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Research report

Incubation of saccharin craving and within-session changes in responding for a cue previously associated with saccharin ☆

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ABSTRACT

Time-dependent increases in cue-induced sucrose seeking after forced abstinence have been described in rats with a history of sucrose self-administration, suggesting sucrose craving "incubates". In the present study, we examined whether the incubation of craving generalizes to the artificial sweetener, saccharin. Thirty-one male Long-Evans rats lever pressed for 0.3% saccharin solution 1 h/day for 10 days. On either Day 1 or 30 of forced abstinence, rats responded for 1 h for presentation of a tone + light cue previously presented with every saccharin delivery during self-administration training. Rats responded more during this cue-reactivity test session following 30 vs. 1 day of forced abstinence ("incubation of craving"). This result is the first demonstration of the "incubation of saccharin craving" and suggests that a post-ingestive caloric consequence of self-administration is not a necessary condition for the development of incubation of sucrose craving. We also examined the time course (within-session decreases) of active-lever responding during the 1-h cue-reactivity test session. Rats in the Day 30 group responded more than rats in the Day 1 group from the beginning of the test session. In addition, within-session decreases in responding were shallower in slope in the Day 30 than the Day 1 group. These results indicate that "incubation of saccharin craving" enhances the persistence of seeking behavior.

Introduction

Incubation of drug craving has been identified in rodents, in which time-dependent increases in cue-induced drug seeking are observed after abstinence from drug self-administration (see Pickens et al., 2011, for review). Craving has been operationally defined as rats responding for (seeking) a cue associated with reinforcement (Grimm, Kruzich, & See, 2000; Markou et al., 1993). Recently, incubation of craving has also been observed in rats with a history of oral sucrose self-administration (e.g., Grimm et al., 2013; see Grimm, 2011, 2012 for reviews). In these studies, rats self-administer sucrose during 10 daily sessions in which each sucrose delivery into a liquid drop receptacle is paired with a discrete tone + light cue. Responding for a cue previously associated with sucrose increases from 1 to 30 days following self-administration training. Examining the incubation of sucrose craving is informative to the question of how craving changes over time, and could reveal treatment strategies for chronic relapsing disorders such as eating disorders.

Non-caloric, high-intensity sweeteners are substituted for added sugars in a variety of foods. However, to the best of our knowledge, no study has investigated whether artificial sweetener seeking increases over time away from the sweetener. The first question of this study was, then, whether the incubation of craving generalizes to the artificial sweetener, saccharin. If incubation of craving generalizes to saccharin, this would mean that the post-ingestive caloric consequences of sucrose self-administration are not a necessary condition for the development of incubation of sucrose craving. In addition, it has been repeatedly demonstrated that consuming non-caloric high-intensity sweeteners results in an increase, rather than a decrease, in body weight (Davidson, Martin, Clark, & Swithers, 2011; de Matos Feijo et al., 2013; Swithers, Baker, & Davidson, 2009; Swithers & Davidson, 2008; Swithers, Martin, Clark, Laboy, & Davidson, 2010; see Swithers, Martin, & Davidson, 2010 for review). Therefore, understanding of seeking behaviors for a non-caloric sweetener could also be important to improve weight control.

The second purpose of this study was to analyze the within-session pattern of responding for a cue previously associated with the primary reinforcer (i.e., saccharin). Such a cue is considered a secondary reinforcer. It has been well documented that responding for

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primary reinforcers does not remain constant within experimental sessions, even when no obvious independent variable is manipulated (e.g., McSweeney & Murphy, 2009). Rate of responding (number of responses per unit time) may increase, decrease, or increase and then decrease during sessions of operant conditioning (McSweeney, Hinson, & Cannon, 1996). It is not known if there are similar within-session changes in responding for a secondary reinforcer. Analysis of within-session patterns is important because it provides us with information about the persistence of behavior. For example, if within-session decreases in responding are steeper in a 1-day abstinence condition than in a 30-days abstinence condition, the data may mean that "incubation of craving" enhances the persistence of seeking behavior. Craving is one of the definitional characteristics of substance use disorders (American Psychiatric Association, 2013), and thus examining persistence of seeking behavior, an animal model of craving, is important in gaining an understanding of the nature of saccharin craving and food craving in general.

Several attempts have been made to describe within-session response curves by best-fit equations (e.g., Bousfield, 1935; Killeen, 1995; McCleery, 1977; McSweeney et al., 1996; Skinner, 1932). In all cases, behavior was treated as a function of time elapsed within a session. As an alternative, Aoyama (1998) treated within-session decreases in responding as a function of the cumulative number of reinforcer deliveries within a session. It was shown that a linear equation adequately described within-session decreases in responding during a continuous reinforcement (CRF) schedule. That is, rates of responding decreased proportionally to the increase in cumulative number of reinforcer deliveries.

This relationship can be described as Eq. (1).

$$Rr = a - bIc \quad (1)$$

where *Ic* is cumulative amount of intake (i.e., cumulative number of reinforcer deliveries), *a* and *b* are free parameters, and *Rr* is response rate. Response rate stands for number of responses per unit time. Parameter *a* is the *y*-axis intercept of the regression line. This parameter indicates response rate at the beginning of the session. Parameter *b* is the slope of the regression line. This parameter represents the rate of response decrease produced by a single presentation of a reinforcer. There is another parameter that can be calculated using Eq. (1): the *x*-axis intercept of the regression line. This parameter represents the cumulative number of reinforcer deliveries that will reduce the response rate to zero. Although it has been repeatedly demonstrated that Eq. (1) can describe responding for primary reinforcers (e.g., Aoyama, 2007, 2012; Aoyama & McSweeney, 2001), Eq. (1) has never been applied to responding for secondary reinforcers. Thus, in this study, Eq. (1) was applied to the within-session responding for the cue previously associated with saccharin. In addition, effects of incubation of craving on the parameters of Eq. (1) were examined. This was the third purpose of this study.

Methods

Subjects

Thirty-one male Long-Evans rats (3 months old at the start of study; Simonsen-derived) bred in the Western Washington University vivarium were housed individually on a reverse day/night cycle (lights off at 7 AM) with nutritionally balanced Purina Mills Inc. Mazuri Rodent Pellets (Gray Summit, MO, USA) and water available *ad libitum* except as noted below. Mazuri Rodent Pellets is a complete diet that is specially formulated for rodents. The ingredients of the diet include de-hulled soybean meal, ground corn, wheat middlings, and ground wheat. The concentrations of carbohydrates in the chow are 0.21% for fructose, 0.19% for glucose, 3.13% for sucrose and 26.08% for starch. During the forced-abstinence period (described in the procedure section), saccharin solution was not given but the chow was provided *ad libitum*. Thus, rats could obtain some sweet substance from the chow during the forced-abstinence period. Rats were weighed each Monday, Wednesday, and Friday for the duration of the experiment. Sixty-five hours (about 2.5 days) before the first day of training, 48 h of a saccharin habituation period started. During this period, rats were deprived of plain water and provided saccharin solution. The concentration of saccharin solution was 0.1% for the first 24 h and 0.3% for the last 24 h of saccharin habituation. This procedure was conducted to acclimate rats to the saccharin solution to facilitate subsequent saccharin self-administration (SA). Immediately prior to the SA training phase, the animals were deprived of water, as well as saccharin solution, for 17 h to encourage SA on the first day of training. Rats were then returned to *ad libitum* water access. All procedures followed the guidelines outlined in the "Principles of Laboratory Animal Care" (National Institutes of Health publication No. 86-23) and were approved by the Western Washington University Institutional Animal Care and Use Committee.

Apparatus

Operant training and testing took place in operant conditioning chambers (30 × 20 × 24 cm; Med Associates) containing two levers (one stationary and one retractable), a tone generator (2 kHz, 15 dB over ambient noise), a white stimulus light above the retractable lever and a red house light on the opposite wall. An infusion pump delivered saccharin into a reward receptacle to the right of the active lever for oral consumption. Four photobeams crisscrossed the chamber. Operant conditioning chambers were enclosed in sound-attenuating cabinets with ventilation fans.

Procedure

Training phase

Rats spent 1 h/day for 10 consecutive days in the operant conditioning chambers where they were allowed to press the retractable (active) lever for a 0.2 ml delivery of 0.3% saccharin into the receptacle to the right of the lever. This concentration was chosen as a separate group of rats from this vivarium were found to prefer 0.3% over 0.1 or 0.2% in a 2 bottle preference test pilot study (data not shown). An active lever response also activated a compound stimulus consisting of the tone and the white light. The compound stimulus lasted for 5 s and was followed by a 40-s time out, during which presses on the active lever were recorded but had no programmed consequence. A response on the inactive (stationary) lever did not have a programmed consequence, but responses were recorded. The total number of photobeam breaks was recorded during all phases of the study. At the end of each session, rats were returned to home cages.

Forced-abstinence phase

There were 2 groups of rats in the study created by the independent variable of forced-abstinence period (1 or 30 days). The 1- or 30-day forced-abstinence phase began as the first day ("Day 1") following the 10th day of the training phase. Rats were housed in home cages for the duration of forced abstinence. At the end of the training phase, rats ($n = 14$ rats/group) were assigned to one of the forced-abstinence periods (1 or 30 days). Active lever