) with a primary food reward (US, e.g., delivery of a milkshake). Such setups have shown that incentive salience can be transferred from the rewarding US to the arbitrary CS, as indicated by increased liking [20, 21] and craving [22–24]. On the neural level, reward-related responses in the striatum also transfers from the US to the CS, a process called temporal difference learning [25–27]. Furthermore, reward-related responses in the amygdala correlated with incentive salience, indicated by the strength of pavlovian-to-instrumental transfer [28]. Thus, after appetitive de-novo conditioning, the arbitrary and abstract CS elicits behavioral and neural responses similar to those elicited by real life food images [29, 30].

The most direct and naturalistic way to induce appetitive conditioning, however, is to couple one feature of an object (e.g. its visual appearance), with another feature of that very same object (e.g. its taste), a learning paradigm also termed “object learning” [31]. Compared to classical denovo-conditioning procedures with arbitrary CS that are unrelated to the US, naturalistic conditioning procedures afford higher ecological validity, because in appetitive conditioning outside the lab CS and US are often related. In animal research, naturalistic “object learning” has led to particularly rapid and robust learning effects [31]. In humans, the coupling of the flavor of a drink (i.e. the CS) with its calorie content (i.e. the US) led to stronger liking of that drink. This increase in incentive salience correlated with increased insular responses to that drink [32]. In this tradition of linking CS and US within the same food, we developed a single-trial de-novo appetitive conditioning paradigm. During the acquisition phase, participants viewed unknown objects made of marzipan. When eating a small part of them, participants learned that they were edible and sweet. This single coupling of gustatory (i.e., US) and visual features (i.e., CS) of the objects was sufficient to generate conditioning effects of both subjective pleasantness ratings and electroencephalographic (EEG) responses in our previous study on this paradigm [Blechert et al., 2016 33]. Specifically, appetitive conditioning changed electrocortical responses in early (N1) and late (LPP) event related potentials (ERPs). The decreased N1 may represent changes in early attentional processes, such as efficient object perception while the increased LPP may point to changes in late motivational processes, such as emotional appraisal of the craved object [33].

While EEG allowed us to map the cognitive processing stream from attentional to motivational processes, it does not speak to the precise neural source regions in deeper brain structures such as the mesolimbic brain. Thus, the aim of the current study was to identify the neural changes that potentially underlie such rapid and efficient Pavlovian appetitive conditioning processes. To achieve this, we used functional magnetic resonance imaging (fMRI) to capture mesolimbic responses after a single trial of Pavlovian appetitive conditioning. After conditioning, we expected that images of conditioned marzipan objects (i.e., CS+), compared to images of inedible control objects (i.e., CS-) should trigger stronger activity in the amygdala, insula, caudate, nucleus accumbens (NAcc), and OFC, regions identified by the meta-analysis of [9]. We also aimed to replicate the conditioning effect on ‘desire-to-eat’ and ‘palatability’ ratings, and we expected to find that higher ‘palatability’ and ‘desire-to-eat’ ratings relate to stronger neural effects of conditioning.

2. Methods

2.1. Participants

Twenty-seven undergraduate students of the University of Salzburg, Austria, participated in exchange for course credit or € 20. To be included, participants had to indicate on a 5-point scale that they “like” (4) or “really like” (5) marzipan. Further inclusion criteria were right-handedness and the absence of neurological diseases or current psychological disorders. FMRI-related exclusion criteria were metal implants, contraceptive coils, and claustrophobia. Four participants were excluded from further analysis due to technical problems. Complete datasets were available for 23 participants (n = 11 women) aged 23.5 (SD = 3.48) with a BMI of 22.1 (SD = 2.81).

Sample size was determined based on the large conditioning effects on pleasantness ratings (η_p^2 = .291), and LPP-amplitude (η^2 = .326) in our previous EEG-study [33]. By using G*Power 3.1 (t-test for two dependent means) [34], the parameters were set as follows: effect size d = 0.80 (large effect), alpha level = 0.05, power = 0.90. The calculation indicated a minimum sample size of 15. We recruited more participants than the power analysis suggested to perform our fMRI-analysis.

To characterize the sample, we used the German versions of the Food Craving Questionnaires, FCQ-T and FCQ-S [35] to assess trait food craving (Mean = 104, SD = 25.9) and state food craving (Mean = 37.7, SD = 9.96). Furthermore, we used the Dutch Eating Behavior Questionnaire, DEBQ [36], to assess emotional eating (Mean = 22.1, SD = 7.82), external eating (Mean = 35.0, SD = 5.13), and restrained eating (Mean = 25.5, SD = 10.9). Finally, we used the Hunger Scale [37], to assess current state of hunger (Mean = 5.95, SD = 1.38), which indicated moderate hunger across subjects.

The study was approved by the university's ethics committee and participants signed an informed consent form before participating.

2.2. Stimulus material

Production of the US objects. The edible stimulus set comprised six different geometric objects made out of marzipan by a Salzburg confectioner. Each marzipan object was produced in two colors (yellow and coral red). The inedible control set were made from the same objects, but the marzipan was dried and coated to look and smell like plastic (see Fig. 1).

Production of the CS images. To generate CS images, the marzipan objects were photographed with a Canon IXUS 9015 camera (resolution 3648 × 2736 pixels). The images were given a transparent background and resized to 600 × 450 pixels.

Counterbalancing. Color and shapes were counterbalanced in four different combinations of edible (CS+) and inedible (CS-) objects. Each participant was presented with six objects of six different shapes, in two different colors. The three shapes of the one color (e.g. yellow) served as CS+, while the three shapes of the other color (e.g. red) served as CS- (see Fig. 1).

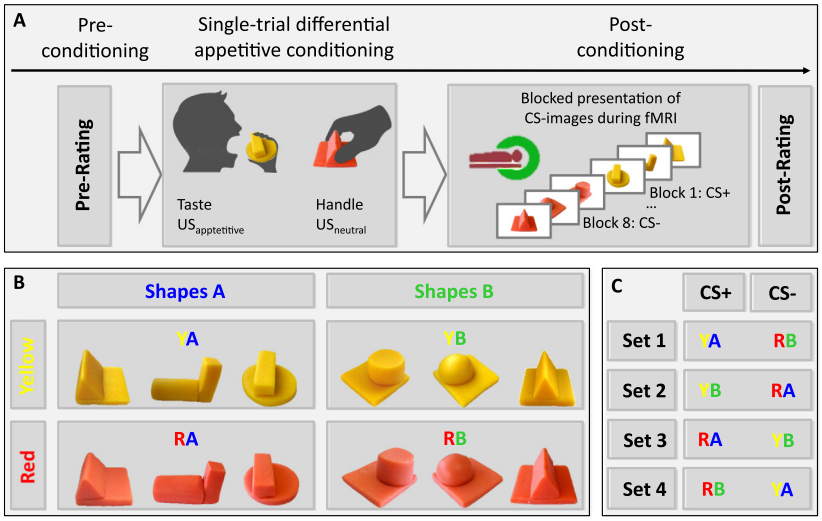

Fig. 1. (A) Experimental design (B) Stimulus material of six different shapes in two colors, (C) counterbalanced to eliminate confounding conditioning effects of color and shape.

2.3. Procedure

Participants were asked to abstain from eating for about 3 h prior to the experimental session. Participants signed the informed consent form and completed the questionnaires on eating behavior and current hunger. They also completed an eating protocol to confirm compliance with the instructed food restriction. One participant had his last meal two and a half hours before the session, but was not excluded because his hunger rating of 6.5 was comparable to the rest of the sample. Prior to the present task, and unrelated to the present study, all participants completed another MRI study involving social stimuli [38], and ten participants additionally saw blocks of food images and blocks with tool images. Afterwards, participants started with the present experiment, including pre-rating, conditioning procedure, fMRI-session and post-rating. All participants were exposed to the same conditions and all comparisons were made within subjects.

Pre-scanning rating in the scanner. Participants rated the images of the three yellow and three red objects on a 9-point visual-analog-scale on 'palatability' ("How appetizing is this object?", from 'not appetizing' to 'very appetizing') and 'desire-to-eat' ("How much would you like to eat this object now?", from 'not much' to 'very much').

Conditioning procedure outside the scanner. The six objects, that had just been rated, were served on a plate. Participants were told which color indicates that an object is made of marzipan. The marzipan objects had to be smelled and tasted. Stimuli of this color represented the CS+ condition. The three inedible control objects of the other color had to be smelled and touched. Images of this color represented the CS- condition. The inspection/consumption of the CSs took about five minutes.

Image viewing in the scanner. Participants were then placed in the scanner. This was followed by blocked passive picture viewing, with eight blocks in total. Four blocks consisted of images from the CS+ condition, the other four blocks consisted of images from the CS- condition. Block order was counterbalanced across participants. Each block consisted of six pictures, hence each image of a specific object was presented twice. Picture order within one block was pseudo-randomized. Each image was presented for 2 seconds, with an inter-stimulus-interval of 1 second. Between blocks a break of 20 seconds was indicated by a fixation cross. The passive picture viewing task was completed after 48 pictures, with a total run time of 4.7 minutes.

Post-scanning rating in the scanner. Finally, participants were asked to rate the six objects again for 'palatability' and desire 'desire-to-eat', under the same conditions as prior to scanning (see Fig. 1).

2.4. Behavioral data analysis

Due to high correlations between 'palatability' and 'desire-to-eat' ratings (CS+ pre: $r = 0.94$, $p < .001$; CS+ post: $r = 0.91$, $p < .001$; CS- pre: $r = 0.86$, $p < .001$; CS- post: $r = 0.90$, $p < .001$), the two scales were merged to one 'craving' rating. To test whether the single-trial conditioning procedure achieved the desired Pavlovian conditioning effect, 'craving' ratings were submitted to a Stimulus type (CS + vs. CS-) by Time (pre vs. post conditioning) repeated measures analysis of variance (rmANOVA), with a significance level of $p < .05$. Significant interactions were followed by post-hoc *t*-tests for repeated measures to specify conditioning effects, with a significance level of $p < .05$. (Figure 2)

An individual 'craving' score was computed for each participant by calculating the difference in 'craving' ratings of CS+ and CS- post conditioning. This score was used as a covariate in the analysis of imaging data.

2.5. Neural data analysis

MRI data were acquired on a 3 T system (Siemens Magnetom Trio Tim Syngo) with a 12-channel head coil. Functional images representing blood oxygenation level (BOLD) contrast were acquired with a T2* weighted echo-planar imaging (EPI) sequence (echo-time: 30 ms, repetition-time: 2250 ms, flip angle: 70°, slice thickness: 3.0 mm, field of view: 64 × 64 matrix with in plane resolution: 3.44 × 3.44 mm). Magnetization-prepared rapid-acquisition T1 weighted gradient echo

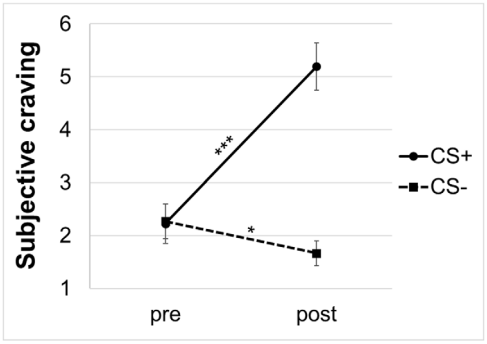

Fig. 2. Pre-conditioning and post-conditioning 'craving' ratings of eatable (CS +) and uneatable (CS-) objects (mean, standard error). 'Craving' ratings were aggregated from 'desire to eat' and 'palatability' ratings. *** p < .001 * p < .05

(MPRAGE) structural images (voxel size of 1 × 1 × 1.2 mm) were acquired for co-registration.

Data was pre-processed and analyzed using SPM12 (Wellcome Department of Cognitive Neurology, London, UK). Functional images were slice time corrected to the onset of the middle slice and co-registered to the high-resolution structural image. Functional images were spatially normalized to Montreal Neurological Institute space using the normalization parameters obtained from the segmentation procedure and subsequently smoothed with a Gaussian kernel of 8 mm full-width at half-maximum.

Voxel-based statistics were computed with a two stage mixed effects model. In the subject-specific first level, each block was convolved with a canonical hemodynamic response function and its first temporal derivative. Realignment parameters were included as nuisance regressors to minimize residual variance caused by head movements. The functional data were high-pass filtered with a cutoff of 128 seconds. Based on this general linear model, parameter estimates were calculated and used to build a contrast between conditions (CS+ > CS-).

In the group-related second level, the subject-specific contrasts were entered into a one-sample *t*-test. Additionally, to probe for effects of 'palatability' and 'desire-to-eat', the composite 'craving' score was included as a covariate to the second level analysis in SPM. For exploratory purposes, a whole brain analysis was conducted, with significance threshold at $p < .001$, uncorrected, and with a minimum cluster size of $k > 5$. A-priori hypothesized regions of interest were used for small volume correction (SVC), which allows for a more focal examination of identified neural areas. For this purpose, AAL masks of the WFU PickAtlas [39] were applied, with significance threshold at $p < .05$, family-wise error (FWE)-corrected at cluster-level. Additionally, results were compared with previous studies justifying our hypotheses, using a sphere (r = 10 mm) centered on the local maximum from previous findings ($p < .05$, cluster-level FWE-corrected).

3. Results

3.1. Behavioral results

The single-trial conditioning procedure resulted in the expected learning effect on individual craving (aggregated 'desire to eat' and 'palatability' rating). Main effects of Stimulus type ($F_{(1, 22)} = 33.4$, $p < .001$, $\eta_p^2 = .603$) and Time ($F_{(1, 22)} = 12.9$, $p = .002$, $\eta_p^2 = .369$) were significant, as well as the interaction between these factors ($F_{(1, 22)} = 28.9$, $p < .001$, $\eta_p^2 = .568$). The appetitive conditioning procedure evoked higher 'craving' ratings for the CS+ ($t_{(22)} = -4.90$, $p < .001$, $d = 1.01$) but lower 'craving' ratings for the CS- ($t_{(22)} = 2.26$, $p = .034$, $d = 0.57$). The low pre-conditioning ratings confirmed that the objects were constructed in a way that they were not previously associated with food (Fig. 2).

3.2. Neural results

The single-trial appetitive conditioning procedure induced effects represented on a neural level. For the whole brain, the *t*-contrast (CS+ > CS-) was significant ($p < .001$ uncorrected) in three clusters ($k > 5$), located in the left caudate (extending to the putamen) and superior and middle frontal gyri (Table 1). Based on the WFU PickAtlas, SVC was applied to the left caudate, which was activated more strongly by the CS + than by the CS- ($p_{svc} = .041$, cluster-level FWE-corrected) (Fig. 3). This finding is comparable with a study by Stoeckel, Weller [17] ($p_{svc} = .027$, cluster-level FWE-corrected). The authors identified the left caudate (peak [x, y, z]: −14, 8, 22) to be more strongly activated by high-calorie foods compared to low-calorie foods.

By including the individual 'cravings' scores as covariate, an interaction right amygdalar activation was found as the only significant cluster ($k > 5$) on whole brain level ($p < .001$ uncorrected). SVC confirmed that the conditioning effect in the right amygdala was stronger in individuals scoring high on 'craving' ($p_{svc} = .020$, cluster-level FWE-corrected) (Fig. 4). This finding is comparable with a study by Fuhrer, Zysset [40] ($p_{svc} = .023$, cluster-level FWE-corrected). The authors identified the right amygdala (peak [converted to MNI x, y, z]: 17, -6, -29) to be more strongly activated by food compared to non-food images, in hunger but not in satiation.

Table 1
Results from the whole brain analysis CS+ > CS-, and the positive covariation of the same contrast with craving (statistical threshold: p < 0.001 (uncorrected), k > 5 voxels).

Contrast	Brain Area	Voxels	MNI [x, y, z]	T_{max}
CS+ > CS-				
	L Caudate	13	-21, -1, 16	4.69
	L Superior frontal gyrus	7	-18, 32, 37	4.13
	L Middle frontal gyrus	6	-36, 47, 10	4.06
CS+ > CS- × craving				
	R Amygdala	6	21, -4, -20	4.12

4. Discussion

For the present study, we adapted a naturalistic single-trial Pavlovian appetitive conditioning procedure from our previous EEG study [33] to determine appetitive conditioning effects in reward-related brain structures. The conditioning procedure comprised tasting of unknown objects made of marzipan and touching of inedible control objects. This single coupling of the visual appearance of the marzipan object (i.e., CS) with its sweet taste (i.e., US) was sufficient for appetitive conditioning effects on the behavioral and neural level. From pre- to post-conditioning, ratings of 'palatability' and 'desire-to-eat' increased for images of the marzipan figures (i.e., CS+), but not for images of the inedible objects (i.e., CS-), precisely replicating our prior study with this task [33]. Neuraly, the left caudate (extending to the putamen) was activated more strongly by the CS+ images than by the CS- images. Furthermore, the right amygdala responded more strongly to the CS+ compared to the CS- in individuals with stronger conditioning effects on subjective craving.

4.1. Dorsal striatum and single trial cue-reward-coupling

The current dorsostriatal conditioning effect, represented by a stronger activation of the left dorsal caudate and putamen by marzipan images (i.e., CS+) than by control images (i.e., CS-), is in line with several previous studies. Traditionally, the dorsal striatum has been associated with goal-directed behavior and habit formation [41]. However, dorsostriatal responses to food cues have also been found during passive picture viewing, i.e. without any required behavioral response [9]. The dorsal striatum is more strongly activated, when the viewed foods are high-caloric [17], as well as when the foods are made directly available during scanning [42]. Furthermore, the caudate responds more strongly in obese individuals, an effect that might be explained by stronger input from the amygdala and dysfunctional cortical control [16, 17, 43]. Food-cue induced activation in the dorsal striatum may thus represent the identification of food-rewards, as an early process in the formation of eating habits [16].

Appetitive de-novo conditioning studies indicate that the dorsal striatum is involved in reward learning: Burger and Stice [29] coupled images of abstract fractals (CS+) with delivery of milkshake (US), and found cue-induced activation in the caudate, which increased with the number of cue-reward couplings. After the cue-reward association has been consolidated, the dorsal striatum can be activated during prediction errors, that is, when the anticipated reward was not delivered as expected [25, 26]. These dorsostriatal responses during appetitive conditioning dovetails with findings on striatal dopamine (DA) in the regulation of eating behavior [44]. Low-affinity D1 receptors determine

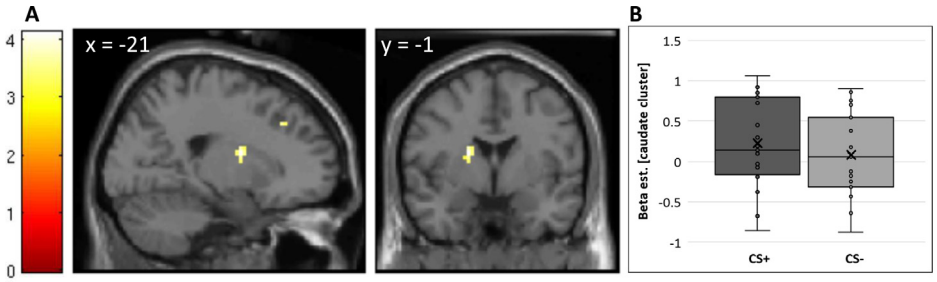

Fig. 3. (A) Results of the CS+ > CS- contrast ($p < .001$ (uncorrected), $k > 5$ voxels). (B) Average cluster parameter estimates of the caudate cluster, under CS+ and CS- conditions.

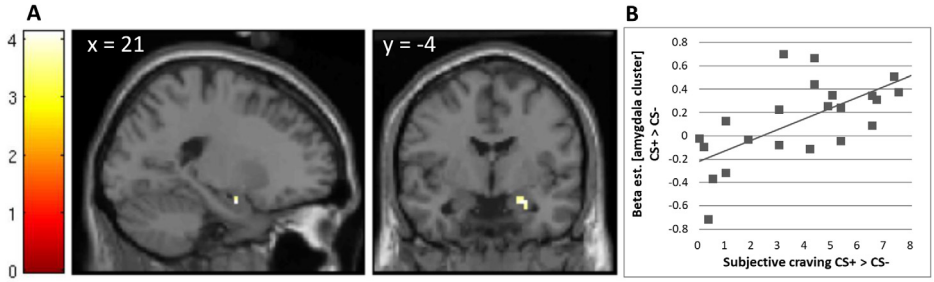

Fig. 4. (A) Results of the CS+ > CS- contrast with individual 'craving' scores as covariate, ($p < .001$ (uncorrected), $k > 5$ voxels). (B) Correlation between individual 'craving' scores and average cluster parameter estimates of the contrast, derived from amygdala cluster ($r = .669$, $p < .001$)

phasic responses triggered by reinforcing stimuli. These cue-induced phasic responses might represent the strengthening of memory traces in the consolidation of reinforcement, as they become stronger with the number of cue-reward couplings [45]. The present dorsostriatal response may therefore represent how strongly the CS+ (i.e. visual appearance) and its rewarding properties (i.e. sweet taste or energy intake) are coupled. This coupling may be an early process in the attribution of incentive salience and the formation of habits.

Alternatively, the current findings may relate to striatal high-affinity D2 receptors. These receptors determine dopaminergic background tone and represent metabolic need, which seems critical for hunger and satiety, as well as for compulsive food intake [46, 47]. In this regard, the current dorsostriatal findings may represent higher metabolically driven eating motivation; however, there was no relationship between the dorsostriatal conditioning effect and individual 'craving' ratings, like we found in the amygdala. Therefore, it seems more plausible that the present dorsostriatal effect is related to the consolidation of cue-reward-coupling, independent of whether the reward is appealing to the participant at that time.

4.2. Amygdala and individual differences in incentive salience

By modeling 'craving' ratings as a covariate, we found a positive relationship between individual craving and conditioned activation of the right amygdala. Traditionally, the amygdala is seen as a pivotal hub for fear conditioning (reviewed by Rosen [48]). However, a growing body of research has identified the amygdala as also being central for appetitive conditioning and food choices [9, 49–51]. Remarkably, the responsiveness of the amygdala has been found to depend on the current biological significance of the reward. Food-deprived participants showed increased cue-induced activation in the amygdala, compared to satiated participants [12, 40, 52]. The amygdala also responds more strongly to food cues when palatable food is in the attentional focus [18] or is immediately available [42]. Beaver, Lawrence [53] showed that amygdalar responsivity correlates positively with trait reward sensitivity, which may explain individual differences in vulnerability to intense food craving and overeating. Amygdalar responses to appetizing food images predicted choices for high-caloric foods [15], and this amygdalar impact on food choices is stronger in obese people [17, 43]. Taken together, the amygdala seems to be a critical structure in the individual, need-dependent evaluation of a current food-reward.

The amygdala, and in particular its basolateral nucleus (BLA), integrates the sensory properties of a CS with the current value of the rewarding US, ensuring that whenever the value of the reward increases, so does the incentive-salience of the CS [51]. In animals, BLA lesions did not affect acquisition of conditioned responses (S-R associations), but they did prevent encoding of detailed need-dependent outcome representations (S-O association), like outcome devaluation after selective satiation [54]. In humans, resection of the anterior temporal lobe (including the amygdala) was found to impair preference conditioning: patients did not prefer the CS coupled most often with reward, as normal control participants did [55]. This suggests that the amygdala represents individual differences in need-dependent incentive salience.

The current findings complement the results of our previous EEG-study, at which we used the same conditioning paradigm [33]. The conditioning effects on the LPP-amplitude (previous study), and on amygdalar activation (present study) both covaried with individual differences in craving (state cravings in the previous study, image ratings in the present study,). That the LPP and amygdalar response are linked is in line with a correlation of LPP-amplitude and amygdalar responsiveness to pleasant images findings during simultaneous EEG and fMRI recordings [56]. Thus, both brain responses may represent the sensitivity of evaluative response systems, which might explain why some individuals are more susceptible to develop intense cravings than others. However, EEG source modelling studies or representational similarity analysis [57] might give more insight in the spatio-temporal processing cascade here.

4.3. Limitations and future directions

Our single-trial conditioning paradigm is designed to capture only the first traces of appetitive conditioning, and thus represents a 'proof of principle' study. The current conditioning effect on palatability and desire can be seen as just a first step in the association of craving to the unknown objects. Yet, by showing these, we provide evidence for

conditioning based etiological accounts of food craving [58]. However, real-life conditioning usually involves more frequent couplings and probably more brain regions, such as the insula, NAcc or OFC [9]. Hence, it is worth investigating whether multiple couplings of a de-novo stimulus with a reward (e.g. repeated consumption at home) have a conditioning effect on these brain regions as well [32]. Future studies might also include additional outcome measures like cephalic phase responses (e.g. salivation) or consummatory behavior [59]. Incentive salience, as acquired here, might impact implicit approach behaviors or attentional biases [60]. Our previous study showed that conditioning affected early, attentional potentials in addition to later, appraisal related potentials [33]. A more applied future direction would be to study how extinction training affect condition responding, to link into the recent literature of novel treatments for craving related conditions [61].

5. Conclusion

In conclusion, the current study showed that a single appetitive conditioning trial was sufficient to induce reliable neural and behavioral conditioning effects, lending credibility to the conditioning account of craving and the concept of incentive salience. Our ecological perspective on “object learning” [31] and the application of more naturalistic conditioning paradigms may inspire future clinical research to investigate maladaptive appetitive conditioning processes in eating disorders and obesity.

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