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Anesthesia-inducing drugs also induce conditioned taste aversions☆

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ABSTRACT

Animals learn to reduce their intake of a tastant when its ingestion is followed by the administration of an anesthesia-inducing drug. To determine the nature of this intake suppression, the current study examined whether ketamine/xylazine (Experiment 1) and pentobarbital (Experiment 2) also conditionally reduce taste palatability. Using lick pattern analysis, we found that pairing saccharin with either drug reduced total licks, lick cluster size, and initial lick rate. Given that both lick cluster size and initial lick rate are indices of palatability, this pattern of results indicates that anesthesia-inducing drugs also induce conditioned taste aversions.

1. Introduction

Palatability, or hedonic value, is one of the most important determinants of food intake (e.g., [12,29]). However, palatability is not a fixed property of a food. Rather, taste palatability can be modulated by postingestive consequences. In particular, when consumption of a novel food is followed by an aversive systemic experience (e.g., gastrointestinal malaise) the food can become unpalatable or disgusting. This learned reduction in palatability is termed a conditioned taste aversion (CTA) and is considered to result from the acquisition of a Pavlovian association between the taste of the food (conditioned stimulus or CS) and the aversive post-ingestive consequence (unconditioned stimulus or US; e.g., [23,24]; for reviews see [6,11,44]). By conditionally lowering taste palatability, CTA learning protects us from the repeated ingestion of poisonous foods (for reviews see [33,34]).

Garcia et al. [25] proposed that another form of aversive taste learning, based on the operation of a qualitatively different mechanism, could also lead to food rejection. These investigators evaluated the effect of pairing a taste CS with either an internal malaise-inducing US (i.e., lithium chloride, LiCl) or an external pain-inducing US (i.e., footshock). Post-conditioning tests revealed that the LiCl-induced CTA was not context dependent. On the other hand, the footshock-paired taste was avoided in the conditioning context but not elsewhere (e.g., home cage). This pattern of results encouraged Garcia and colleagues to advocate a distinction between CTA (which involves a reduction in both CS palatability and intake) and taste avoidance learning (TAL; which involves a reduction in CS intake only). According to their analysis of TAL, the taste CS is avoided because it has become a signal for impending danger. Supportive of this interpretation, Pelchat et al. [42] reported evidence that footshocks, unlike lithium toxicosis, do not cause a downshift in the palatability of the associated taste CS.[1]

The present article is concerned with the nature of the taste learning supported by anesthesia-inducing drugs. Although having a pharmacological action that is different from those of poisons and toxins, these drugs can function as a US to reduce intake of a taste CS. The aversion-avoidance dichotomy suggests that the nature of aversive taste learning depends upon the type of US – poisons (e.g., LiCl) support CTA whereas external pain-inducing stimuli (e.g., footshocks) support TAL. Anesthesia-inducing drugs may support taste suppression learning via either mechanism. To investigate this issue, we employed lick pattern analysis methodology because it allows simultaneously measurement of intake and palatability during the voluntary drinking trials of the standard taste learning protocol [31,33].

When voluntarily drinking, rats make runs of licks that are interrupted by pauses. These runs of licks are termed clusters and the number of licks in a cluster defines the size of a cluster. Among the various measures that can be extracted from lick pattern analysis, lick cluster size accurately reflects taste palatability while other measures (e.g., number of licks, number of clusters, inter-cluster interval) are

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[1] There were some notable procedural differences between the LiCl and footshock experiments in the Pelchat et al. study. For example, the LiCl experiment involved two conditioning trials, each spaced 3days apart, in which 10-min access to sucrose preceded LiCl administration by intragastric gavage. The footshock experiment, on the other hand, involved one 10-min conditioning trial per day, for a total of 20–25days. On each of these trials rats had access to two stimulus bottles (sucrose and water), and a footshock was delivered within a few seconds of the start of each bout of sucrose drinking; no shocks were delivered for drinking water. Although procedural differences may play some role, the distinctive nature of the USs has been considered the dominant cause contributing to the development of CTA or TAL (e.g., [26,40,42]).

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correlated with amount consumed or post-ingestive consequences (for reviews see [20,33]). For example, lick cluster size displays a positive monotonic relationship with the concentration of palatable solutions like sucrose and polycose (e.g., [15,18]) but a negative monotonic relationship with increasing concentrations of unpalatable solutions like quinine [27,47]. Importantly, lick cluster size is not directly related to amount consumed, which bears an inverted U-shaped function to concentration (i.e., sucrose intake peaks at intermediate concentrations and diminishes as concentration increases; [18]). Like cluster size, initial lick rate also reflects taste palatability [16]. Furthermore, initial lick rate is considered to represent palatability that is purely conveyed by the orosensory aspect of the taste stimulus (e.g., [17]) because the brief sampling period (first 1 to 3 min of licking) limits the influence of post-ingestive feedback. Accordingly, we used lick cluster size and initial lick rate as dependent measures for taste palatability.

In this study, ketamine/xylazine and pentobarbital were the anesthetics that served as the USs because they are known to reduce the intake of a taste CS (ketamine/xylazine: [1,35]; pentobarbital: [48]). Furthermore, the two USs differentially induce emesis: whereas ketamine/xylazine (xylazine in particular) can cause vomiting [22,28], pentobarbital appears to attenuate nausea and vomiting (e.g., [49]). The aversion-avoidance dichotomy states that only nausea/vomiting-inducing USs can induce CTAs whereas other USs support TAL ([42]; for reviews see [39,40]; [50]). Accordingly, evaluating the effects of ketamine/xylazine and pentobarbital on taste learning is theoretically important as it refines our understanding of the distinction between CTA and TAL.

In Experiment 1, two doses of ketamine/xylazine (10/0.5 and 20/1.0 mg/kg) were tested, both of which are lower than that used to induce general anesthesia (100/10 mg/kg). These lower doses were chosen because in a preliminary study we found nearly complete intake suppression after a single conditioning trial with a 100/10 mg/kg ketamine/xylazine, which prevented the collection of sufficient data for meaningful lick pattern analysis. Experiment 2 evaluated the conditioning effect of pentobarbital, a barbiturate anesthetic, on taste-evoked consummatory behavior. As in Experiment 1, we used sub-anesthetic doses of the US (15 and 30 mg/kg) to prevent rapid intake suppression. In both experiments water-deprived rats were allowed to consume 0.1% saccharin for 15 min and, 5 min later, were injected with saline or the drug US. Based on the aversion-avoidance account, we expect that ketamine/xylazine, but not pentobarbital, will support CTAs. That is, both taste intake and palatability are predicted to decline over repeated pairings when ketamine/xylazine serves as the US. On the other hand, we expect to find a significant reduction only in intake but no change in taste palatability (TAL learning) following contingent pentobarbital injections.

2. Materials and methods

2.1. Subjects

Experimentally naïve male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were used as subjects. On arrival, the rats were individually housed in plastic cages (Ancare, Inc., Bellmore, NY) in a vivarium that was maintained at ~70 °F with a 12:12 light:dark cycle (light on at 7:00 am). Food and water were available ad libitum until the experiment started. At that time, the rats were placed on a water deprivation schedule (see Procedure section); lab chow (Teklad global 18% protein; Envigo, IN) was always available in the home cages. The University of Illinois at Chicago Animal Care and Users Committee approved the procedures employed in the current study. At all times, rats were treated in accord with the guidelines recommended by the American Psychological Association [2] and the National Institutes of Health [37].

2.2. Apparatus

Eight identical drinking chambers (Med Associates, St. Albans, VT; 30.5 cm × 24.0 cm × 29.0 cm) were employed, each housed in a sound resistant cubicle fitted with a ventilation fan. The sidewalls of each chamber were made of aluminum, the front wall, back wall and ceiling were clear Plexiglas and the floor consisted of 19 stainless rods. A house light and a white noise generator (providing a masking noise level of 80 dB) were mounted at the top of the left wall in the chamber. Fluid was presented in a retractable sipper tube accessible through an oval hole (1.3 cm × 2.6 cm) in the middle of the right wall. In its extended position, the tip of the sipper tube was ~3 mm outside the wall. Each chamber was equipped with a lickometer circuit that allowed collection of lick times with a resolution of 10 ms. Stimulus presentation and data collection from the drinking chambers were carried out on-line with a computer running Med-PC software (Med Associates) located in an adjacent room.

2.3. Procedure

2.3.1. Experiment 1

Following 7 days of environmental acclimation, the rats were deprived of water by giving 15-min water access in the morning in the drinking chambers and 15-min access each afternoon in the home cages. Once morning water intake stabilized, the experiment began. Counterbalanced by baseline water intake, the rats were assigned to three groups based on the dose of the US, which was a mixture of ketamine (ketamine HCl; Hospira Inc., Lake Forest, IL) and xylazine (Lloyd Inc., Shenandoah, IA). Training occurred in 3-day cycles. On Day 1 of each cycle, rats were allowed to consume saccharin (0.1%, w/v) for 15 min in the morning followed, 5 min later, with an IP injection of physiological saline (Group KX0; $n = 9$), 10/0.5 mg/kg (Group KX-d1; $n = 10$) or 20/1.0 mg/kg (Group KX-d2; $n = 9$) of ketamine/xylazine; all injections were 1 ml/kg. On Day 2, following the 15-min morning water access, Groups KX-d1 and KX-d2 received saline injections while Group KX0 was given ketamine/xylazine injections ($n = 4$ and 5 for high and low doses, respectively). On Day 3, rats were treated as Day 2, except there were no injections. This 3-day cycle was repeated once and followed, 24 h later, by a single taste-only test trial (see Table 1).

2.3.2. Experiment 2

A new set of naïve male rats was obtained from Charles River Laboratory. The conditioning procedure was identical to that of Experiment 1, except that the US was pentobarbital (Lundbeck Inc., Deerfield, IL). The US doses were 15 and 30 mg/kg for, respectively, Group P-d1 ($n = 7$) and Group P-d2 ($n = 9$). As in Experiment 1, Group P0 ($n = 9$) received contingent injections of saline.

2.4. Dependent measures and data analysis

To characterize the nature of taste-guided learning, each experiment included three dependent variables: total licks, lick cluster size, and initial lick rate (total licks in the 3 min that followed the first lick). To afford comparability with prior studies that examined contingent palatability changes following US administrations (e.g., [21,32]), a cluster was defined as a run of licks separated by inter-lick intervals of < 0.5 s. Data were analyzed with 2-way mixed design analyses of

Table 1
Experimental design.

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	Sac-Sal	Water-US	Water	Sac-Sal	Water-US	Water	Sac
Experimental	Sac-US	Water-Sal	Water	Sac-US	Water-Sal	Water	Sac

Note. Sac = saccharin CS; Sal = saline; US = unconditioned stimulus.