Taste reactivity responses in rats: influence of sex and the estrous cycle

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Clarke, Sharon N. D. A., and Klaus-Peter Ossenkopp. Taste reactivity responses in rats: influence of sex and the estrous cycle. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R718–R724, 1998.—Gonadal hormones (e.g., estradiol) may regulate feeding by producing a shift in the taste or palatability of food items. This study examined the impact of endogenous gonadal hormones on palatability by investigating sex differences in taste responsivity, as well as the effect of the estrous cycle on taste responsivity, in a rodent model. In the taste reactivity test, male and female Long-Evans rats received a brief (1 min) intraoral infusion of one of three tastants: sucrose (0.3 M), quinine (0.0003 M), and a sucrose-quinine mixture (0.3 M sucrose and 0.0003 M quinine). Statistical analyses indicated that female rats tested during diestrus or proestrus produced significantly more ingestive responses than did male rats and fewer aversive responses than did both male rats and female rats tested during estrus or metestrus (P < 0.05). These results indicate a sex difference in taste responsivity in the rat that is modulated by the reproductive status of female rats. This finding implies a role of gonadal hormones in the regulation of taste responsivity in the rat.

Taste reactivity test; ingestive responses; aversive responses; estradiol; ingestive behavior

Behavioral investigations of taste responsivity in the rat have found sex differences in taste preference, as well as fluctuations in taste preference and acuity that are related to natural changes in hormonal levels that occur during the estrous cycle and pregnancy (1, 10). These observations suggest that gonadal hormones such as estradiol may modulate taste responsivity in the rat, via organizational and activational mechanisms.

Some of the earliest evidence of a possible sex difference in taste responsivity in the rat is based on sex differences in food intake (1). Generally, male rats weigh more and eat more than do female rats, particularly after puberty (3, 26). According to Laviano et al. (17), this sex difference in food intake is attributable to a disparity in the number and the size of meals consumed by both sexes. Assessment of the food intake of rats (Fischer 344) over a period of 44 days showed that male rats had a relatively constant level of food intake, characterized by an increase in meal size but a decrease in meal number over time. In contrast, female rats displayed fluctuations in both meal number and size that covaried with the different stages of their estrous cycles (17).

Gonadal hormones, especially estradiol, appear to be largely responsible for the sex difference in ingestive behavior in rats. For example, both central and peripheral administration of estradiol suppress food intake (2, 23). In addition, ovariectomy results in increased food intake, which is reversed by the administration of estradiol. Furthermore, during the estrous cycle food intake is elevated when estradiol levels are basal (e.g., metestrus) and is reduced when estradiol levels are high (e.g., proestrus) (9, 24).

Estradiol may regulate ingestive behavior in the rat by modifying the taste (palatability) system. Support for this hypothesis is derived from two sources. First, anatomic investigations have shown that estradiol is located in areas of the central nervous system that affect both eating and taste preferences, including the ventromedial hypothalamus (12) and the medial amygdala (15). Second, behavioral investigations have revealed sex differences in taste preferences acuity for “sweet,” “salty,” and “bitter” tastants, which are highly correlated with changes in the hormonal status of female rats during the estrous cycle (11). Of course, the characterizations of tastants as “bitter,” “salty,” or “sweet” is based on human perception of these taste qualities.

Using a choice paradigm, Valenstein et al. (25) reported that female rats consumed more of a nutritive, 3% glucose solution and a nonnutritive, 0.25% Na-saccharin solution in comparison to male rats. This sex difference in level of intake was interpreted as a sex difference in taste preference for the “sweet” glucose and Na-saccharin tastants. Using a similar measure of preference, other studies have determined that the female preference for such tastants appears after puberty, is eliminated by ovariectomy, but can be reinstated with the administration of estradiol (27). Thus the sex difference in taste preferences for “sweet” taste qualities in rats appears to be regulated by gonadal hormones, particularly estradiol.

Female rats also exhibit a greater preference for saline solutions relative to males, but this taste preference appears to be modulated by testosterone. Krecsk (16) observed that female rats consumed more of a 3% NaCl solution than did males. Administration of testosterone (1 mg/kg) to 2-day-old females resulted in a male-like pattern of intake of the saline solution. In contrast, administration of testosterone to 12-day-old females did not affect the sex difference in salt intake. This observation provides further support for the modulatory role of gonadal hormones in taste preferences in rats.

Investigations of sex differences in aversions to quinine and saccharin-quinine tastants have yielded mixed results. Nance et al. (18) reported that male rats displayed stronger aversions than did female rats to a saccharin-quinine solution (0.25% saccharin solution and quinine sulfate). Wade and Zucker (30) did not observe such a sex difference but did report that intact
females had stronger aversions to quinine than did ovariectomized females. The inconsistencies in these observations may be due to the difference in the tastants which were investigated. Nance et al. (18) focused on a saccharin-quinine mixture whereas Wade and Zucker (30) used only a quinine tastant. Despite the differences, however, these observations suggest that aversions to such tastants might also be modulated by gonadal hormones.

Zucker (31) hypothesized that sex differences in taste preferences are due to the impact of ovarian hormones on the taste system of the female rat, and to a lesser extent the impact of testosterone on the taste system of the male rat. This is evidenced by changes in taste preferences across the estrous cycle. Female rats exhibit strong preferences for saccharin and strong aversions to quinine, both of which are altered during periods of marked changes in hormonal levels (e.g., pregnancy, ovariectomy) (27–30). This correlation between changes in taste preferences and aversions, and hormonal levels suggests an impact of ovarian hormones on taste responsivity.

Data obtained from electrophysiological examinations of taste responsivity in the rat also support findings obtained with simple intake measures. For example, Di Lorenzo and Monroe (6) reported that single unit recording in the parabrachial nucleus (PbN) revealed that pregnant and nonpregnant rats showed greater responses to sweet stimuli (0.5 M sucrose, 0.004 M Na-saccharin) relative to male rats. Furthermore, there was a greater proportion of units in these female rats that responded best to sweet stimuli. This observation suggests that there might be a sex difference in the neural sensitivity to sweet tastants in the rat. In another study, these authors reported that unit recordings in the PbN of ovariectomized rats showed a greater response to quinine (0.1 M) in comparison to the responses produced by male and intact (diestrus) female rats (7). Collectively, the observations made with electrophysiological investigations of taste and palatability support the hypothesis that these constructs are affected by the sex of the animals and their reproductive status.

Traditional behavioral investigations of sex differences in taste responsivity and changes in taste responsivity during the estrous cycle have typically relied on level of intake as an indirect measure of these phenomena (e.g., Ref. 21). In the standard intake paradigm, a taste stimulus is presented either alone or in conjunction with a neutral stimulus, usually water. The relative level of consumption of the taste stimulus, especially in comparison to water, is then interpreted as evidence of preference for, or aversion to, that taste quality. Intake measures, however, can be influenced by factors other than taste (e.g., hunger, satiety, fear), which brings into question their validity as a direct measure of taste responsivity (4, 8, 13).

The taste reactivity test (TRT), developed by Grill and Norgren (14), circumvents problems associated with intake tests by providing a behavioral measure of the palatability of a tastant. This test is based on quantification of species-specific orofacial and somatic responses to a tastant. In the standard TRT a taste stimulus is infused directly into the mouth of a rat through an intraoral cannula. The somatic and orofacial behavioral responses elicited by the infused tastant are videotaped and analyzed for frequency of occurrence. Three categories of taste reactivity responses have been used in the TRT: ingestive responses consisting of behaviors resulting in consumption of the taste stimulus; rejection or aversive responses, which result in the active removal of the tastant from the oral cavity; and a passive-aversive response (passive drip), which results in the passive removal of the taste stimulus from the oral cavity during the forced-exposure procedure. Thus the TRT is able to assess both the consumption and rejection of tastants by quantifying the number, duration, and pattern of taste reactivity responses, and in this manner provides an assessment of the palatability of a taste stimulus.

There are additional advantages offered by the TRT test in the assessment of taste responsivity. Because it is a forced-exposure procedure it does not require any food- or fluid-deprivation and is usually conducted in food- and fluid-replete rats. Thus any effects that deprivation conditions might have on taste responsivity are eliminated. In addition, only small amounts of the test fluid are infused at a controlled rate during the TRT, providing the ability to assess preingestional responses to the tastant and minimizing or eliminating the effect of postingestional consequences on taste responsivity. Finally, the TRT allows for the assessment of different taste qualities, as well as varying concentrations of the same taste quality, in the same subject and easily lends itself to the investigation of conditioned responses to taste (e.g., Refs. 4, 19, 20).

The taste reactivity paradigm has been used to investigate sex differences in salt preference in rats (10). The results of this study indicated that female rats exhibited a stronger two-bottle preference for NaCl solutions ranging from 0.03 M to 1.0 M than did age-matched controls. In terms of taste reactivity responses, however, female rats displayed more ingestive responses to a 0.15 M and 0.3 M NaCl and more aversive responses to 1.0 M NaCl, relative to age-matched males. Thus the observations obtained with the taste reactivity paradigm supported the results obtained with the more traditional methods, but they also revealed subtle differences not apparent with these intake-based procedures.

Previous research, based on traditional intake paradigms, has suggested that sex differences in ingestive behavior in the rat may reflect sex differences in taste responsivity. Because the TRT provides a direct, behavioral measure of taste responsivity and palatability, this paradigm was used to investigate sex differences in taste reactivity responses, as well as the relationship between the taste reactivity behaviors and natural changes in gonadal hormone levels during the estrous cycle in the laboratory rat.
MATERIALS AND METHODS

Subjects. Seventy-two naive, adult, Long-Evans hooded rats (Charles River) were used in this study (30 males, 275–350 g, and 42 females, 250–275 g). They were housed individually in stainless steel cages and were kept in a temperature-controlled room (21 ± 1°C), with a 12:12-h light-dark cycle (lights on between 0800 and 2000). Rat chow (Agway) and tap water were available ad libitum.

Intraoral cannulation. One week after arriving in the laboratory, all of the rats were implanted with intraoral cannulas. Rats were anesthetized with pentobarbital sodium (Somnotol: males, 50 mg/kg ip; females, 35 mg/kg ip). A 15-gauge, thin-walled, stainless steel needle was inserted through the skin in the dorsal midneck region, brought subcutaneously behind the ear along the inside of the cheek, and exited through the soft part of the cheek behind the first molar. With the needle in place, polyethylene tubing (PE-90) was inserted through the barrel of the needle, the needle was then withdrawn, and the tubing was secured at the neck by a 20-gauge intramedic adapter and by a plastic washer (5 mm in diameter), which was secured in the mouth by heat flaring the end of the tubing with a soldering iron. To prevent infection, rats received a single injection of penicillin (Pen- long 20,000 U im) immediately after surgery. Rats were given 5 days to recover from surgery before behavioral testing began.

TRT. Training and testing were conducted in a transparent Plexiglas chamber (29 x 25 x 29 cm). At the start of each session, the intraoral cannulas were flushed with distilled water and rats were placed individually in the test chamber. All intraoral infusions were delivered via an infusion hose (PE-90 tubing, 1 m in length) and an infusion pump (model 341-A; Sage Instruments, Cambridge, MA) at a constant rate of 0.78 ml/min. A mirror mounted at a 45° angle below the transparent floor of the test chamber provided a view of the ventral surface of the rat. Orofacial and somatic responses to the end of the tubing with a soldering iron. To prevent infection, rats received a single injection of penicillin (Pen-long 20,000 U im) immediately after surgery. Rats were given 5 days to recover from surgery before behavioral testing began.

Taste reactivity scoring. The videotapes of the taste reactivity test were scored at one-fifth regular speed to facilitate the identification of behaviors. Taste reactivity responses were scored according to the categories described by Grill and Norgren (14) and elaborated on by Ossenekopp and Eckel (19). Total ingestive responses consisted of the combined frequency of two ingestive behaviors: tongue protrusions (midline and lateral extensions of the tongue) and rhythmic mouth movements (movements of the mouth without extensions of the tongue). Total aversive responses included the combined frequency of four behaviors: chin rubs (forward projection of the head with the chin rubbing against the substrate), gapes (triangular, wide opening of the mouth), forelimb flails (rapid shaking of the forelimbs), and head shaves with fluid expulsion. The passive-aversive response consisted only of the frequency of passive drips (number of drops of the test solution that dripped from the mouth to the floor when the rat was not actively rejecting the solution).

Testing procedure. Rats were habituated to the TRT procedure on three consecutive days. First, the intraoral cannulas were gently flushed with distilled water, and then rats were placed in the test chamber for 15 min followed by a 1-min infusion (0.78 ml/min) of distilled water.

On day 4, rats were assigned to experimental groups based on sex and infused tastant. Three tastants were used in this study: sucrose (0.3 M), quinine (0.003 M), and a sucrose-quinine mixture (0.3 M sucrose and 0.003 M quinine). Tastants were made using reagent-grade chemicals and distilled water. Each rat received a single infusion of one of the three tastants (depending on group assignment) at the rate of 0.78 ml/min. All testing was conducted between 9:00 and 11:00 AM.

The concentrations selected were based on previous investigations in our laboratory, which found that they elicit reliable patterns of ingestive and aversive taste reactivity responses (5). In addition, the rate of infusion used in this study approximated the infusion rate originally used by Grill and Norgren (14).

Estrous cycle. The estrous stage of each female rat was determined by taking a vaginal smear just before taste reactivity testing (22). Cells types in the smear were subsequently examined under a microscope to determine the estrous stage. Proestrus was identified by the scattered distribution of predominantly cornified cells and the presence of nucleated cells. Estrus was identified by the dumping and dense distribution of cornified cells. Metestrus was identified by the presence of leukocytes and fewer cornified cells, and diestrus by the reduction in cell number and the presence of leukocytes.

Data analysis. The data were analyzed using analysis of variance procedures. Post hoc comparisons were performed using Tukey's honestly significant differences test. All hypothesis tests used α = 0.05 as the criterion for significance.

RESULTS

Estrous stage. Examination of the vaginal smears obtained just before taste reactivity testing indicated that 50% (n = 21) of the females used were tested the morning of estrus or metestrus, when estradiol levels are reported to be low (22). The remainder were tested the morning of diestrus or proestrus, when estradiol levels are reported to be high (22). Thus statistical comparisons were made between male rats, female rats tested during estrus/metestrus (E/M), and female rats tested during diestrus/proestrus (D/P). Data from the following groups were analyzed: Sucrose-Males (n = 12), Sucrose-E/M Females (n = 8), Sucrose-D/P Females (n = 8), Quinine-Males (n = 10), Quinine-E/M Females (n = 8), Quinine-D/P Females (n = 8). Error bars represent SE.
Females (n = 56), Quinine-D/P Females (n = 57), Sucrose-Quinine-Males (n = 58), Sucrose-Quinine-E/M Females (n = 7), Sucrose-Quinine-D/P Females (n = 6).

Group comparisons: ingestive score. The mean frequency of total ingestive responses (mouth movements and tongue protrusions) elicited by all three tastants is depicted in Fig. 1. Statistical analysis identified a significant main effect of group \[ F(2,63) = 4.05, P = 0.02 \]. Post hoc comparisons indicated that D/P females exhibited significantly more ingestive responses than did males. There was also a main effect of tastant \[ F(2,63) = 90.89, P = 0.001 \]. As expected, the sucrose solution elicited the most ingestive responses and the quinine solution the least, with the sucrose-quinine solution intermediate. The group \times\ tastant interaction failed to reach significance \[ F(4,63) = 2.34, P = 0.07 \].

Individual ingestive responses. For mouth movements, there was a main effect of group \[ F(2,63) = 4.93, P = 0.01 \]. E/M females displayed more mouth movements than did D/P females. There was also a main effect of tastant \[ F(2,63) = 8.40, P = 0.001 \], but the group \times\ tastant interaction was not significant \[ F < 1; \text{ Fig. 2A} \].

Analysis of tongue protrusion responses identified a significant main effect of group \[ F(2,63) = 5.20, P = 0.008 \], a main effect of tastant \[ F(2,63) = 63.4, P = 0.001 \], as well as a significant group \times\ tastant interaction \[ F(4,63) = 4.06, P = 0.005; \text{ Fig. 2B} \]. Post hoc comparisons revealed that D/P females produced significantly more tongue protrusions during the infusion of the sucrose solution than did the other two groups.

Passive drips. For the passive drip data (Fig. 3B), there was no significant group main effect \[ F(2,63) = 2.30, P = 0.10 \], but there was a significant tastant main effect \[ F(2,63) = 40.27, P = 0.001 \]. As expected, the sucrose solution produced the lowest frequency of passive drips, the quinine solution the highest, and the sucrose-quinine solution intermediate levels of this response. The group \times\ tastant interaction failed to reach significance \[ F(4,63) = 2.44, P = 0.056 \].

Aversive score. Analysis of the total aversive responses revealed a significant group main effect \[ F(2,63) = 3.37, P = 0.04; \text{ Fig. 3A} \]. D/P females exhibited significantly fewer total aversive responses relative to the E/M females and males. There was also a tastant main effect \[ F(2,63) = 20.13, P = 0.001 \]. As expected, the sucrose tastant elicited the lowest level of
aversive responding, and the quinine solution the highest, with the sucrose-quinine tastant intermediate. The group × tastant interaction, however, was not significant (F < 1).

Individual aversive responses. Analysis of the data for individual aversive responses of chin rubs, head shakes, and forelimb flails failed to show any significant group main effects or group × tastant interactions (P values > 0.05) (Fig. 4, A, C, and D). However, a significant group main effect was obtained for gapes [F(2,63) = 4.27, P = 0.02; Fig. 4B]. D/P females exhibited significantly fewer gapes than did the other two groups. The group × tastant interaction for gapes was not significant [F(4,63) = 1.80, P = 0.14].

DISCUSSION

This study demonstrated that there are sex differences in the taste reactivity responses of rats that are predicated on the stage of the reproductive cycle of the female rats. In the present study, the pattern of taste reactivity responses displayed by the D/P females (with presumed high levels of estradiol) differed significantly not only from males (hence the sex difference) but also from the E/M females (with presumed low levels of estradiol). These observations strongly suggest that any discussion of sex differences in taste responsivity in the rat must take into consideration the stage of the estrous cycle of the female rats at time of testing.

Previous studies, relying on intake paradigms as indirect measures of taste responsivity, have reported changes in taste preferences during the estrous cycle of the rat (e.g., Refs. 29, 30), putatively related to fluctuations in levels of estradiol. Briefly, high levels of estradiol produce an increase in the preference for palatable solutions, whereas the inverse is true for low levels of estradiol (28, 29). The results of the present study are in part consistent with these previous observations, within the context of the estrous cycle. For example, the D/P females produced significantly more total ingestive responses (mouth movements plus tongue protrusions) than did males. In contrast, there was no significant difference between the frequency of total ingestive responses produced by the E/M females and the male rats. Because ingestive responses index the preference of a tastant, the findings of our study suggest that the D/P females exhibited a greater preference for the infused tastants in comparison to the other two groups, which did not differ from each other.

Fig. 4. Group mean frequencies of individual aversive responses elicited by a 1-min infusion of sucrose, sucrose-quinine, and quinine. A: chin rubs. B: gapes. C: head shakes. D: forelimb flails. Error bars represent SE.
Analysis of the individual ingestive responses of mouth movements and tongue protrusions further highlights the putative effect of fluctuations of hormonal levels on taste responsivity in the rat. Notably, E/M females (with presumed low levels of estradiol) produced significantly more mouth movements overall than D/P females. On the other hand, D/P females produced significantly more tongue protrusions than did E/M females and males, but only to the sucrose tastant. These observations reinforce the argument that discussions of sex differences in taste responsivity in the rat must take into account the stage of the estrous cycle of females at time of testing.

Examination of the aversive taste reactivity responses also provide evidence of the effect of the estrous cycle on taste responsivity in the rat. The D/P females produced less aversive responses (primarily gapes) than did both the E/M females and males to both the sucrose-quinine and quinine solutions. Thus D/P females responded less negatively to the quinine and sucrose-quinine solutions than did E/M females and males. According to previous research, high levels of estradiol produce an increase in aversion to unpalatable solutions like quinine (e.g., Refs. 28, 29). Clearly these previous observations are at variance with the data of this study. However, the observed pattern of aversive taste reactivity responses complements the observed pattern of ingestive taste reactivity responses in the present study. The D/P females displayed the highest level of ingestive responses, and the lowest level of aversive responses to the infused tastants.

Furthermore, much of the evidence of the effect of estradiol on taste preferences has been garnered from studies that rely on magnitude of intake as an indirect measure of palatability. It is possible that high levels of estradiol might produce an avoidance of a taste stimulus that could be based on factors other than palatability, such as fear or satiety. The data from the present taste reactivity test suggest such a distinction, but clearly this issue warrants further investigation. Finally, as has been hypothesized, it is possible that the strong taste preferences and reduced food intake that characterize periods of elevated estradiol reflect a hedonic mechanism designed to regulate diet selection.

Examination of the individual aversive responses reinforces the conclusions drawn from analysis of the composite aversive score. There were no significant differences in the frequencies of chin rubs, head shakes, and forelimb flails among the E/M females, the D/P females, and the males. In contrast, both the E/M females and the males produced significantly more gapes to the sucrose-quinine and quinine solutions than did the D/P females.

It has been proposed that taste reactivity responses can be ordered along a unidimensional scale of palatability, with tongue protrusions reflecting extreme acceptance and gapes and forelimb flails representing extreme rejection of a taste stimulus (4). Examination of the pattern of individual taste reactivity responses is informative in terms of reflecting motivational states (4). In the present study, D/P females, with presumed high levels of estradiol, produced significantly more tongue protrusions and fewer gapes than did E/M females and males. Conversely, E/M females produced more mouth movements than did D/P females. This pattern of taste reactivity responses suggests that the infused tastants were more palatable or less aversive to the D/P females than to the E/M females and the males.

In summary, this study indicates that taste responsivity in the rat is modulated by the stages of the estrous cycle and the associated variations in hormonal levels. Whereas E/M females (with presumed low levels of estradiol) tended to produce a pattern of taste reactivity responses comparable to that produced by males (e.g., fewer tongue protrusions to sucrose but more gapes to quinine or sucrose-quinine), the D/P females (with presumed high levels of estradiol) tended to differ from both of these groups, especially the males. This observation suggests a possible modulating influence of estradiol on palatability. Thus any examination of sex differences in intake and taste responsivity in the rat must take into consideration the stage of the estrous cycle of the females at time of testing.

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