

Sex Differences in Electrophysiological and Behavioral Responses to NaCl Taste

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We tested the hypothesis that sex differences in preference for NaCl are attributable to estrogen-mediated alterations in gustatory processing. Electrophysiological responses of the chorda tympani nerve to NaCl were blunted by estrogen treatment in ovariectomized female rats, suggesting that females are less sensitive to concentrated NaCl solutions during high estrogen conditions. In contrast, after a taste aversion was conditioned to 150-mM NaCl, estrogen- and oil-treated ovariectomized rats generalized the aversion to a lower concentration of NaCl than did males, suggesting that females are more sensitive to the taste of dilute NaCl solutions regardless of estrogen. Thus, sex differences in NaCl preferences may be attributable to differences in NaCl taste processing that involve both acute and developmental effects of estrogen.

Keywords: estrogen, gustation, sodium chloride, electrophysiology, detection threshold

Males and females differ in their food preferences (Curtis, Davis, Johnson, Therrien, & Contreras, 2004; Frye & Demolar, 1994; Valenstein, Kakolewski, & Cox, 1967; Wansink, Cheney, & Chan, 2003), and accumulating evidence suggests a role for reproductive hormones in these differences. Many women report that their preference for and consumption of specific foods, including salt-rich foods, varies across the menstrual cycle (Bowen & Grunberg, 1990; Frye & Demolar, 1994; Kuga, Ikeda, & Suzuki, 1999; Than, Delay, & Maier, 1994), an effect that also occurs in rats during estrous cycling (Danielson & Buggy, 1979; Kananka, Dua-Sharma, & Sharma, 1979). Sex differences in NaCl intake by rats are well known (Chow, Sakai, Witcher, Adler, & Epstein, 1992; Flynn, Schulkin, & Havens, 1993; Krecek, Novakova, & Stibral, 1972; Wolf, 1982) and become pronounced at sexual maturity (Chow et al., 1992; Krecek et al., 1972; Scheidler, Verbalis, & Stricker, 1994), suggesting a role for gonadal hormones. However, assessment of the role of specific reproductive hormones in sex differences in NaCl intake and preference by rats has been complicated by conflicting findings. There is disagreement in regard to whether the differences are attributable to estrogen (Curtis et al., 2004; Danielson & Buggy, 1979; Kensicki, Dunphy, & Ely, 2002; Scheidler et al., 1994) or to testosterone (Chow et al., 1992; Krecek, 1973). In fact, even among investigators who agree that estrogen affects NaCl intake by rats, there is no consensus on whether the effect is stimulatory (Curtis et al., 2004; Kensicki et al., 2002; Wolf, 1982) or inhibitory (Danielson & Buggy, 1979; Scheidler et al., 1994; Stricker, Thiels, & Verbalis, 1991).

Methodological considerations may contribute to these contradictory findings, particularly during long-term tests over the course of hours or days. Normal reproductive cycling (or estrogen replacement) is associated with alterations in fluid balance (Stachenfeld, Silva, Keefe, Kokoszka, & Nadel, 1999), cardiovascular function (Pechere-Bertschi & Burnier, 2004), and gastrointestinal transit (Bond, Heitkemper, & Perigo, 1996). These alterations may influence the initial NaCl intake and/or affect the magnitude or duration of postingestive consequences after the initial ingestion, thereby influencing NaCl consumption later in the test. Clearly then, results obtained during long-term tests that have been the mainstay of most examinations of sex differences in NaCl intake and preference must be interpreted cautiously. Moreover, estrogen effects on NaCl intake by rats depend, to a large extent, on whether NaCl is consumed during ad libitum conditions or after experimental manipulations that produce NaCl intake. Sex differences in physiological responses to experimentally induced sodium loss would be expected to result in predictable differences in NaCl intake. However, despite reports that estrogen augments hormonal and renal responses to changes in body sodium (Ota, Crofton, Liu, Festavan, & Share, 1994; Wang et al., 1995) and that treatment with the diuretic–natriuretic drug Furosemide produces less urinary sodium loss in females (Wolf, 1982), female rats nonetheless consume more NaCl solutions after Furosemide treatment than do males (Chow et al., 1992; Scheidler et al., 1994; Wolf, 1982). To date, therefore, the role of estrogen in sex differences in NaCl intake and preference by rats is unclear, and the underlying mechanism in such sex differences remains to be determined.

Flynn and colleagues (1993) reported that, in taste reactivity tests, female rats showed fewer aversive responses as well as more positive, ingestive responses to the taste of concentrated NaCl solutions than did males. Moreover, in our recent study using very brief (10-s) behavioral tests to minimize postingestive consequences (Curtis et al., 2004), we found that, unlike male rats, female rats did not decrease licking to concentrated NaCl solutions that were mixed in dilute sucrose. These observations of sex

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differences in behavioral responses to the taste of NaCl, in conjunction with the potent role of taste in ingestion, suggest that sex differences in NaCl taste processing may contribute to differences in NaCl intake. Surprisingly, only a few studies (e.g., Di Lorenzo & Monroe, 1989, 1990; Kuga et al., 1999) have investigated the possibility of sex differences in sensory processing of NaCl taste. We focused on peripheral mechanisms to address this possibility and tested the hypothesis that estrogen-dependent sex differences in gustatory neural responses to NaCl in rats underlie sex differences in NaCl preference and consumption.

General Method

Animals, Surgical Procedures, and Hormone Treatment

Adult Sprague–Dawley rats were individually housed in a temperature- and light-controlled colony room with food and water available ad libitum except as noted. Female rats were bilaterally ovariectomized (OVX) under sodium pentobarbital anesthesia (nembutal sodium; Abbott Laboratories, North Chicago, IL; 50 mg/kg body weight ip) and permitted to recover for ≥ 7 days before being given estradiol benzoate (EB; Sigma, St. Louis, MO; 10 $\mu\text{g}/0.1$ ml sc) or the oil vehicle (OIL; 0.1 ml sc) on an intermittent schedule as in our previous studies (Curtis et al., 2004; Curtis, Stratford, & Contreras, 2005). The Florida State University Institutional Animal Care and Use Committee approved all procedures.

Statistics

Data are presented as group means (\pm SEM). Statistical comparisons were made using one-, two-, or three-factor analysis of variance (ANOVA), with repeated measures (RM) where appropriate (Statistica; StatSoft, Tulsa, OK). Pairwise comparisons of statistically significant ($p < .05$) main effects or interactions were evaluated using Student–Newman–Keuls tests and specific planned comparisons were made using Bonferroni corrections.

Experiment 1: Chorda Tympani (CT) Whole Nerve Electrophysiology

Salt taste information is conveyed primarily by the CT branch of the facial nerve, which innervates the anterior two thirds of the tongue. The CT nerve is highly responsive to salt taste stimuli, responding in a concentration-dependent manner across a wide range of NaCl concentrations (e.g., Contreras & Frank, 1979; Nachman & Pfaffmann, 1963). The Na⁺ channel blocker amiloride reduces, but does not eliminate, CT responses to NaCl (Heck, Mierson, & DeSimone, 1984; Pittman & Contreras, 2002), suggesting that NaCl taste transduction involves an amiloride-sensitive component and an amiloride-insensitive component of the CT response. Accordingly, we recorded whole nerve electrophysiological activity from the CT in male and OVX rats with or without EB replacement to determine whether there are estrogen-mediated sex differences in gustatory neural responses to NaCl. In addition, to determine whether any sex differences were selective to the amiloride-sensitive component of the CT response, we recorded whole nerve activity during coadministration of amiloride and NaCl.

Method

Electrophysiological activity was recorded from the CT in urethane anesthetized (2.5 mg/kg ip) male ($n = 15$) and OVX rats. OVX rats were

given EB (OVX–EB; $n = 13$) or OIL (OVX–OIL; $n = 14$) on 2 consecutive days and were tested 48 hr after the second injection. NaCl solutions (75, 150, 300, 450, and 600 mM) were delivered across the anterior tongue for 10 s at 50 $\mu\text{l}/\text{s}$ using a custom fluid delivery system and computer software. The electrophysiological recording procedures, fluid delivery system, and computer software have been described in detail in our recent publication (Pittman & Contreras, 2002). Each NaCl stimulus was presented once with deionized water rinses (50 $\mu\text{l}/\text{s}$ for ≥ 60 s) before and after each stimulus to ensure that differences in responses were not attributable to differing levels of salivary Na⁺ (Contreras & Catalanotto, 1980). To avoid the potential complication of normalizing nerve responses to standard taste stimuli that also may have been affected by estrogen or differed by sex, CT responses to NaCl were expressed as increase from baseline (see, e.g., Contreras & Frank, 1979) and were calculated as area under the curve (AUC). CT responses also were evaluated using a multiple regression analysis (Statistica; StatSoft), using group (male, OVX–OIL, and OVX–EB) and NaCl concentration (converted to log values) as regressors.

Whole nerve activity was recorded during coadministration of amiloride (100 μM) and NaCl in subgroups of these rats (male, $n = 11$; OVX–OIL, $n = 10$; OVX–EB, $n = 9$). Amiloride inhibition of the CT response to each NaCl concentration was calculated as $[\text{NaCl response (AUC)} - \text{NaCl} + \text{amiloride response (AUC)}] \div [\text{NaCl response (AUC)}] \times 100$.

Results

Figure 1 shows CT responses to lingual NaCl stimulation from both a representative male and an OVX–EB rat. As illustrated in Figure 2, which shows mean CT responses expressed as increase from baseline and calculated as AUC, CT responses for all groups increased with increasing NaCl concentration. Moreover, the mean CT responses to all concentrations of NaCl were lowest in OVX–EB rats and greatest in male rats. Multiple regression analysis revealed that, overall, the relationship between CT responses and NaCl concentration and group was significant, $F(2, 207) = 28.34$, $p < .001$. CT responses showed a significant positive relationship to NaCl concentration, $t(207) = 6.86$, $p < .001$, and a significant negative relationship to group, $t(207) = 3.09$, $p < .01$. Consistent with the results from multiple regression analysis, a two-way RM ANOVA revealed that, independent of group, CT responses depended on concentration, $F(4, 156) = 61.75$, $p < .001$, with significantly greater responses to each NaCl concentration ($ps < .01$ – $.001$), as expected. Given the significant relationship between group and CT response revealed by the multiple regression analysis, it is surprising that neither the main effect of group nor the Group \times Concentration interaction were statistically significant; however, specific planned comparisons showed that CT responses to 600-mM NaCl in OVX–EB rats were significantly less than those in both OVX–OIL ($p < .05$) and male ($p < .001$) rats. Although responses in OVX–OIL rats to 600-mM NaCl tended to be intermediate between male and OVX–EB rats, they were not statistically different from those in males. One-way ANOVA revealed that baseline CT activity did not differ among the groups (see Table 1).

A two-way RM ANOVA revealed that amiloride inhibited CT responses to NaCl in all three groups (see Table 1). Overall, the amiloride inhibition of CT responses depended on NaCl concentration, $F(4, 108) = 4.60$, $p < .01$. Independent of group, the inhibition of CT responses to 600-mM NaCl was less than that to all other concentrations ($ps < .05$) except 450-mM NaCl, which also was less than that to 150-mM NaCl ($p < .05$). There was no

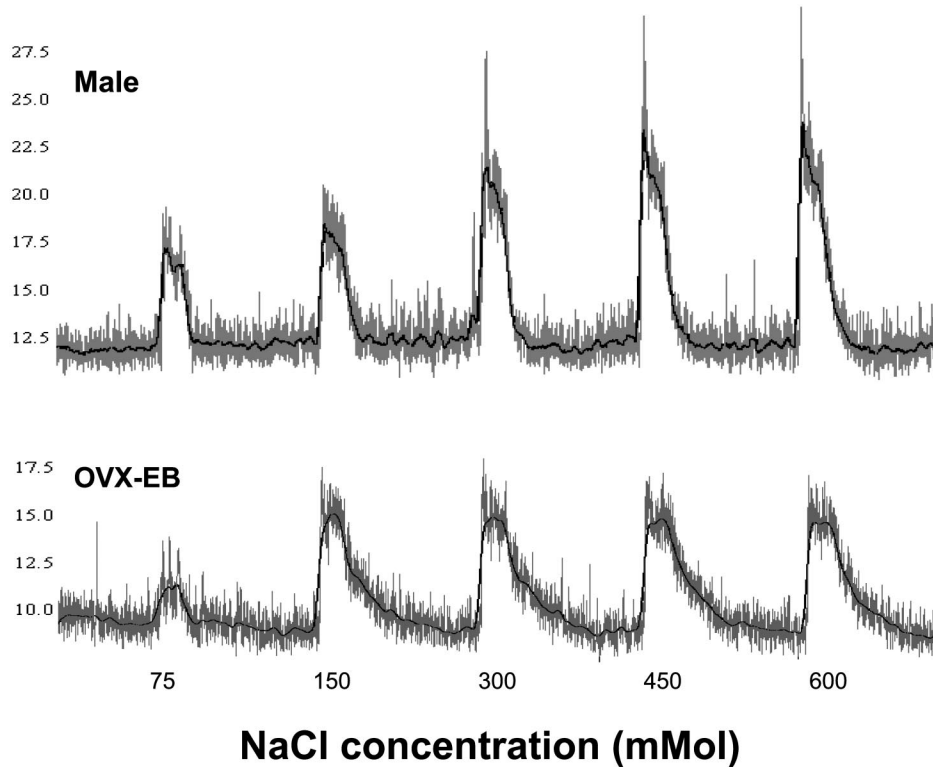


Figure 1. Chorda tympani whole nerve electrophysiological activity in a male rat (top) and an estradiol-benzoate-treated ovariectomized (OVX-EB; bottom) rat in response to 10-s lingual stimulation with ascending NaCl concentrations. Gray lines = raw nerve activity; black lines = rectified, integrated nerve activity.

effect of group and no Group \times Concentration interaction. In fact, the amiloride-induced inhibition of nerve responses was similar in all groups at all NaCl concentrations.

Discussion

CT responses to NaCl were concentration dependent overall; however, the relationship between CT responses and NaCl concentration was also influenced by sex and, more specifically, by estrogen. Whole nerve responses to NaCl were attenuated in OVX-EB rats, and the effect was particularly pronounced at 600-mM NaCl. At 600-mM NaCl, CT responses were greatest in male rats and lowest in OVX-EB rats. Given that the magnitude of the CT response is related to the intensity of NaCl taste stimuli, these observations are consistent with results from our recent behavioral study (Curtis et al., 2004) in which we observed an inverse pattern of estrogen-dependent sex differences in intake of a very concentrated (500 mM) NaCl solution (see also Kensicki et al., 2002; Wolf, 1982).

The sex differences in CT responses to NaCl were not due to baseline differences in activity levels (see Table 1). Moreover, the proportion of CT responses to NaCl that were inhibited by amiloride was similar in all groups despite the attenuated responses in OVX-EB rats. In other words, reduced CT responses to NaCl in estrogen-treated OVX rats are not attributable to a selective decrease in the amiloride-sensitive component of the CT response or, by implication, in the amiloride-insensitive component. Rather,

sex differences in CT responses are attributable to a generalized decrease in CT responses to NaCl in female rats that is attributable to high levels of estrogen. We opted to analyze CT responses over a specific range of NaCl concentrations because of their behavioral relevance. Accordingly, we cannot rule out the possibility that estrogen causes a rightward shift in the NaCl concentration-response curve, nor the possibility that this shift may influence preference for or intake of very low concentrations of NaCl as well as those for highly concentrated NaCl solutions.

Experiment 2: Detection of NaCl Taste

Decreased CT responses to NaCl in OVX rats may signal less intense salt taste and, thereby, contribute to sex differences in the ingestion of concentrated NaCl solutions. However, the full extent of behavioral effects resulting from the reduction of both the amiloride-sensitive and amiloride-insensitive components of CT responses is unknown. In male rats, amiloride increases the detection threshold for the taste of NaCl in behavioral tests (Geran & Spector, 2000) but does not affect the shape of the NaCl preference-aversion curve (Brot, Watson, & Bernstein, 2000). Interestingly, amiloride impairs the ability of male rats to discriminate between Na^+ and non- Na^+ salts (Spector, Guagliardo, & St. John, 1996). These observations suggest that the amiloride-insensitive component may be sufficient to distinguish among NaCl concentrations, but that the amiloride-sensitive component is necessary for normal NaCl detection or recognition. Thus, the

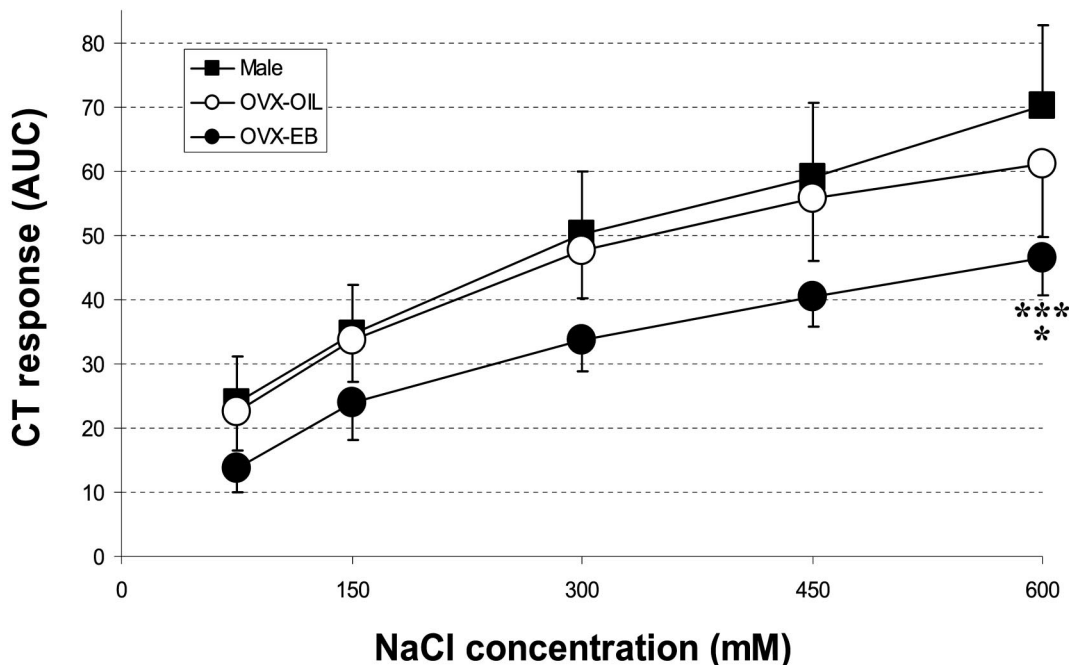


Figure 2. Mean chorda tympani (CT) responses expressed as increase from baseline and calculated as area under the curve (AUC) in male (squares), oil-vehicle-treated ovariectomized (OVX-OIL; white circles), and estradiol-benzoate-treated ovariectomized (OVX-EB; gray circles) rats in response to 10-s lingual stimulation with ascending NaCl concentrations. Error bars represent standard error. * $p < .05$ vs. OVX-OIL; *** $p < .001$ vs. male.

reduction of both components of the CT response to NaCl, as was observed in OVX-EB rats in the present study, might be expected to interfere with the ability to distinguish among NaCl solutions. Consistent with this idea, we recently reported sex differences in the ability to distinguish among concentrated NaCl solutions (Curtis et al., 2004). In addition, our findings of a generalized decrease in CT responses in OVX rats that was especially pronounced after EB treatment raises the possibility that there are sex differences in the detection of NaCl taste and that these differences also may depend on estrogen. To address this possibility, we conditioned an aversion to the taste of 150-mM NaCl and then evaluated the generalization of the conditioned taste aversion (CTA) to dilute

NaCl solutions, including those previously shown to be at the detection threshold in male rats (Clarke, Koh, & Bernstein, 2001).

Method

Male ($n = 8$) and OVX rats were trained to consume fluid from graduated drinking tubes by restricting daily access to water to 10 min in the morning (~0900) and 30 min in the afternoon (~1600). Stable 10-min water intakes were established within 5 days. On the conditioning day, rats were injected with LiCl (3 mEq/kg ip) immediately after 10-min access to 150-mM NaCl, and, on the following day, the CTA was verified in 10-min, 2-bottle (NaCl and water) tests. OVX rats then were treated with EB ($n = 9$) or OIL ($n = 9$) on 2 consecutive days. The generalization of the CTA

Table 1

Mean Baseline Chorda Tympani (CT) Whole Nerve Activity in Male and Ovariectomized Rats Given Estradiol Benzoate (OVX-EB) or the Oil Vehicle (OVX-OIL) and the Inhibition of CT Responses to Lingual NaCl (Expressed as % Decrease) During Co-Application of Amiloride (100 μ M) and NaCl

Condition	Male	OVX-OIL	OVX-EB
Baseline (mV)	9.27 \pm 0.90	8.97 \pm 1.02	9.10 \pm 0.86
Amiloride inhibition (%)			
75-mM NaCl	59.88 \pm 8.40	61.62 \pm 9.32	63.25 \pm 8.44
150-mM NaCl	64.70 \pm 7.16	65.62 \pm 9.44	60.99 \pm 8.46
300-mM NaCl	51.87 \pm 5.15	62.71 \pm 5.08	65.78 \pm 5.25
450-mM NaCl	50.60 \pm 4.79	54.21 \pm 4.12	55.03 \pm 4.64
600-mM NaCl	47.72 \pm 3.63	49.51 \pm 4.37	51.06 \pm 4.91

to 50-, 5-, and 1-mM NaCl was assessed in 10-min, 2-bottle tests beginning on the day of the second EB or OIL treatment; the CTA to 150-mM NaCl was verified on completion of these generalization tests. In separate control groups (male, $n = 9$; OVX-OIL, $n = 8$; OVX-EB, $n = 9$), 150-mM NaCl was paired with injection of the saline vehicle (SAL; 0.15-M NaCl) prior to assessment of NaCl preferences. Preferences for NaCl solutions were calculated as NaCl intake \div (NaCl intake + water intake).

Results

As expected, preference for 150-mM NaCl (see Table 2) depended on drug, $F(1, 46) = 176.39, p < .001$, with preference scores after LiCl significantly less than those after SAL. There also was a significant Drug \times Time interaction, $F(1, 46) = 12.74, p < .001$. The LiCl-induced CTA, indicated by preference scores less than 0.045, was robust prior to the generalization tests and persisted thereafter; however, preference for 150-mM NaCl increased ($p < .001$) in rats that had been injected with SAL. Preference for 150-mM NaCl also was affected by group, $F(1, 46) = 3.90, p < .05$. Regardless of whether 150-mM NaCl had been paired with LiCl or SAL, males had significantly lower preference scores than did OVX-OIL or OVX-EB ($ps < .05$).

All groups generalized the CTA to dilute NaCl solutions as evidenced by the significantly lower preference scores in LiCl-injected rats compared with those in SAL-injected rats (see Figure 3), $F(2, 46) = 114.88, p < .001$. However, the preference for dilute NaCl solutions depended on both group, $F(2, 46) = 5.41, p < .01$, and concentration, $F(2, 46) = 10.90, p < .001$. Specific comparisons revealed that OVX-OIL and OVX-EB rats generalized the CTA to 1-mM NaCl ($ps < .001$; LiCl vs. SAL), but males showed no difference in their preference for 1-mM NaCl relative to water whether previously injected with SAL or with LiCl ($p = .196$).

We also evaluated preferences for NaCl solutions during hormone treatment (or the corresponding time, for male rats) separately in rats that had been given the SAL injection and found that preferences depended on group (see Figure 4), $F(2, 23) = 5.33, p < .05$. Male rats had lower preference scores than did OVX-OIL and OVX-EB rats ($ps < .05$), regardless of NaCl concentration.

Table 2

Mean Preference Scores for 150-mM NaCl in 10-Min, 2-Bottle (NaCl and Water) Tests by Male and Ovariectomized Rats Given Estradiol Benzoate (OVX-EB) or the Oil Vehicle (OVX-OIL)

Condition	Male	OVX-OIL	OVX-EB
Before			
SAL	0.251 \pm 0.079	0.630 \pm 0.084	0.484 \pm 0.108
LiCl	0.000 \pm 0.000	0.000 \pm 0.000	0.031 \pm 0.023
After			
SAL	0.623 \pm 0.116	0.775 \pm 0.130	0.839 \pm 0.069
LiCl	0.016 \pm 0.016	0.019 \pm 0.013	0.044 \pm 0.023

Note. Scores were calculated as NaCl intake \div (NaCl intake + water intake). 150-mM NaCl was paired with LiCl or with 0.15-M NaCl (SAL) control injection, and preferences were evaluated prior to (Before) and at the conclusion of (After) generalization testing.

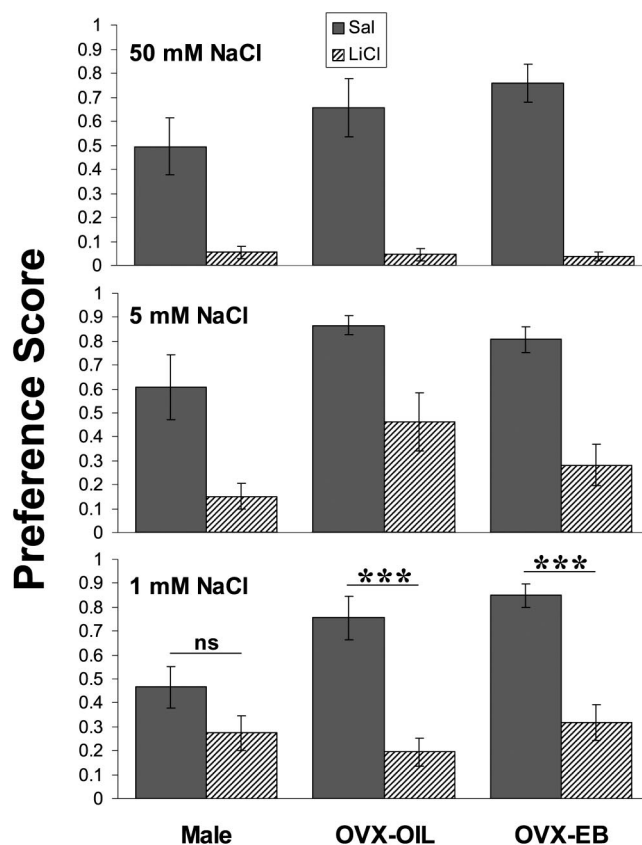


Figure 3. Mean preference scores for NaCl solutions—calculated as NaCl intake \div (NaCl intake + water intake)—in male, oil-vehicle-treated ovariectomized (OVX-OIL), and estradiol-benzoate-treated ovariectomized (OVX-EB) rats. 150-mM NaCl was paired with injection of vehicle (SAL; solid bars; male, $n = 9$; OVX-OIL, $n = 8$; OVX-EB, $n = 9$) or LiCl (hatched bars; male, $n = 8$; OVX-OIL, $n = 9$; OVX-EB, $n = 9$). Ten-min, two-bottle generalization tests to more dilute NaCl solutions were conducted on consecutive days and the aversion to 150-mM NaCl was verified at the end of the generalization tests. Error bars represent standard error. ns = not significant. *** $p < .001$.

Discussion

The pairing of 150-mM NaCl induced a robust LiCl-induced CTA in all groups that generalized to 50- and 5-mM NaCl in all groups. Thus, neither sex nor estrogen altered the CTA or the ability to generalize the CTA to NaCl concentrations within the detectable range (Clarke et al., 2001). As expected (Clarke et al., 2001), the generalization to 1-mM NaCl was greatly attenuated in male rats but, surprisingly, both groups of OVX rats generalized the CTA to 1-mM NaCl (see Figure 3). Although it is possible that subtle differences in the strength of the original CTA contribute to these sex differences, we think this is unlikely as the CTA was quite robust in all groups. In addition, the persistence of a robust CTA in all groups after the generalization testing (see Table 2) indicates that these group differences were not attributable to differential extinction of the CTA. Finally, OVX rats that had been given SAL injections showed a greater preference for all NaCl solutions than did SAL-injected male rats, and the difference was especially pronounced for 1-mM NaCl (see Figure 4). In fact, the

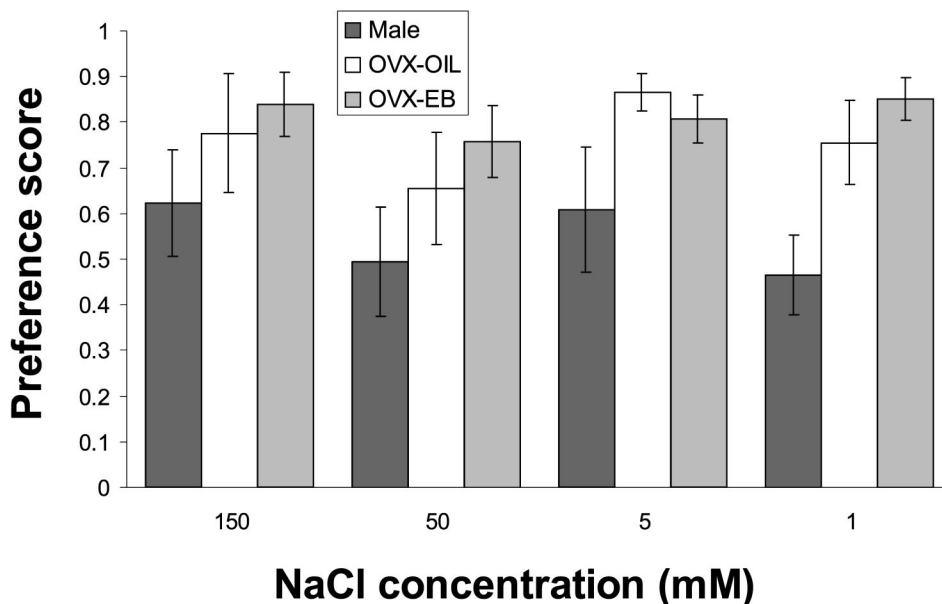


Figure 4. Mean preference scores for NaCl solutions by male (black bars; $n = 9$), oil-vehicle-treated ovariectomized (OVX-OIL; white bars; $n = 8$), and estradiol-benzoate-treated ovariectomized (OVX-EB; gray bars; $n = 9$) rats that previously had been injected with saline. Error bars represent standard error.

preference for 1-mM NaCl by SAL-injected male rats decreased to levels not significantly different from those in LiCl-injected males, which had increased slightly, further supporting the idea that males are not able to detect the taste of 1-mM NaCl (see also Clarke et al., 2001). These sex differences suggest that, independent of estrogen, female rats have a greater preference for isotonic and hypotonic NaCl solutions that may be attributable to a lower gustatory detection threshold for NaCl.

General Discussion

The present results indicate that, compared with male rats, female rats are less sensitive to the taste of concentrated NaCl solutions during high estrogen conditions but are more sensitive to the taste of dilute NaCl solutions regardless of estrogen status. On the surface, these findings are consistent with sex differences in preferences for dilute NaCl solutions observed in this study and for concentrated NaCl solutions reported in many other studies. When considered together, however, our observations that female rats have a greater preference for dilute NaCl and a lower NaCl taste threshold seem counterintuitive given the generalized attenuation of CT responses to NaCl in EB-treated OVX rats.

A similar paradox exists in the relationship between CT activity and salt intake in male rats that have been maintained on an Na-deficient diet: suppressed CT activity (Contreras & Frank, 1979) but increased intake of and preference for both dilute and concentrated NaCl solutions (Contreras & Catalanotto, 1980; Curtis, Krause, & Contreras, 2001). Herness (1992) found that CT responses to NaCl were affected by the sodium conserving hormone aldosterone. Thus, for both sodium deficiency and high estrogen conditions, hormones may underlie the change in CT responses to NaCl above isotonic concentrations; however, the basis of the increased behavioral sensitivity to dilute NaCl solu-

tions in both groups of OVX rats remains to be determined. The glossopharyngeal and greater superficial petrosal nerves also respond to NaCl taste (Frank, 1991; Sollars & Hill, 1998), suggesting the relative weight of sensory input from gustatory nerves may be important mediators of behavioral responses (Sollars & Bernstein, 1994; Tabuchi, Uwano, Kondoh, Ono, & Torii, 1996). Whatever the mechanism, however, it seems clear that hormones indicative of physiological status modulate gustatory processing.

Increasing NaCl intake is critical to restore sodium balance in the face of experimental manipulations that produce sodium loss. Thus, a hormonal signal of sodium loss—aldosterone—may promote compensatory increases in NaCl ingestion. In contrast, the advantages of increased NaCl consumption during high estrogen conditions are less obvious; however, one possibility is suggested by reports that preferences for and ingestion of NaCl are especially pronounced in many species during pregnancy or lactation (Clarke & Bernstein, 2001; Duffy, Bartoshuk, Striegel-Moore, & Rodin, 1998; Stricker et al., 1991). NaCl intake may compensate for the fluid and electrolyte loss that occur during lactation or the increased vascular volume that accompanies pregnancy. Thus, the estrogen signal of reproductive conditions conducive to fertilization and pregnancy and the concomitant changes in fluid and electrolyte balance may promote increased NaCl consumption and, ultimately, benefit both mother and offspring.

It should be noted that the detection threshold for the taste of NaCl in male rats was greater than that in OVX rats regardless of EB treatment (see Figure 3). These observations raise the possibility that the male gonadal hormone testosterone contributes to sex differences in gustatory responses to NaCl. Although we cannot rule out this possibility, two observations argue against it. First, CT activity in male and OVX-OIL rats was virtually indistinguishable in response to NaCl concentrations ≤ 300 mM, and

although CT responses to 600-mM NaCl tended to be greater in males, they did not differ significantly from those in OVX-OIL rats (see Figure 2). Second, dramatic peaks in estrogen occur in female rats during the early postnatal period as well as at the onset of puberty. We suggest that elevated estrogen levels early in development set the tone for gustatory input related to NaCl taste, as is known to occur for sex-specific reproductive behaviors, and that sex differences in behavioral and sensory responses to concentrated NaCl due to these early developmental effects are enhanced by acute estrogen fluctuations in adults.

In conjunction with reports of the influence of estrogen in other sensory systems (Cox, 1980; Doty, Snyder, Huggins, & Lowry, 1981; Swanson & Dengerink, 1988), our findings of estrogen effects on gustatory processing suggest that there are sex differences in neurobiological processes as fundamental as sensation and perception. Thus, regardless of sex differences in higher order neural processing, behavioral differences may occur, in part, because males and females literally sense the world differently. In the case of taste-driven behaviors and, more specifically, of behaviors related to salt taste, two differences are noteworthy. First, dilute NaCl appears to be more readily detected and, subsequently, more preferred by females regardless of estrogen status, as shown in Experiment 2. Second, suprathreshold concentrations of NaCl (and perhaps non-Na⁺ salts) appear to be perceived as less intense during conditions of elevated circulating estrogen, as suggested by the results of Experiment 1. Less intense salt taste may be more palatable, and, consistent with this idea, EB-treated OVX rats consume more of concentrated NaCl solutions in long-term tests compared with male rats or with OVX rats treated with oil (Curtis et al., 2004; Kensicki et al., 2002). In both cases, sex differences in sensory processing related to salt taste may underlie the enhanced preference for and ingestion of salt-rich foods by females. Therefore, given the importance of body sodium regulation for successful reproduction and, indeed, for the maintenance of normal function, the behavioral consequences of subtle differences in gustatory input related to NaCl taste may well have enormous physiological relevance.

But are sex differences in gustatory input specific to salt taste? Although this question has not been addressed directly, our ongoing behavioral studies show that, compared with male rats, female rats have a lower detection threshold for the taste of linoleic acid, a fatty acid that is a major component in many dietary fats. In addition, there are sex differences in sucrose taste detection thresholds in humans (Than et al., 1994). Interestingly, in these behavioral studies, sucrose detection thresholds were decreased in reproductively cycling women during high estrogen conditions, whereas estrogen increased the detection threshold in OVX rats in our recent study (Curtis et al., 2005). Methodological or species differences may underlie the differences, and additional studies will be necessary to address these issues. Nonetheless, it seems clear that there are sex differences in taste detection of fatty acids and sucrose. Not surprisingly, females of many species, including human and rat, also have greater preferences for fats and sweets compared with males (Valenstein et al., 1967; Wansink et al., 2003).

Changes in estrogen levels influence food intake and body weight (e.g., Curtis et al., 2004; Geary, 2001) along with hormones known to be involved in feeding and body weight regulation, such as leptin (Shimizu et al., 1997). Interestingly, leptin has been

reported to suppress gustatory nerve responses to sweet taste stimuli (Kawai, Sugimoto, Nakashima, Miura, & Ninomiya, 2000), raising the possibility of indirect effects of estrogen on peripheral gustatory processing. Finally, estrogen receptors are located within numerous areas of the central nervous system, including those involved in gustatory processing, suggesting that the peripheral effects of estrogen to influence gustatory input may be augmented by the actions of estrogen on receptors in central gustatory pathways.

The unsettling prospect, therefore, is that taste-driven sex differences in preferences for salts, fats, and sweets may contribute to greater consumption of calorically dense, salt- or fat-rich foods by females and, as a consequence, to the greater incidence of obesity and obesity-related health complications among females (Paeratakul, Lovejoy, Ryan, & Bray, 2002).

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