

Estrogen increases the taste threshold for sucrose in rats

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Abstract

Anecdotal and empirical evidence suggests that females' preferences for sweet foods are affected by hormonal fluctuations across the reproductive cycle. In rats, the preference for sweet foods may involve estrogen-mediated changes in response to the taste of sweets. Our recent work showed that ovariectomized female rats lick less to dilute sucrose solutions when given estrogen than when given the oil vehicle. These findings suggest that estrogen decreases the preference for less concentrated sucrose solutions; however, an alternative explanation is that estrogen interferes with the ability to detect dilute sucrose solutions. To distinguish between these possibilities, we conditioned a taste aversion to 0.2 M sucrose in ovariectomized rats by pairing it with injection of LiCl and then examined the generalization of that taste aversion to 0.075 and 0.025 M sucrose solutions during estrogen or oil treatment. Oil-treated rats generalized the LiCl-induced aversion conditioned to 0.2 M sucrose to both 0.075 and 0.025 M sucrose. Estrogen-treated rats generalized the LiCl-induced taste aversion to 0.075 M sucrose but not to 0.025 M sucrose. Moreover, two weeks later, when estrogen had cleared the system, both groups generalized the aversion to both 0.075 and 0.025 M sucrose. These results show that estrogen affects the ability to discriminate dilute sucrose from water and suggest that estrogen may have short-term effects on the detection threshold for sucrose taste in rats.

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1. Introduction

Anecdotal and empirical evidence in humans suggest that there are sex differences in food preferences and that reproductive hormones may be important in these differences. For example, preferences for a variety of foods, including sweets, differ between males and females, and sweet preferences change across the menstrual cycle and during pregnancy [9,22,14]. Sex differences in food intake also have been reported in animal models, including rat [15,25,28], and reproductive hormones appear to be important in these differences as well. Feeding fluctuates across the estrus cycle in rats, with lowest levels of food intake associated with high levels of estrogen [15,25]. Moreover, ovariectomized rats consume more food than do intact female rats, and this effect is reversed by estrogen replacement [13,15,28]. However, tests evaluating the consumption of standard laboratory chow provide little

information about differences in taste responses, as laboratory chow is not particularly palatable. In the real world, foods are more varied—and likely better tasting.

Are there sex differences in taste responses to palatable foods by laboratory rats? And, if so, does estrogen play a role in those differences? Little work has examined sex differences in taste preferences in animal models. Some studies have attempted to address the issue using long-term tests [17,28]; however, estrogen influences metabolism and gastro-intestinal function [1,3], making these results difficult to interpret. Another study examined the issue more directly using taste reactivity procedures [4] and found that estrogen does not affect taste reactivity measures to sweet tastes. However, this study used only one highly concentrated sucrose solution, and relied upon the characterization of responses during intraoral infusion of solutions, rather than during choice tests.

Our recent study [5] used a range of sucrose concentrations in short-term tests of behavioral taste responses and showed that estrogen affects licking by female rats to dilute, but not to concentrated, sucrose solutions. Specifically,

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when rats were ovariectomized (OVX) and then treated with estrogen, the rate of licking to dilute, 0.025 M sucrose was comparable to the rate of licking to water, whereas OVX rats that were treated with the oil vehicle licked at greater rates to 0.025 M sucrose than to water. These results may be explained by two possibilities. First, dilute sucrose may be less palatable or less rewarding than concentrated sucrose when OVX rats are treated with estrogen. In other words, estrogen may decrease the preference for dilute sucrose. Alternatively, OVX rats that are treated with estrogen may have an elevated detection threshold for sucrose that affects the ability to detect dilute sucrose solutions. The goal of this experiment was to examine the basis for estrogen-mediated sex differences in behavioral responses to sweet tastes in rats by determining whether estrogen affects the detection of dilute sucrose solutions.

2. Methods

2.1. Animals and surgical procedures

Adult female Sprague–Dawley rats (Charles River) were individually housed in a temperature controlled colony room on a 12:12 dark:light cycle. Rats were bilaterally ovariectomized (OVX) under sodium pentobarbital anesthesia (Nembutal Sodium; Abbott Laboratories, North Chicago, IL; 50 mg/kg BW, ip) using a ventral approach. The abdominal muscles then were sutured, wound clips were applied to the skin, and rats were permitted to recover for seven days.

2.2. Behavioral testing

Rats then were placed on a schedule during which they had access to water for 30 min in the afternoon. During the morning, deionized water was given in graduated drinking tubes, and intakes were recorded after 10 min. Stable 10-min water intakes were established by 6 days. On the following day (Day 1), all rats were given 10-min access to 0.2 M sucrose in graduated drinking tubes. Intakes were recorded and immediately thereafter rats were injected with 0.15 M LiCl (3 mEq/kg BW, ip; $n=16$) or the 0.15 M NaCl vehicle (ISO; 20 ml/kg BW, ip; $n=16$). The conditioned taste aversion to 0.2 M sucrose was verified in 10-min, 2-bottle (0.2 M sucrose and water) tests conducted on Day 2. On Day 3, rats were given 10-min access to water. Two-bottle (sucrose and water), 10-min tests were conducted on subsequent days to evaluate the generalization of the taste aversion conditioned to 0.2 M sucrose to less concentrated sucrose solutions. Rats were given 0.025 M sucrose and water on Day 4, 0.075 M sucrose and water on Day 5, and 0.025 M sucrose and water on Day 6.

Rats were given ad libitum access to water from Day 7–17 before again restricting access to water as described. Ten-min morning water intakes were stable by Day 19. On Day 20, an additional 10-min, 2-bottle (0.075 M sucrose and

water) test was conducted; on Day 21, a final 10-min, 2-bottle (0.025 M sucrose and water) test was conducted.

To minimize the possibility of effects attributable to position preference related to training and/or conditioning, we alternated the position of the drinking tubes so that for each rat, tubes containing sucrose solutions were equally likely to be in the ‘conditioning’ position or in the ‘non-conditioning’ position on any test day.

2.3. Hormone replacement

On Day 3 and Day 4, rats treated with LiCl were given sc injections of estradiol benzoate (EB; 10 μ g/0.1 ml oil; Sigma, St. Louis, MO; $n=7$) or the oil vehicle (OIL; 0.1 ml; $n=9$). Rats treated with ISO also were given sc injections of EB ($n=8$) or OIL ($n=8$). This estrogen replacement schedule frequently is used in studies of ingestive behaviors by rats [12,13,15,18] and mimics the pattern of fluctuations of estrogen levels in the intact, cycling female rat [30]. Moreover, in a study using this schedule and dosage, Woolley and McEwen [30] also showed that plasma estrogen concentration decreases to levels present in non-cycling, OVX rats within ~nine days after the second injection. Thus, a second series of 2-bottle tests was conducted ~2 weeks after hormone replacement.

2.4. Statistical analysis

Preferences for sucrose solutions during 2-bottle tests were expressed as preference scores, calculated as [intake of sucrose (ml)/total intake (ml)]. Scores ≥ 0.6 indicate a preference for sucrose; scores ≤ 0.4 indicate an aversion to sucrose; scores between 0.4–0.6 indicate indifference to sucrose. During EB or OIL treatment, intake of 0.025 M sucrose and water and preference for 0.025 M sucrose were calculated by averaging the intakes and preference scores on Days 4 and 6 for each rat. Data are presented as group means \pm S.E.M.

Statistical comparisons of the taste aversion conditioned to 0.2 M sucrose were made using two-factor (Drug \times Hormone) analysis of variance (ANOVA; Statistica, StatSoft, Tulsa, OK). Comparisons of the generalization of the conditioned aversion to 0.075 and 0.025 M sucrose solutions were made using four-factor (Drug \times Hormone \times Concentration \times Time) repeated-measures ANOVA. Pairwise comparisons of statistically significant ($p < 0.05$) main effects or interactions were evaluated using Student Newman–Keuls tests. Additional planned comparisons were made using Bonferroni corrections.

3. Results

3.1. Conditioned taste aversion

Rats that were injected with LiCl showed a robust conditioned taste aversion to 0.2 M sucrose, regardless of

hormone condition, whereas rats that were injected with ISO showed a robust preference for 0.2 M sucrose regardless of hormone condition (Fig. 1). The main effect of drug was statistically significant ($F(1,28)=355.62$, $p<0.001$), with preference scores after LiCl injection significantly less than those after ISO injection. There was no effect of hormone and no interaction between hormone and drug.

3.2. Generalization of conditioned taste aversion

Fig. 2 shows mean preference scores for 0.075 M (left) and 0.025 M (right) sucrose by OVX rats after previous access to 0.2 M sucrose that was paired with ISO injection or with LiCl injection. Preference scores during EB or OIL treatment are shown in panel A; preference scores two weeks after EB or OIL treatment are shown in panel B.

Overall, preference scores by rats that previously had been injected with LiCl were less than those by rats that previously had been injected with ISO, as evidenced by the statistically significant main effect of drug ($F(1,28)=140.65$, $p<0.001$). Thus, independent of hormone condition, sucrose concentration, or time (during or after hormone treatment), the LiCl-induced taste aversion to 0.2 M sucrose generalized to both 0.075 and 0.025 M sucrose. In addition, preference scores depended on the concentration of the sucrose solution ($F(1,28)=4.98$, $p<0.05$), independent of hormone condition, drug, or time. Overall, preference scores for 0.025 M sucrose were greater than those for 0.075 M sucrose.

Neither the main effect of hormone nor the main effect of time was statistically significant; however, there were significant interactions between hormone and drug ($F(1,28)=4.30$, $p<0.05$), between drug and concentration ($F(1,28)=6.51$, $p<0.05$), and between hormone, drug, and time ($F(1,28)=5.56$, $p<0.05$). Further examination of these latter differences revealed that, after previous ISO injection, preference scores by OIL-treated rats were greater than those by EB-treated rats during ($p<0.01$), but not after ($p=0.724$) OIL or EB treatment. In contrast, after previous LiCl injection, preference scores did not differ between the groups

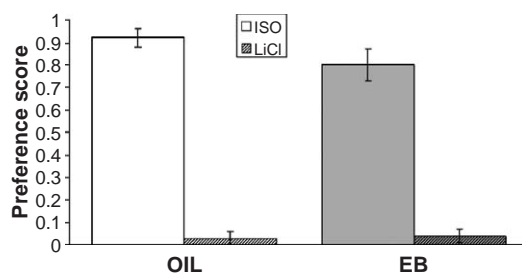


Fig. 1. Preference scores for 0.2 M sucrose by ovariectomized female rats that were subsequently treated with estradiol benzoate (EB; right bars) or the oil vehicle (OIL; left bars). Twenty-four hours prior to the two-bottle (sucrose and water) test, 0.2 M sucrose had been paired with injection of 0.15 M NaCl (ISO; solid bars) or 0.15 M LiCl (hatched bars).

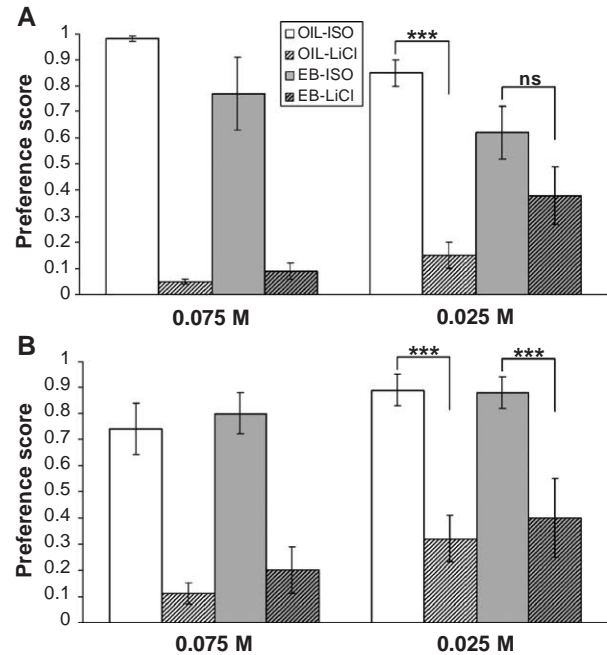


Fig. 2. Preference scores for 0.075 M sucrose (left bars) or 0.025 M sucrose (right bars) in two-bottle tests conducted after 0.2 M sucrose previously had been paired with ISO (solid bars) or LiCl (hatched bars). (A) Preference scores during OIL (white, white/black hatched bars) or EB (gray, gray/black hatched bars) treatment. (B) Preference scores ~ two weeks after OIL or EB treatment. *** = $p<0.001$; ns = not significant.

either during ($p=0.091$) or after ($p=0.350$) OIL or EB treatment.

Finally, we made specific comparisons of the preference for 0.025 M sucrose after previous ISO or LiCl injection during and after OIL or EB treatment. These comparisons revealed that preference scores by OIL-treated rats after previous LiCl injection were significantly less than those after ISO injection, both during and after OIL treatment ($ps<0.001$). In contrast, during EB treatment, preference scores by rats that previously had been injected with LiCl did not differ from those by rats that previously had been injected with ISO ($p=0.120$). After EB treatment, however, preference scores after previous LiCl injection were significantly less than those after ISO injection ($p<0.001$).

4. Discussion

Evidence from studies of humans [9,22,14] suggests that the preference for sweet tastes is affected by reproductive hormones such as estrogen. In contrast, most research using rats report that estrogen does not affect behavioral responses to sweet tastes [17,28,4]; however, these studies typically used very concentrated sucrose solutions. Our recent study showed estrogen-mediated sex differences in licking to sucrose that occurred only with comparatively dilute sucrose solutions [5]. In fact, during EB treatment, licking by OVX rats to 0.025 M sucrose did not differ from licking to water. These findings raise the possibility that estrogen elevates the

threshold for gustatory detection of sucrose. The present study addressed this possibility by evaluating the effect of estrogen on the generalization of a taste aversion conditioned to 0.2 M sucrose to dilute sucrose solutions.

As expected, rats showed a pronounced aversion to 0.2 M sucrose after it had been paired with LiCl injection and a robust preference for 0.2 M sucrose after it had been paired with ISO injection (Fig. 1). This initial pairing and conditioned taste aversion testing was conducted prior to OIL or EB treatment. Thus, there was no pre-existing group difference in the taste aversion conditioned to 0.2 M sucrose or in the preference for the concentrated sucrose solution when it had not been paired with LiCl.

When 0.2 M sucrose previously had been paired with LiCl, rats showed a pronounced aversion to 0.075 M sucrose regardless of whether they were treated with OIL or EB (Fig. 2A, left). Moreover, when 0.2 M sucrose previously had been paired with ISO, preference scores for 0.075 M sucrose were similarly high regardless of whether rats were treated with OIL or EB (Fig. 2A, left). Together, these results suggest that EB did not affect the ability to generalize a taste aversion conditioned to 0.2 M sucrose to a less concentrated sucrose solution, nor did it alter the preference for the less concentrated sucrose solution when 0.2 M sucrose previously had been paired with ISO.

Previous pairing of 0.2 M sucrose with LiCl also produced a pronounced aversion to 0.025 M sucrose, but only in rats that were treated with OIL (Fig. 2A, right). Similarly, when 0.2 M sucrose previously had been paired with ISO, preference scores for 0.025 M sucrose were high, but only in rats that were treated with OIL. Regardless of whether 0.2 M sucrose previously had been paired with LiCl or with ISO, rats that were treated with EB showed a similar preference for 0.025 M sucrose (Fig. 2A, right). Moreover, 0.025 M preference scores by both groups of EB-treated rats were greater than that by OIL-treated rats that previously had been injected with LiCl, but less than that by OIL-treated rats that previously had been injected with ISO. Thus, rats that were treated with OIL generalized the LiCl-induced taste aversion to 0.025 M sucrose but showed a robust preference for 0.025 M sucrose after previous ISO injection. In contrast, EB not only disrupted the generalization of a conditioned taste aversion to 0.025 M sucrose, but also decreased the preference for 0.025 M sucrose. In fact, mean preference scores for 0.025 M sucrose during EB treatment were in the range (0.4–0.6), attributable to chance and indicative of indifference. These findings suggest that during EB treatment rats are unable to discriminate 0.025 M sucrose from water and thus are consistent with an estrogen-mediated elevation in the detection threshold for sucrose.

Additional two-bottle tests conducted approximately two weeks after EB or OIL treatment showed that when 0.2 M sucrose had been paired with ISO, preference scores for both 0.075 and 0.025 M sucrose were comparably high regardless of whether rats previously had been treated with

OIL or EB (Fig. 2B, left, right). Similarly, when 0.2 M sucrose had been paired with LiCl, all rats showed an aversion to both 0.075 and 0.025 M sucrose (Fig. 2B, left, right). These tests were conducted when EB levels likely had decreased to levels comparable to those in non-cycling, OVX rats [30], suggesting that the elevated threshold for sucrose detection observed during EB treatment is attributable to increased circulating levels of EB. The time frame of the present study does not allow us to assess whether the comparatively short-term alterations in sucrose detection are a consequence of the genomic or non-genomic effects of estrogen [11]; nonetheless, it is clear that these estrogen-mediated alterations are short-lived.

Two points are worthy of additional mention. First, during EB treatment, 0.025 M sucrose preference scores by rats that previously had been injected with ISO tended to be greater than those by EB-treated rats that previously had been injected with LiCl, although the differences were not statistically significant (Fig. 2A, right). We chose 0.025 M sucrose as the ‘subthreshold’ concentration based on our recent findings [5]. However, previous studies using male rats showed that, whereas the effective sucrose concentration for discrimination tasks involving reward is 0.013 M [19], detection thresholds in tasks involving negative consequences are substantially lower, ranging from 0.0015–0.001 M [23,2]. These observations raise the possibility that estrogen effects on the generalization of a conditioned taste aversion may be more pronounced with even more dilute sucrose solutions. A thorough assessment of the shift in the sucrose detection threshold during EB treatment will require additional studies using a range of dilute sucrose solutions. Ultimately, additional approaches that allow finer resolution, such as operant threshold testing (see e.g., Refs. [2,10]) will be necessary to determine the effect of estrogen on the absolute threshold for sucrose detection.

Second, when 0.2 M sucrose previously had been paired with LiCl, preference scores for both 0.075 and 0.025 M sucrose tended to increase over time (Fig. 2A and B) regardless of whether rats had been treated with OIL or EB. Thus, the generalization of the conditioned aversion to both 0.075 and 0.025 M may have decreased somewhat over time, which typically is associated with extinction of a conditioned taste aversion. Interestingly, preference scores increased to a greater degree in EB-treated rats. This observation suggests that estrogen may facilitate extinction, and is consistent with a recent report that conditioned eye-blink responses extinguish more rapidly in EB-treated OVX rats [20]. It is important to note that although extinction may be more rapid in rats that had been treated with EB, preference scores indicate that rats that had previously been injected with LiCl continue to show a robust aversion to 0.075 M sucrose in tests conducted ~two weeks after EB treatment. Thus, it is unlikely that enhanced extinction accounts for EB effects on the generalization of taste aversions conditioned to sucrose. Moreover, when rats

previously had been injected with ISO, the preference for 0.025 M sucrose was virtually unchanged two weeks after OIL treatment (during OIL: 0.85 ± 0.05 ; after OIL: 0.89 ± 0.06), but increased substantially two weeks after EB treatment. Preference scores indicate relative indifference to 0.025 M sucrose during EB treatment (0.62 ± 0.10), but a robust preference two weeks later (0.88 ± 0.06), further supporting an EB-mediated shift in the sucrose detection threshold.

The results from the present study, therefore, suggest that estrogen produces a short-term increase in the sucrose detection threshold. But is this effect specific to the taste of sucrose or does it involve other sweet tastes? A number of investigators have reported sex differences in preferences for saccharin and glucose by rats [29,27,12] that appear to be mediated by estrogen [12]. However, at present it is unknown whether differences in sweet preferences, particularly when evaluated in long-term tests, are attributable to a difference in the threshold for detection of sweet tastes. In either case—whether specific to sucrose or involving sweet tastes in general—the mechanism underlying the estrogen-mediated increase in detection threshold is unknown.

Given the release of estrogen from the ovaries into systemic circulation (or elevated circulating levels during estrogen replacement), it seems reasonable to assume that estrogen may act on peripheral components of the gustatory system; however, the effect of estrogen on lingual taste receptors has not been examined. Our pilot data suggest that estrogen does not affect the numbers of taste papillae (data not shown), but this gross measure does not provide information about specific types of taste receptors or ion channels that may be affected by estrogen. The idea of hormone effects on taste receptors is not unprecedented, as the adrenal hormone, aldosterone, increases the number of amiloride-sensitive Na^+ channels in taste receptors, as well as the responsiveness of isolated taste receptor cells [21]. There also is precedent for hormone effects on gustatory nerve responses [16], suggesting that estrogen-mediated differences in sucrose detection threshold may involve peripheral neural signals. In this regard, our recent study and preliminary data [5,6] showed sex differences in the behavioral responses to the taste of NaCl, as well as estrogen-mediated differences in whole-nerve chorda tympani responses to NaCl. At present, however, nothing is known about the effect of estrogen on gustatory nerves, such as the greater superficial petrosal nerve, that carry sweet taste information to the central nervous system.

Sex differences in response to sucrose taste that may involve estrogen have been reported in electrophysiological studies of the pontine parabrachial nucleus [7,8]. These observations of altered neuronal activity in a central gustatory relay nucleus are consistent with ‘upstream’ effects of estrogen-mediated changes in peripheral gustatory input related to sweet taste; however, estrogen is a steroid hormone that readily can access the brain. Moreover, estrogen receptors have been reported in a number of brain

areas, including those involved in gustatory processing (e.g., [24]), and estrogen is known to affect gene expression within the central nervous system (e.g. [11]). It is therefore possible that estrogen-induced increases in the sucrose detection threshold are attributable, in part, to direct estrogen effects on central gustatory pathways, and ongoing studies are investigating this possibility.

In summary, in the present study conducted to examine the possibility that estrogen elevates the threshold for gustatory detection of sweet taste, we found that EB-treated OVX rats generalized a taste aversion conditioned to 0.2 M sucrose to 0.075 M sucrose, but did not generalize the aversion to 0.025 M sucrose. Two weeks later, when EB likely had cleared the system [30], rats generalized the aversion to both 0.075 and 0.025 M sucrose. Thus, it appears that rats are unable to discriminate 0.025 M sucrose from water during EB treatment, which suggests that there are short-term, estrogen-mediated increases in the detection threshold for sucrose taste. It should be noted that Than et al. [26] reported that, in reproductively cycling human females, high estrogen levels are associated with lower sucrose detection thresholds. These findings conflict with the results of the present study, as well as with our recent report [5] and suggest the possibility of species differences in the effect of estrogen on the sucrose detection threshold. Alternatively, methodological differences such as testing procedures or differences in the timing of estrogen fluctuations may contribute to the discrepant findings. Additional studies will be necessary to determine the mechanism by which estrogen alters the detection threshold for sucrose, whether the detection of other sweet tastes also is affected by estrogen, and whether there are species-specific effects of estrogen on the detection of sweet tastes.

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