Physiology & Behavior 164 (2016) 407–416

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Higher-order conditioning of taste-odor learning in rats: Evidence for the association between emotional aspects of gustatory information and olfactory information

Takuya Onuma [a,b], Nobuyuki Sakai [a,*]

[a] Department of Psychology, Graduate School of Arts and Letters, Tohoku University, Kawauchi 27-1, Aoba-ku, Sendai 980-8576, Japan
[b] Division for Interdisciplinary Advanced Research and Education, Tohoku University, Aramaki aza Aoba 6-3, Aoba-ku, Sendai 980-8578, Japan

HIGHLIGHTS

- Associative structure of taste-odor learning was examined in rats.
- Second-order conditioning of taste-odor learning was acquired.
- Alternatively, sensory preconditioning of the learning was not.
- Odors may be mainly associated with the emotion evoked by tastes.

ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 11 June 2016
Accepted 18 June 2016
Available online 21 June 2016

Keywords:
Taste-odor learning
Higher-order conditioning
Association

ABSTRACT

Previous studies have shown that rats prefer an odor paired with saccharin solution to an odor paired with quinine solution (taste-odor learning). However, it remains unclear whether the odors are associated with the emotional (i.e., positive and/or negative hedonics) or qualitative (i.e., sweetness and/or bitterness) aspects of gustatory information. This study aimed to examine this question using higher-order conditioning paradigms: second-order conditioning (SOC) and sensory preconditioning (SPC). Adult Wistar rats were divided into SOC and SPC groups. Food flavors, purchased from a Japanese market, such as melon (0.05%), lemon (0.1%), vanilla (0.1%), and almond (0.1%), were randomly used as odors A, B, C, and D for each rat. The SOC group was exposed to 0.005 M saccharin solutions with odor A and 0.02 M quinine solutions with odor C in the first 5 days of learning. Additionally, they were exposed to water with a mixture of odors A and B, and water with a mixture of odors C and D in the next 5 days of learning. The order of these two learning sessions was reversed in the SPC group. We hypothesized that if odor was associated with the emotional, or qualitative, aspects of gustatory information, the SOC, or SPC groups, respectively, would prefer odor B to odor D. Our results showed that the SOC group preferred odor B to odor D, whereas the SPC group did not show any such preference. This suggests that odors may be primarily associated with emotion evoked by gustation in taste-odor learning.

1. Introduction

We often perceive an odor, such as vanilla or lemon, as 'sweet' or 'sour' smelling, respectively. Nevertheless, gustation and olfaction are discrete perceptual systems. Taste-odor synesthesia—perception of an odor as having some taste-like property—is thought to be acquired and modulated by daily food experience, and consequently has been described as "learned synesthesia" [1,2]. For instance, Stevenson, Prescott, and Boakes [3] showed that after repeated pairing of the sweet taste of sucrose with unfamiliar odors such as lychee or water chestnut, these odors were judged as smelling sweeter. This perceptual change of odor is thought to be based on classical conditioning [2]. First, when an unfamiliar odor (e.g., lychee)—a conditioned stimulus (CS)—is paired with a taste (e.g., sucrose)—an unconditioned stimulus (US)—association between these two stimuli (CS-US) is acquired. Once this association has been acquired, the odor (CS) always activates the representation of the taste (US), and thus, the lychee odor is

* Corresponding author at: Department of Psychology, Graduate School of Arts and Letters, Tohoku University, Kawauchi 27-1, Sendai, Miyagi 980-8576, Japan.
E-mail addresses: t.onuma@dc.tohoku.ac.jp (T. Onuma), nob_sakai@m.tohoku.ac.jp (N. Sakai).

http://dx.doi.org/10.1016/j.physbeh.2016.06.025

perceived as smelling sweet. Similar results were obtained in subsequent studies using human participants [4], with taste-odor associations thought to be more robust than others, such as taste-color associations [5,6].

Although researchers carefully select odor stimuli, which are unfamiliar to most of their participants, humans have such diverse histories with their own food experiences that the effects of these variables in the experiments cannot be controlled. Therefore, animal studies are needed to better elucidate and understand the mechanism of taste-odor learning. For instance, Fanselow and Birk [7] showed that rats acquired a preference for an odor that had been paired with saccharin solution, and an avoidance of another odor that had been paired with quinine solution. This result appeared to suggest that the rats acquired an association between the odor (CS) and sweetness (or bitterness) of the taste (US). However, it is possible that positive or negative hedonics, and not just sweet or bitter taste quality, could elicit the same preference or avoidance behavior [2,8]. To elucidate this point, taste-odor learning was tested with brain-lesioned rats [9]. The results showed that rats with lesions in the amygdala, a region involved in processing emotional aspects of gustatory information [10], showed rapid extinction of the preference for the saccharin-associated odor. On the other hand, rats with lesions in the insular cortex, a region involved in processing qualitative aspects of gustatory information [11,12], showed normal acquisition of the preference. This result suggested that the rats mainly acquired an association between olfactory information and the emotional aspects (i.e., positive and/or negative hedonics) but not the qualitative aspects (i.e., sweetness and/or bitterness) of gustatory information.

To further elucidate this finding, we introduce higher-order conditioning paradigms as non-invasive tools that investigate processes involved in taste-odor associative learning and memory [13]. Higher-order conditioning paradigms consist of second-order conditioning (SOC) and sensory preconditioning (SPC), whereby a CS (CS2) acquires the ability to elicit a conditioned response (CR) by being paired with another CS (CS1), rather than being directly paired with a US (Table 1). Pairing of CS1 and the US is followed by pairing of CS2 and CS1 in SOC, whereas the order of pairing is reversed in SPC. In any case, CS2 acquires the ability to elicit a CR even though it is never directly paired with the US.

It is suggested that there are critical differences between the SOC and SPC paradigms. The most important difference for the present study is that in the SOC paradigm, CS1 is thought to be associated mainly with the emotional and motivational states evoked by the US. Conversely, in the SPC paradigm, CS2 is thought to be associated mainly with the representation of CS1, and thus the US [13]. In the SPC paradigm, CS2 and CS1 are paired before CS1 is paired with the US. Therefore, the association between the representations of CS2 and CS1, and the association between the representations of CS1 and the US, are acquired. Rizley and Rescorla [14] confirmed this assumption: repeated non-reinforcement of CS1 (CS1 was presented without the US) caused extinction of the CR, not only to CS1, but also to CS2 in the SPC paradigm. These results indicate that CS2 is associated with the representation (i.e., perceptual, qualitative information) of CS1 and the US in the SPC paradigm. Alternatively, in the SOC paradigm, CS2 and CS1 are paired after development of the strong association between the US and CS1. Rizley and Rescorla [14] showed that repeated non-reinforcement of CS1 did not cause extinction of the CR to CS2 in the SOC paradigm. Therefore, the associations acquired in the SOC paradigm seem to be different from those acquired in the SPC paradigm.

Furthermore, Holland and Rescorla [15] showed that devaluation of the US (e.g., making animals sated in food appetitive conditioning) reduced conditioned response to CS1, but did not reduce them to CS2 in SOC. Holland [16] also showed that light (CS1) paired with food (US) elicited a rearing response, whereas tone (CS2) paired with light (CS1) elicited a startle-like response. Therefore, it has been suggested that CS2 is associated with emotional and motivational states elicited by the US or CS1 in SOC [13,16].

Taken together, these findings suggest that CS2 acquires the ability to elicit a CR through the development of an association with the emotional states elicited by the US in SOC, whereas in SPC, this occurs through an association with the representations of CS1 and US. In other words, CS2 seems to be mainly associated with the emotional information of the US in SOC, whereas CS2 is associated with the qualitative information of the US in SPC. If learning is based primarily on the development of the associations between representations of CSs and emotional aspects of the US, SOC would be applicable to the learning. However, if learning is based mostly on the association between the representations of CSs and the US, SPC would be more applicable to learning. Herein, by examining whether SOC or SPC paradigms are acquired successfully, we can determine which aspects (emotional or qualitative) of the US are associated with those of the CSs in taste-odor learning.

The present study consisted of three behavioral experiments. In Experiment 1, we aimed to replicate previous taste-odor learning findings from studies that used first-order conditioning [7,9], and to select and validate our odor stimuli. In Experiments 2 and 3, we aimed to examine whether odors were associated with the emotional or qualitative aspects of gustatory information using higher-order conditioning of taste-odor learning.

2. Experiment 1

2.1. Materials and methods

2.1.1. Subjects

Twelve adult Wistar male rats (300–360 g body weight) were used. The rats were housed in individual home cages in a temperature-controlled (23 ± 2 °C) and humidity-controlled (50 ± 5%) room on a 12:12 light/dark cycle, where they had free access to food (dry pellets, Oriental Yeast Co., Ltd., Japan) and deionized water, except when deprived for training, learning, and testing as described below. This study was reviewed and approved by the ethics committee of the Center for Laboratory Animal Research, Tohoku University.

2.1.2. Stimuli

Sodium saccharin (0.005 M) and quinine hydrochloride (0.02 M) were used as taste stimuli. The odor stimuli were food flavors (Narizuka Corporation, Japan) and consisted of melon (0.05%), lemon (0.1%), vanilla (0.1%), and almond (0.1%). Our pilot research revealed that odor stimuli in these concentrations were estimated to be of the same intensity by a panel of human judges. Two of these odor stimuli were presented as odor A and odor B, differently in each rat (counterbalanced). Stimuli were presented in the manner shown in Table 2.

Table 1
Procedural difference between first-order conditioning and higher-order conditioning (second-order conditioning and sensory preconditioning) (revised from Gewirtz and Davis [13]).

	Phase 1	Phase 2	Test
Classical conditioning (first-order conditioning)	CS-US		CS ?
Higher-order conditioning			
Second-order conditioning (SOC)	CS1-US	CS2-CS1	CS2 ?
Sensory preconditioning (SPC)	CS2-CS1	CS1-US	CS2 ?

Table 2
Flow chart of the sessions and stimuli presented in Experiment 1.

Training	Learning (Days 1–5)	Test (Days 6–10)
w	As vs. Bq	A vs. B

w: deionized water, As: saccharin solution with odor A, Bq: quinine solution with odor B, A: deionized water with odor A, B: deionized water with odor B.