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Saccharin pre-exposure enhances appetitive flavor learning in pre-weanling rats

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Abstract

In adult rats, data suggest that consumption of sweet tastes that do not deliver anticipated caloric consequences using high-intensity, non-caloric sweeteners, such as saccharin, interferes with learned relations that may contribute to energy balance. The goal of the present study was to assess the development of learning about sweet taste and calories by assessing whether pre-exposure to saccharin solutions reduces cue competition in pre-weanling rats. The results demonstrated that rats pre-exposed to saccharin and then trained with a novel grape flavor paired with a glucose-sweetened solution consumed more of the novel grape flavor presented alone than rats that had been pre-exposed to saccharin and given the grape flavor paired with water alone. No differences in intake of the novel grape flavor were observed in groups given pre-exposure to water or glucose solutions. Thus, by 15 days of age, rats appear to have established an association between sweet tastes and calories, and this association can be weakened by exposure to saccharin.

Keywords

Appetitive learning; rat; cue competition; energy balance; sweeteners

Previous research shows that rats learn to use sweet taste cues to predict caloric consequences (e.g., Capaldi, Owens, & Palmer, 1994; Harris, Gorissen, Bailey, & Westbrook, 2000). Other work shows that such learning may promote the performance of conditioned responses that help to maintain energy homeostasis (e.g. Power & Schulkin, 2008; Woods & Ramsay, 2000). One implication of these findings is that disrupting the predictive relationship between oral taste cues and their postingestive caloric or nutritive outcomes should interfere with the ability of animals to maintain energy balance. Consistent with this idea, we have reported that adult rats given access to high-intensity and non-caloric sweeteners such as saccharin show increased body weight gain, increased adiposity and an impaired ability to compensate for calories provided in novel sweetened diets (Davidson & Swithers, 2004; Swithers, Baker, & Davidson, 2009; Swithers & Davidson, 2008; Swithers, Martin, Clark, Laboy, & Davidson, 2010; Swithers, Martin, & Davidson, 2010).

While this earlier work provided evidence that consuming saccharin could lead to energy dysregulation and body weight gain, it did not directly assess the notion that consuming saccharin had this effect as a result of weakening the predictive relationship between sweet tastes and calories. A recent study from our lab addressed this question. We (Davidson, Martin, Clark, & Swithers, 2011) exploited the well-established principle (Dwyer, Haselgrove, & Jones, 2011; Rescorla & Wagner, 1972; Urushihara & Miller, 2009; Wagner, 1969) that manipulations which reduce the salience or predictive validity of a stimulus will

also weaken the ability of that cue to compete with other cues for association with an unconditioned stimulus (US). For example, learning to associate a novel stimulus with a US can be reduced or prevented (i.e., blocked), if the novel cue is trained in compound with another stimulus that had been previously established as a strong signal for that US (Kamin, 1969). In other words, a novel cue is unable to compete with a previously trained cue for association with the US. In addition to blocking, cue competition can also be influenced as a consequence of latent inhibition (e.g., Lubow & Moore, 1959). Latent inhibition occurs when repeated presentation of a stimulus in the absence of US retards conditioning of that cue when it is trained as a signal for delivery of that US. Thus, such a latently inhibited cue would be less able to compete with a novel stimulus for association with the US.

We reasoned that consuming saccharin could extinguish pre-experimentally formed sweet taste-calorie associations thereby reducing the ability of sweet taste to block the associations of other stimuli with a caloric US or could latently inhibit the formation of sweet taste-calorie associations that might be formed during experimental training. In either case, the validity of sweet taste as a signal for a caloric US would be reduced and the ability of sweet taste to compete with other cues for association with that US would be diminished.

We evaluated this possibility by first pre-exposing one group of rats to a saccharin solution and another group to plain water for 14 days. This was followed by a 20-day training phase in which each group received one flavor (cherry or grape) mixed in solution with glucose and the other flavor mixed with equicaloric polycose. Our rationale was that prior exposure to saccharin, relative to water, would reduce the ability of the sweet taste of glucose to compete with the novel flavor for association with the caloric US produced by glucose. In contrast, because polycose does not appear to taste sweet to rats (Sclafani, Nissenbaum, & Vigorito, 1987), prior exposure to saccharin was not expected to alter the ability of the taste of polycose to compete with the other novel flavor for association with the caloric US produced by polycose.

These predictions were tested by measuring the intake of each flavor when it was presented without glucose or polycose. If prior saccharin exposure weakened the ability of sweet taste to compete with a flavor cue for association with the caloric US, then rats previously exposed to saccharin would consume more of the flavor that had been paired with glucose than would rats previously exposed to only water. Furthermore, intake of the flavor that had been paired with polycose would not differ for the rats previously exposed to saccharin compared to the rats exposed only to water because weakening the association between taste and calories should not interfere with the predictive validity of the non-sweet “poly” taste.

The results of this study confirmed both predictions. In addition, a second experiment showed that prior exposure to saccharin had the selective effect of promoting intake and body weight gain for rats maintained on a diet sweetened with glucose, but not on an equicaloric diet mixed with polycose (Davidson, et al., 2011). This outcome linked weakening of the sweet taste-calorie association to a reduced ability to regulate intake of sweet, high-calorie foods.

The present paper set out to assess whether rats that were pre-exposed, trained, and tested at a much earlier, pre-weanling, stage of development would also exhibit a similar reduction in the validity of sweet taste cues. Little is currently known about the ontogeny of the association between sweet taste and calories. However, there is evidence that adult rats may enter experimental situations with an association between sweet tastes and calories already established. It is conceivable, if not likely, that this association is formed earlier in life based on exposure to sweet tastes that predict caloric consequences outside of experimental contexts (Davidson, et al., 2011; Rescorla, 2008).

Constraints associated with the age of the pups and the short length of the pre-weanling period necessitated several changes relative to our previous study. For example, the rat pups were implanted with an oral cannula to provide uniformity in the delivery of experimental solutions and enhance consumption. Also, the amount of solution (1 ml compared to 30ml) and the duration of solution presentations (11 min compared to overnight) within pre-exposure and training sessions were greatly reduced compared to our previous study in adults. Further, in addition to groups that were pre-exposed to saccharin solution or water, the present study also included a group that was given a glucose solution to serve as a positive control for pre-exposure to the sweet taste-calorie relationship.

The duration of the pre-weaning period necessitated other changes in our experimental design (Table 1). First, the length of the pre-exposure phase was reduced from 14 days to 2 days. Second, we trained each pup for two, instead of 10, days with only one instead of two flavors. Thus, training with different flavor-nutrient compounds was conducted using a between-subjects design in this study rather than a within-subjects design. To maximize the between-group difference in training compounds, half the rats that had been assigned to each pre-exposure condition were given the novel grape flavor mixed in water with glucose (designated as the “plus” condition in Table 1) and the remaining half received the same novel grape flavor mixed only in water (designated as the “minus” condition). On a final test day, intake of the grape flavored solution mixed only with water was recorded for all pups.

If pre-exposure to saccharin reduces the validity of sweet taste to compete with a novel flavor to signal a caloric US, then rat pups pre-exposed to saccharin and trained with a novel flavor (Group Saccharin Plus) should consume more of that flavor during testing than their untrained controls (Group Saccharin Minus). More importantly, the difference between the Saccharin Plus and Saccharin Minus groups should be larger than the difference between the trained and untrained groups that were pre-exposed to glucose (Groups Glucose Plus and Glucose Minus) or water (Groups Water Plus and Water Minus), none of which experienced sweet taste without a caloric outcome.

Methods

Subjects were offspring of primiparous and multiparous Sprague-Dawley rats, bred in our colony. Male and female rats were mated for 1 week, then females were separated from males and housed in pairs until the week of parturition. Cages were checked daily at 1700 for births and the day of birth was designated as P0. Litters were culled to 10 pups (5 males and 5 females when possible) on the day after birth and remained with the dam undisturbed except for routine maintenance until 15 days of age (P15). On P15, all pups were removed from the dam and anterior oral cannulas constructed of PE-10 tubing were inserted under anesthesia as previously described (Hall, 1979). The placement of the cannulas allowed animals to consume liquids infused through the cannula through active licking and lapping; fluids could be rejected actively or by passively allowing them to drip out of the mouth. Following cannula placement, animals were housed with littermates in a warm, humid incubator for 3-4 hr prior to testing. Immediately prior to testing, pups were stimulated to urinate and defecate, and then weighed. Body weights collected immediately following the termination of testing were used as estimates of intake; data from animals that urinated or defecated during testing were discarded. Immediately following the conclusion of solution presentations each day, animals were carefully cleaned to remove any solutions and then returned to the dam. Oral cannula were checked daily for patency, and replaced as necessary.

The study was divided into Pre-exposure, Training, and Testing phases and animals were assigned to one of six groups that differed with respect to the treatment they received during

the Pre-exposure and Training phases (Table 1). Male and female rats from each of 14 litters were distributed across the conditions with no more than two animals from each litter used in the same condition. Infusions were delivered by attaching the PE-10 tubing to lengths of PE-50 tubing attached to syringes housed in syringe pumps. During all phases of Pre-Exposure, Training and Testing, animals were housed in individual test containers located in a warm, humid incubator. During the Pre-Exposure and Training phases, animals received approximately 1 ml of solution delivered over 11 min each day; pumps were turned on for 30 sec, then off for 10 sec throughout Pre-exposure and Training.

During the Pre-exposure phase (P15 and P16), infusions of 0.3% saccharin, 20% glucose or water were delivered through the anterior oral cannulas implanted under the pup's tongue. During the Training phase, on P17 and P18, pups were given an oral infusion of a solution that combined a novel flavor (0.1% Grape Kool-Aid) with either a sweet-tasting, caloric solution (20% glucose; Plus groups) or a solution that neither tasted sweet nor delivered calories (water; Minus groups).

During Testing, on P19, the effects of prior exposure to saccharin, glucose or water on learning about the Grape flavor were assessed. Animals were given infusions of the Grape flavor in water alone, and during testing, infusion pumps were turned on for 30 sec, then off for 10 sec as in Pre-Exposure and Training sessions. However, during Testing, to avoid ceiling effects on intake during the limited 11 min exposure, infusions were continued until animals rejected the solution (either passively by allowing the fluid to drip out of the mouth without any active mouthing movements, or actively by ejecting the fluid through head shaking or chin scraping) during 2 consecutive infusion periods. Testing was terminated at this time, and amount consumed was estimated by weighing the animals. To minimize experimenter bias, animals were identified only by number, which did not provide information about group assignment, and to the extent possible, during testing experimenters were blind to the animal's previous pre-exposure history, training history and/or the experimental hypothesis.

Intake during Pre-exposure, Training and Testing was determined by changes in body weight and expressed as percent body weight $[(\text{Body Weight after} - \text{Body Weight Before}) / \text{Body Weight Before}] * 100$. Animals that did not consume at least 1.5% of their body weight during the Pre-exposure phase ($n = 19$) or 2.5% of their body weight during the Training phase ($n = 6$ additional animals) were excluded from analysis. Body weights on the first day of pre-exposure differed significantly between males and females (Means + SEM = 35.9 ± 0.4 for males and 33.4 ± 0.3 , respectively; $F_{1, 89} = 22.02$, $p = 0.00001$), therefore, the animal's starting body weight was used as a Covariate in all analyses. Intake data on Pre-exposure, Training, and Testing days were analyzed separately with Three-Way (Sex X Pre-Exposure X Training) ANCOVAs. Post-hoc tests were done using Duncan's tests where indicated with a $p < 0.05$ taken as significant in all tests.

Results

Results indicated that during Pre-exposure, intake of the solutions was affected by the solution infused (Figure 1A; Main effect of solution; $F(2, 7) = 31.53$, $p < 0.0000001$). Post-hoc analyses revealed that intake was significantly different across all three Pre-exposure conditions. During Training, intake of the solutions was by whether the Grape solution contained glucose or not (Main effect of Conditioning group; $F_{2, 78} = 12.88$, $p = 0.000058$), with animals in the Plus conditioning groups consuming more than animals in the Minus groups during Training (Figure 1B).

During testing, intake of the solution was affected by the solution experienced during the Pre-exposure phase as well as the Training condition (Figure 1C; Pre-Exposure solution X Conditioning group, $F [2, 7] = 4.43$, $p = 0.015$). Animals given saccharin during the Pre-exposure phase and then given Grape flavor paired with glucose during Training consumed more of the Grape flavor during Testing than animals given saccharin during Pre-exposure and then Grape flavor paired with water during Training. In contrast, no differences in intake of the Grape flavor during Testing were observed in animals given glucose or water during the Pre-exposure phase.

Discussion

These data confirm and extend previous findings that prior exposure to saccharin can increase the ability of a novel flavor cue to compete with sweet taste for association with a caloric or nutritive outcome when both type of cues have been trained in compound as a signal for that US (Davidson et al., 2011). Our results showed that pre-weanling rats are sensitive to this effect of saccharin pre-exposure, and that this sensitivity develops following very brief periods of exposure and following a very limited amount of compound training with flavor and sweet taste cues.

The pattern of data we obtained suggests that training with the flavor and sweet taste compound was not sufficient to produce significant conditioning of the novel flavor for pups that had been pre-exposed to either glucose or water. Pups in these groups showed no more conditioned responding to the flavor previously mixed with sweet-tasting and caloric glucose compared to untrained control groups for which the flavor was presented without sweet taste or calories. Only the pups given this compound training following pre-exposure to saccharin provided evidence for learning about the flavor.

Differences among the pre-exposure conditions may be informative about the ontogeny of learning about sweet taste→calorie predictive relation. A question of interest concerns whether our rat pups learned this relationship based on experiences programmed as part of our experiment, or had formed this association prior to being exposed to our experimental manipulations (e.g., Rescorla, 2008). For example, it may be that the ability of sweet taste to compete with the flavor as a signal for calories was reduced for Group Saccharin Plus as a result of latent inhibition (e.g., (Rodriguez & Hall, 2008) that was produced by prior non-reinforced exposure to sweet taste without a US. On the other hand the rat pups in that group could have entered the experiment with the sweet taste-calorie association already formed. Then, consuming saccharin in the pre-exposure phase might have partially extinguished or weakened this association, thereby reducing the ability of sweet taste to compete with the flavor during training (Bills, Dopheide, Pineno, & Schachtman, 2006; Kamin, 1969).

The test performance of the groups pre-exposed to glucose and water, respectively, seem to favor the latter hypothesis. For example, to account for the absence of learning about flavor by Group Glucose Plus, it could be argued that the pups in that group associated sweet taste with calories in the Pre-exposure phase and this enabled sweet taste to block learning about the flavor and calories during Training (Dwyer, et al., 2011). However, this account does not anticipate the finding that Group Water Plus did not exhibit learning about the flavor paired with sweet taste during Training. Group Water Plus had been given no explicit opportunity to associate sweet taste with calories during the Pre-exposure phase and therefore learning about sweet taste in the Pre-exposure phase could not have blocked learning about the flavor during Training. It also might be argued that the minimal evidence of learning in the Glucose Plus and Water Plus groups indicates a generalization decrement in these groups since they had been exposed to the Grape flavor associated with sweet taste and calories during training, but during testing only the Grape flavor was presented without sweet taste

or calories. In the Glucose Minus and Water Minus groups, familiarity with the Grape flavor alone could have driven intakes higher. However, this generalization decrement account cannot explain why animals given saccharin during the pre-exposure phase did show evidence of learning, since they would be expected to have similar experience during training and testing with regard to generalization decrement in the Saccharin Plus group. And while it is somewhat surprising that neither the Glucose Plus nor Water Plus animals showed evidence of learning about the relation between the novel grape flavor and either the sweet taste or the calories provided by the glucose during training, this lack of learning may simply reflect the minimal amount of training provided (2 exposures).

One way to account for learning about the flavor by Group Saccharin Plus and the failure of both Groups Glucose Plus and Water Plus to exhibit evidence of flavor learning, is to assume that sweet taste→calorie associations were formed pre-experimentally for the pups in all groups and that this association was weakened by pre-exposure to sweet taste without calories for Group Saccharin Plus. Such associations could be formed either pre-natally or post-natally since there is evidence that rat pups not only learn about cues that predict the delivery of milk (which is high in sugars, and likely sweet-tasting) from the first day of post-natal life (Johanson & Hall, 1979; Johanson, Hall, & Polefrone, 1984), but like human infants (Mennella & Beauchamp, 2005), can form preferences for flavors delivered in utero as well (Chotro & Molina, 1990; Dominguez, Chotro, & Molina, 1993; Dominguez, Lopez, & Molina, 1998; Smotherman, 1982). In addition, the enhancement in alcohol intake stimulated by in utero exposure to alcohol in rats can be extinguished by minimal non-reinforced exposure to alcohol odor during postnatal life (Dominguez, et al., 1993).

In conclusion, the results are consistent with the idea that by the third week of life, rats have been exposed to a predictive relation between sweet tastes and calories, and that this relation can be weakened by exposure to a non-predictive relation such as that provided by consuming high-intensity sweeteners. One implication of this conclusion is that the ability of rats to use sweet taste to signal calories is not only acquired very early in life, but can be disrupted early in life as well. Moreover, this disruption can be a consequence of very limited exposure to high-intensity sweeteners. The implications of these results for the regulation of energy intake and body weight in young animals and later in adulthood remain to be investigated, but if effects are similar to those demonstrated in adult animals, then even very limited exposure to high-intensity sweeteners early in life could prove problematic for later regulation of energy balance.

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Figure 1. Solution Intake (expressed as percent body weight) A) during the Exposure phase was highest in animals given glucose, followed by saccharin, then water; B) during the Training phase was higher when the Grape flavor was combined with Glucose (plus) than when combined with Water (minus); C) during the Testing phase was higher in Saccharin animals trained with Glucose (plus) compared to Saccharin animals trained with Water (minus). Numbers in parentheses indicate the number of animals included in each group. Bars with different letters within each panel are significantly different. * $p < 0.05$ compared to Saccharin Plus animals

Table 1

Experimental Design

Group	Exposure (P15 and P16)	Training (P17 and P18)	Testing (P19)
Saccharin Plus	Saccharin	Grape Flavor + Glucose	Grape Flavor + Water
Saccharin Minus	Saccharin	Grape Flavor + Water	Grape Flavor + Water
Glucose Plus	Glucose	Grape Flavor + Glucose	Grape Flavor + Water
Glucose Minus	Glucose	Grape Flavor + Water	Grape Flavor + Water
Water Plus	Water	Grape Flavor + Glucose	Grape Flavor + Water
Water Minus	Water	Grape Flavor + Water	Grape Flavor + Water