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# The Pavlovian craver: Neural and experiential correlates of single trial naturalistic food conditioning in humans

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## HIGHLIGHTS

- A new single-trial Pavlovian appetitive conditioning paradigm is presented.
- Appetitive conditioning modulated early and late neural responding.
- Pavlovian appetitive conditioning can occur after a single conditioning episode.
- Appetitive conditioning may contribute to food craving and binge eating.

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## ABSTRACT

Present-day environments are replete with tempting foods and the current obesity pandemic speaks to humans' inability to adjust to this. Pavlovian processes may be fundamental to such hedonic overeating. However, a lack of naturalistic Pavlovian paradigms in humans makes translational research difficult and important parameters such as implicitness and acquisition speed are unknown. Here we present a novel naturalistic conditioning task: an image of a neutral object was conditioned to marzipan taste in a single trial procedure by asking the participant to eat the 'object' (made from marzipan). Relative to control objects, results demonstrate robust pre- to post-conditioning changes of both subjective ratings and early as well as late event related brain potentials, suggesting contributions of implicit (attentional) and explicit (motivational) processes. Naturalistic single-trial taste-appetitive conditioning is potent in humans and shapes attentional and motivational neural processes that might challenge self-regulation during exposure to tempting foods. Thus, appetitive conditioning processes might contribute to overweight and obesity.



## 1. Introduction

Humans like to think of themselves as acting deliberately and in a reflected manner whereas non-human species are thought to obey to simple learning rules. However, evidence from many fields of psychology suggest the contrary [1,2]; in fact, Pavlovian rules might apply to many human behaviors just as well. One of the areas in which Pavlovian processes ensure efficiency and automaticity is in the reward system where procurement, detection, selection and consumption of energy rich foods is coordinated and controlled [3]. In today's food environments, characterized by an all-time availability of palatable and energy dense foods, such efficiency contributes to excessive consumption and weight gain, thereby creating one of the largest challenges for today's health care systems.

Exposure to food cues (e.g. sight, smell, taste of foods, conditioned stimulus, CS) – conditioned to the delayed effects of ingestion (energy and nutrient availability, dopamine release, unconditioned stimulus, US) can elicit cephalic phase responses – preparatory neural-appetitive responses such as salience attribution, salivation and gastric secretion – along with the intense urge to consume a specific food (conditioned response, CR) [4–6]. By analogy to drug addiction such intense desires for food in the absence of hunger have been termed *food-cravings* which are highly prevalent in both healthy individuals [7] and those with eating disorders [8,9] and which threaten diet adherence in weight loss programs [10]. However, despite much research on the

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nature and origin of such cravings, only recently have experimental paradigms become available that allow the naturalistic study of appetitive conditioning in humans.

### 1.1. Neural and behavioral responses during appetitive conditioning

A number of recent studies on Pavlovian appetitive learning in humans have documented that cognitive and evaluative subjective responses can readily be conditioned to stimuli predicting a food-US through multiple trials of CS–US pairing [11–15]. However, conditioning is partially implicit in humans and evidence from non-self-report based measures of incentive salience and cephalic phase responses are rare [16–18]. Neuroimaging studies have helped to delineate neural mediators of appetitive conditioning: neutral images served as CSs that predicted an injection of a sweet beverage into the mouth (US). A network of amygdala, orbitofrontal cortex, striatum and anterior cingulate cortex supports appetitive conditioning in humans [19], partially in line with a similar network in mice [4] and the speed of appetitive conditioning in the striatum in this task predicts weight change [20].

But how fast and – by implication – implicit is such a process, assumed to be translational across species? Associative mechanisms can operate in the absence of awareness of CS/US contingencies (e.g. in animals) and thus a rather implicit measure in human conditioning would be helpful. Event related potentials (ERPs) track brain activity with excellent temporal resolution. It is generally assumed that early ERPs (P1, N1) are rather implicit indices of early selective attention, object recognition and categorization [21–23]. Early ERPs have also been linked to an automatic detection of caloric density in images of food items [24,25]. Late components such as the P3 or the late positive potential (LPP), on the other hand, index sustained attention to motivational aspects of the stimulus and meaning evaluation [22,26–28]. These late components seem to be partially under conscious control, as suggested by studies on emotion regulation and craving regulation [29–31]. A recent study further documented appetitive conditioning effects on mid-latency (P2) and late (P3) ERPs [32]. Thus, ERPs are sensitive to conditioning-mediated changes and speak to the level of implicitness of such processes.

### 1.2. Appetitive conditioning in a single trial? The present study

But how efficient is such an appetitive conditioning process, i.e. how fast is differential conditioning acquired? Franken et al. [32] presented 100 trials during which a neutral CS-picture was paired with the injection of a sweet solution in the mouth. Burger and Stice [20] presented 16 pairings of the CS+ image with sweet solution. By contrast, single trial food conditioning has so far only been demonstrated in animals [33,34] and would represent a particularly powerful demonstration of the relevance and efficiency of appetitive conditioning if replicated in humans. Thus, the present study developed a new appetitive conditioning procedure that involved a single 'pairing' of CS and US: colored geometric objects of unfamiliar shape made from marzipan were consumed by participants as $US_{appetitive}$ whereas images of these objects represented the CS+. Thus, we tried to model a naturalistic sequence where a food is perceived (at some distance, visual image = CS), later handled, and then eaten (taste, digestion = $US_{appetitive}$). By using unfamiliar shapes we avoided tapping into pre-existing associations (that most food images have) and ensured neutrality of the CS. Non-edible, otherwise comparable objects served as control objects and their images represented the CS−. Note that this procedure 'merges' CS and US (same object), only the modalities (seeing vs. eating, i.e. visual vs. gustatory modality) distinguish CS and US. This paradigm follows literature showing that naturalistic conditioning protocols using CSs that resemble USs in some aspect produce stronger conditioning [35] and thus might increase chances of successful single trial conditioning. Drawing on the literature reviewed above, single trial appetitive conditioning was predicted to increase late ERPs as well as pleasantness ratings. Furthermore, early ERPs (N1) were assessed as indication of particularly early and possibly implicit conditioning stages, along with changes in state craving for sweet foods before and after conditioning.

## 2. Materials and method

### 2.1. Participants

Twenty-three healthy female psychology students (*Mean Age* = 23.3, *SD* = 3.37 years) of the University of Salzburg, Austria, participated in exchange for course credit or € 10 after providing informed consent, as approved by the ethics committee of the University of Salzburg. Sample size was determined based on previous ERP studies in the food context [36,37]. Since marzipan was used as the appetitive unconditioned stimulus ($US_{appetitive}$), an inclusion criterion was liking marzipan more than 2 on a 0–4 point Likert-type scale (anchor: "not at all", "very much"). To disguise the actual purpose of the study, the marzipan item was included among a larger number of food-preference items in an online questionnaire filled out at least one day before the laboratory session. Only female participants were included since women are more affected by food craving than men [38]. Self-report based exclusion criteria were cardiovascular or neurological diseases, diabetes, psychiatric disorders or acute mental stress, regular use of medication other than contraceptives, age <18 or >30 years, and body mass indices (BMI) outside of the healthy range (<17 or ≥30 kg/m$^2$).

### 2.2. Psychometric measures

Food craving was measured by the German short-form of the Food Craving Questionnaire, State version [FCQ-S: 39, 40], composed of 15 items on a 5-point Likert scale (anchor; "strongly agree", "strongly disagree"). Since we used marzipan taste as the US stimulus, the statements in the questionnaire specifically referred to "*something sweet*". The FCQ-S has excellent psychometric proprieties [39] as confirmed in the present sample (FCQ-S: Pre-conditioning $\alpha = 0.947$; Post-conditioning $\alpha = 0.949$).

### 2.3. Procedure and conditioning task

Participants were instructed to abstain from eating at least three hours prior to testing to reduce between-participant variance in food deprivation and to simulate a typical meal time homeostatic state. Laboratory testing commenced with the signing of the informed consent form. Then weight and height measures were collected (BMI: *M* = 21.5, *SD* = 3.02). Participants were then seated on a comfortable chair in a light- and sound-attenuated room, and EEG electrodes were attached. To avoid explicit associations with marzipan or other types of food belonging to one of the stimulus classes prior to conditioning, instructions emphasized that the aim of the study was on object perception. For the same reason pre- and post-conditioning pleasantness ratings were collected for the stimuli and not wanting or liking appraisals.

Participants then performed the conditioning task, comprised of two picture viewing blocks (pre-conditioning, post-conditioning) with an acquisition phase in between. An *E*-Prime program (Psychology Software Tools, Sharpsburg, PA, USA) presented pictures of geometric objects centrally on a 23″ monitor (120 Hz) at ~0.5 m viewing distance and was used to obtain ratings. Three of these objects were made from fresh, high quality marzipan by a local confectioner in two different colors. Three similar objects were also made from marzipan (in the same two colors) but were dried and finished with clear coat that sealed and hardened the surface and prevented emission of odors, giving it the look and feel of a plastic form. Thus, the color-marzipan assignment could be counterbalanced across participants (see Fig. 1): in one half of the participants, images of yellow objects were used as CS+ and resembled edible marzipan objects (images of red objects being