*Am J Physiol Regul Integr Comp Physiol* 283: R739–R747, 2002;
10.1152/ajpregu.00103.2002.

# Transecting the gustatory branches of the facial nerve impairs $NH_4Cl$ vs. KCl discrimination in rats

LAURA C. GERAN, MIRCEA GARCEA, AND ALAN C. SPECTOR
*Department of Psychology and Center for Smell and Taste,*
*University of Florida, Gainesville, Florida 32611*

Received 19 February 2002; accepted in final form 10 June 2002

**Geran, Laura C., Mircea Garcea, and Alan C. Spector.** Transecting the gustatory branches of the facial nerve impairs $NH_4Cl$ vs. KCl discrimination in rats. *Am J Physiol Regul Integr Comp Physiol* 283: R739–R747, 2002; 10.1152/ajpregu.00103.2002.—Ammonium and potassium chloride share a common taste quality and an amiloride-insensitive route of transduction. An amiloride-sensitive pathway might also be partially activated by these salts, although very few studies have reported effects of amiloride on nonsodium salt perception. This experiment was designed to determine *1*) whether rats could discriminate KCl from $NH_4Cl$ and, if discrimination was evident, whether performance was impaired with *2*) amiloride or *3*) gustatory nerve transection. Rats were trained to discriminate KCl from $NH_4Cl$ ($n = 8$) and NaCl from $NH_4Cl$ ($n = 8$). Amiloride (100 μM) impaired NaCl vs. $NH_4Cl$ but not KCl vs. $NH_4Cl$ performance, whereas both groups showed significant impairments after transection of the chorda tympani (CT) and greater superficial petrosal (GSP) branches of the facial nerve. This suggests that rats can discriminate between KCl and $NH_4Cl$ and that this discrimination does not rely on an amiloride-sensitive mechanism but does depend on the CT and/or GSP nerves. This experiment supports the hypothesis that the facial nerve is important for salt taste recognition and discrimination.

taste; salt; amiloride; greater superficial petrosal; chorda tympani

MAMMALS HAVE EVOLVED A VARIETY of mechanisms to defend hydromineral balance. This includes the specific appetite for sodium salts expressed by herbivores and omnivores such as the rat after sodium depletion (see Refs. 6, 34, 35, 47). A better understanding of the role of taste in cation-specific ingestive behavior can be reached by assessing the rat's ability to detect and discriminate among a variety of salts. Although a number of behavioral studies have focused on the rat's ability to discriminate sodium chloride from nonsodium chloride salts (3, 17, 20, 40, 41, 44, 45), to our knowledge none have investigated whether these animals are also capable of discriminating between two nonsodium salts. It was our goal to test whether nonsodium salt discrimination was possible and, if it was evident, to examine potential physiological bases for this phenomenon.

Nonsodium salt transduction is thought to occur primarily via a relatively nonselective cation pathway in the oral cavity (8, 13, 19, 50). Transduction has been linked to submucosal receptor sites (10, 36, 48, 50) and apical channels (8) and can be activated by a variety of cations including $K^+$, $Na^+$, and $NH_4^+$. Overall, transduction via this less selective route is largely unaffected by the epithelial sodium channel (ENaC) blocker amiloride (13, 18, 31, 50), although chorda tympani nerve (CT) responses to KCl and $NH_4Cl$ have been partially suppressed with amiloride in some cases (2, 19, 21, 23, 26, 31). At low concentrations (<0.3 M), $NH_4^+$ transduction is thought to involve an additional amiloride-sensitive pathway consisting of apically located ion channels (19). To date, behavioral tests in rats have not provided any evidence of taste-guided responses to $NH_4Cl$ being disrupted by amiloride (17) and only one report of amiloride affecting responses to KCl (5).

In addition to sharing a common transduction pathway in the rat, KCl and $NH_4Cl$ also share a common pattern of activity in the nucleus of the solitary tract (NST) and a common taste quality as assessed by generalization tests (11, 27, 29). Given the similarities between these two salts, we hypothesized that if rats were indeed able to discriminate between nonsodium salts, a KCl vs. $NH_4Cl$ discrimination would likely be the most difficult salt discrimination task for them to learn. Accordingly, we tested whether rats could discriminate between KCl and $NH_4Cl$ on the basis of taste. We also trained a second group of rats to discriminate NaCl from $NH_4Cl$ for the purposes of comparison. This task was thought to be easier than the KCl vs. $NH_4Cl$ task as rats fail to generalize between NaCl and $NH_4Cl$ under normal conditions (17). We then tested whether performance on these tasks is disrupted with adulteration of the stimuli with an apparently tasteless (24) concentration of amiloride (100 μM). This concentration of amiloride was also chosen because it is thought to maximally block lingual epithelial sodium channels at NaCl concentrations lower than ~0.5 M (1, 2, 7, 17) and would inhibit potential activation of apical channels by $NH_4^+$ (19).

Address for reprint requests and other correspondence: A. C. Spector, Dept. of Psychology, Box 112250, Univ. of Florida, Gainesville, FL 32611-2250 (E-mail: spector@ufl.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

  

We also explored the necessity of gustatory input from the facial nerve for the maintenance of discrimination performance. The sensory limb of the facial nerve consists of two salt-responsive branches. The CT innervates taste buds in the fungiform papillae of the anterior tongue, whereas the greater superficial petrosal nerve (GSP) innervates taste buds in the soft palate and nasoincisor ducts (25). The CT is most responsive to salts and acids, whereas the GSP is maximally responsive to sugars but responds moderately to salts and acids (15, 30, 38). The sodium responsiveness of both nerves is inhibited significantly by amiloride (2, 7, 31, 38, 49).

Transection of the CT alone or in combination with the GSP substantially disrupts performance on a variety of taste discrimination tasks. In contrast, transection of the glossopharyngeal nerve (GL), which innervates taste buds in the posterior tongue, does not affect any of the discriminations tested so far (see Ref. 46). This has led to the hypothesis that the collective input from the gustatory branches of the facial nerve is more important than the input from the GL for taste recognition and discrimination tasks (see Ref. 46). Therefore, we hypothesized that if rats could discriminate between KCl and $NH_4Cl$, performance would most likely be impaired by combined transection of the CT and GSP nerves.

## METHODS

*Subjects.* Sixteen adult male Sprague-Dawley rats (Charles River Breeders; Wilmington, MA) weighing ~250–300 g at the start of training served as subjects. These animals were placed on a 12:12-h light-dark schedule with lights on at 6:00 AM. The temperature and humidity of the colony room were automatically controlled. Rats were housed individually in hanging wire mesh cages with ad libitum access to laboratory rodent chow (PMI 5001, St. Louis, MO). Water bottles (distilled) were removed from home cages on Sunday and replaced after the last session on Friday. Monday through Friday the rats worked to obtain access to distilled water during a 40-min session. If an animal's weight fell below the 85% ad libitum value calculated each week, supplemental water in the amount of 5 ml was given after the last session of the day.

*General procedure.* All training and testing sessions took place in a taste-testing apparatus (gustometer) designed to deliver small volumes of taste stimuli (reagent grade chemicals; Fisher Scientific, Orlando, FL) and record lever presses in response to these stimuli (39). Rats were allowed to complete as many trials as possible during a 40-min session. Briefly, each trial began when the rat made contact with a dry spout. This contact allowed the rat to obtain access to a sample stimulus for 3 s or 5 licks, whichever came first. Animals were trained to press one lever after a KCl stimulus (or NaCl stimulus depending on group assignment) and the other lever after an $NH_4Cl$ stimulus. On the first day of training, rats were presented with only a single 0.2 M concentration of a particular stimulus, but by the last week of training animals were presented with the four concentrations (0.4, 0.2, 0.1, and 0.05 M) of two salts used for testing. After each correct press, the rat received access to distilled water (40 licks or 10 s). During testing, if the rat pressed the wrong lever or failed to press within 5 s (limited hold), it was punished with a 20-s time out. The limited hold and time out durations were changed over the course of training. Training required a total of 33 sessions with both the KCl vs. $NH_4Cl$ and NaCl vs. $NH_4Cl$ groups advancing from one training phase to the next at the same time. For a more detailed description of the two-lever operant conditioning procedure, see Kopka et al. (20) and St. John et al. (44).

*Discrimination and amiloride testing.* Immediately after training, rats were tested for 5 days using the same stimuli used during training. This was followed by 1 day of testing in which all salt concentrations were dissolved in 100 μM amiloride hydrochloride (Sigma Chemical; St. Louis, MO). Amiloride was prepared the evening before and allowed to spin overnight in a dark room. In addition, the flask was wrapped in aluminum foil to further minimize possible reactions with light. All salt solutions were made the following morning with the amiloride solution as the solvent. At the end of each day, regardless of phase, reservoirs were rinsed thoroughly and stimulus assignments were rotated to reduce the possibility that a nonchemical cue might become associated with fluid delivery from a particular solenoid or reservoir. After amiloride testing, the rats were given an additional week of testing on the original discrimination in the absence of amiloride (postamiloride testing), to determine what effect, if any, experience with amiloride had on discrimination performance.

*Surgery.* After postamiloride testing, rats were anesthetized with an intramuscular injection of ketamine hydrochloride (125 mg/kg body wt) mixed with xylazine hydrochloride (5 mg/kg body wt). One-half of these rats received bilateral transection of both the CT and GSP branches of the facial nerve. An incision was made dorsal to the pinna and the external ear was retracted to expose the ear canal. A small slit was then made in the canal, and the fascia was dissected to expose the auditory meatus. The surrounding musculature was blunt dissected and retracted, and the opening of the bony meatus was enlarged by means of a high-speed pneumatic dental drill. The tympanic membrane, ossicles, and CT were removed followed by removal of the tensor tympani muscle and a small piece of temporal bone using microforceps to expose the GSP. The nerve was transected and the ends were cauterized. The retractors were then removed and the incision was closed with wound clips. The remaining eight rats received sham surgery involving incision, retraction of the pinna and musculature, and puncture of the tympanic membrane. The wound was closed in the same manner as for the nerve-transected group. Each rat received a prophylactic dose of penicillin (30,000 U sc) immediately after surgery and for the next 3 days.

*Postsurgical treatment.* Rats in the nerve-transected group were allowed to recover for 23–24 days after surgery. Sham-transected animals had 22 days between surgery and postsurgical testing. After surgery, all rats were given wet mash (powdered PMI 5001 and distilled water) mixed with a high-calorie dietary supplement (NutriCal, Evsco Pharmaceuticals, Buena, NJ). The rats were also given dilute sweetened condensed milk [354 ml Borden milk: 354 ml distilled water plus 1 ml Poly-Visol multi-vitamin with iron (Mead-Johnson)]. Rats in the combined transection (CTX + GSPX) group were given ad libitum access to the milk diet with intake measured daily. The average milk intake was then calculated each day, and this amount was given to each of the sham-transected rats on the following day to control for exposure to the taste of milk. After 10 days, all rats were taken off the milk diet but continued to receive unsupplemented wet mash for an additional 10 days before being switched to laboratory chow in powdered form. One rat in the CTX + GSPX group failed to recover from surgery and was euthanized.

*Postsurgical discrimination testing and water control test.* After recovery from surgery, rats were tested on the original discrimination for 5 days. Animals were then given a water control test for 1 day. All fluid reservoirs were filled with distilled water on this day, with one-half of these reservoirs assigned to the left lever and one-half to the right lever. This test was performed to determine whether the rats were under stimulus control or if they were capable of making the appropriate response in the absence of chemical cues.

*Experience testing.* After the water control test, sham-transected rats were given 4 days on the original discrimination to ensure that performance had returned to baseline. These rats then received additional testing to determine whether observed differences between the two groups in discrimination performance during testing were due in part to differences in training stimuli. Rats that had originally received NaCl vs. $NH_4Cl$ discrimination training were tested on a KCl vs. $NH_4Cl$ task for 4 days, whereas rats that were trained to discriminate KCl from $NH_4Cl$ were given NaCl and $NH_4Cl$ dissolved in amiloride for the same 4 days.

*Histology.* Animals were deeply anesthetized with pentobarbital sodium and transcardially perfused with physiological saline followed by 10% buffered formalin. Transected rats were killed the day after the water control test, whereas sham-transected rats were not killed until ~25 days later. The tongue, soft palates, and nasoincisor ducts of each animal were removed and stored in 10% buffered formalin. Histological analysis was performed by an observer unaware of the subject's surgical group.

The anterior portion of the tongue from the intermolar eminence to the tip was placed in distilled water for 30 min, then dipped in 0.5% methylene blue until well saturated (~1 min) and rinsed with distilled water. The epithelium was then stripped from the underlying muscle and connective tissue and pressed between two glass slides. The numbers of fungiform papillae both with and without taste pores were then counted under a light microscope (see Ref. 45). The epithelium surrounding the pore appears as a small blue dot near the center of the papilla after methylene blue staining (33). The soft palate and nasoincisor ducts were embedded in paraffin and cut into 10-μm sections on a rotary microtome. These sections were then mounted on slides, stained with hematoxylin and eosin, and coded so that the surgical condition was unknown. Taste buds with pores were then counted under the microscope.

*Data analysis.* Overall discrimination performance was based on the proportion of correct responses on trials with a lever press, collapsed across salt and concentration for each testing phase. A two-way ANOVA was performed for each phase and group to determine the effects of stimulus (NaCl, KCl, and $NH_4Cl$) and concentration on performance. The normal approximation of the binomial distribution was used to determine significant departures from chance responding for both the water control and postsurgical tests (4). A more conservative Bonferroni test was also used on data from the water control and postsurgical tests to adjust for the number of statistical tests performed. In addition, independent *t*-tests were conducted to assess between- and within-subject differences in performance. The statistical rejection criterion for all analyses was set at the conventional $P \leq 0.05$.

## RESULTS

*Discrimination testing.* The overall presurgical performance for all rats, regardless of discrimination group, was >84% collapsed across salt, concentration, and the 5 days of testing (Figs. 1 and 2). An independent *t*-test indicated that the performance of rats on the NaCl vs. $NH_4Cl$ task (94.0 ± 0.01) was slightly but significantly greater ($P < 0.001$) than that of rats on the KCl vs. $NH_4Cl$ task (87.8 ± 0.01). A two-way ANOVA (salt × concentration) indicated a significant main effect of concentration [$F(3,21) = 36.3$, $P < 0.001$] as well as a significant salt × concentration interaction [$F(3,21) = 24.7$, $P < 0.001$] in the KCl vs. $NH_4Cl$ group, apparently due to the fact that KCl performance was relatively stable regardless of concentration, whereas $NH_4Cl$ performance increased with concentration (Fig. 3). In the NaCl vs. $NH_4Cl$ group (Fig. 4), there was a significant effect for concentration only [$F(3,21) = 18.0$, $P < 0.001$].

*Amiloride testing.* Amiloride (100 μM) significantly compromised overall NaCl vs. $NH_4Cl$ performance, but had no effect on KCl vs. $NH_4Cl$ discrimination ($P < 0.001$ and $P > 0.14$, respectively; Figs. 1 and 2). A two-way ANOVA (salt × concentration) revealed a significant main effect of concentration [$F(3,21) = 3.7$, $P < 0.03$] and a salt × concentration interaction [$F(3,21) = 8.1$, $P < 0.002$] for the KCl vs. $NH_4Cl$ group (Fig. 3). In the NaCl vs. $NH_4Cl$ group, however (Fig. 4), only the main effect of concentration was significant

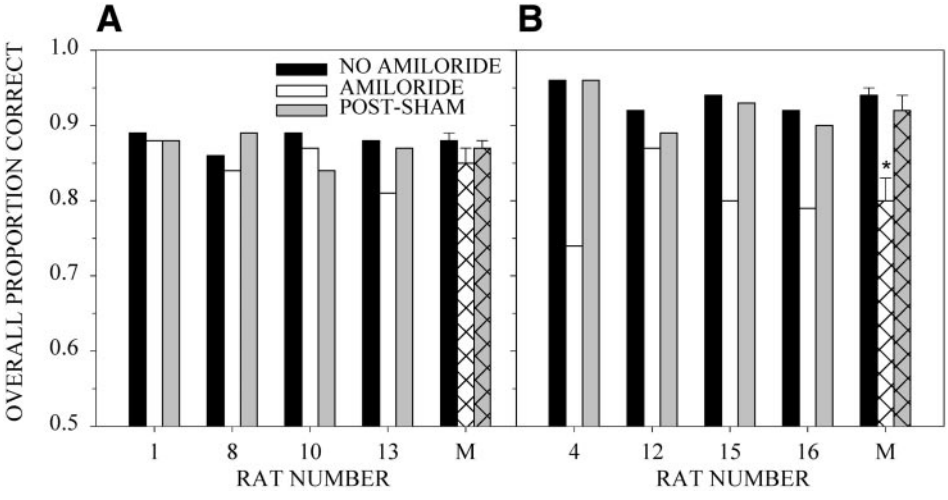


Fig. 1. Overall proportion correct for each sham-operated rat trained to discriminate KCl from $NH_4Cl$ (*A*) and trained to discriminate NaCl from $NH_4Cl$ (*B*) grouped separately. Three phases of discrimination testing are shown for each animal (presurgical testing, black; amiloride testing, white; and postsurgical testing, shaded bars). The mean for each test phase (same color scheme as individual data) is shown at *right* of each panel with crosshatched bars. *Significant decrease in NaCl vs. $NH_4Cl$ discrimination with the addition of 100 μM amiloride (paired *t*-test, $P < 0.05$).

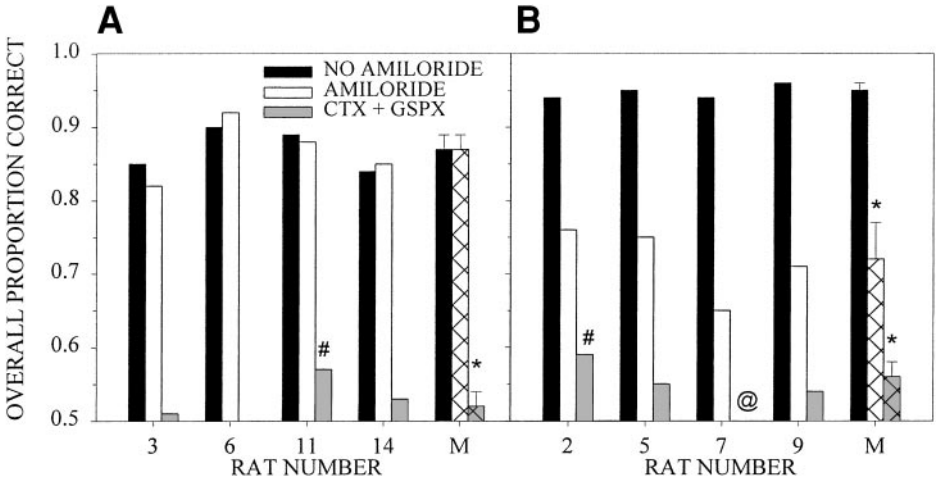


Fig. 2. Overall proportion correct for each chorda tympani (CT) and greater superficial petrosal (GSP) transected (CTX + GSPX) rat trained to discriminate KCl from $NH_4Cl$ (*A*) and trained to discriminate NaCl from $NH_4Cl$ (*B*) grouped separately. Three phases of discrimination testing are shown for each animal (presurgical testing, black; amiloride testing, white; and postsurgical testing, shaded bars). The mean for each test phase (same color scheme as individual data) is shown at *right* of each panel with crosshatched bars. *Significant drop in NaCl vs. $NH_4Cl$ discrimination with the addition of 100 μM amiloride and an even more substantial decrease after CT + GSP transection for both discrimination groups (paired *t*-test, $P < 0.05$). With the use of the normal approximation of the binomial distribution, *rats 11* and *2* performed significantly better than chance (#) after nerve transection ($P < 0.05$). When a Bonferroni adjustment was made, only the performance of *rat 2* remained greater than chance ($P < 0.009$). The postsurgical performance for *rat 6* was equal to 0.5. @*Rat 7* died after surgery.

[$F(3,21) = 8.2$, $P < 0.002$]. Thus ANOVAs revealed the same results in both the presence and absence of amiloride.

*Postsurgical discrimination testing.* The mean overall performance of rats with combined CT and GSP transection dropped to chance levels regardless of discrimination group (Fig. 2). Analysis of individual performance indicated that two CTX + GSPX rats scored better than chance on the postsurgical test (*rat 11*: $z = 1.74$, $P < 0.05$ and *rat 2*: $z = 3.12$, $P < 0.001$) using the normal approximation of the binomial distribution (Fig. 2). When a Bonferroni adjustment was made for these rats, however, only *rat 2* remained significant ($P < 0.009$). Although performance was statistically greater than chance for this animal, it was still <60% compared with >90% correct before surgery. The performance of sham-transected rats was not different from presurgical performance for either discrimination group (Fig. 1). Analysis of the postsurgical performance of the sham-transected rats indicated main effects similar to those observed before surgery.

*Water control test.* Every rat except one (*rat 11*) performed at chance levels when taste stimuli were replaced with distilled water, suggesting that the animals were under stimulus control during discrimination testing. It is highly unlikely that this rat was responding to some extraneous cue due to the fact that postsurgical performance for this animal dropped >30% with transection (see Fig. 2). It is more likely that significance was merely the result of the number

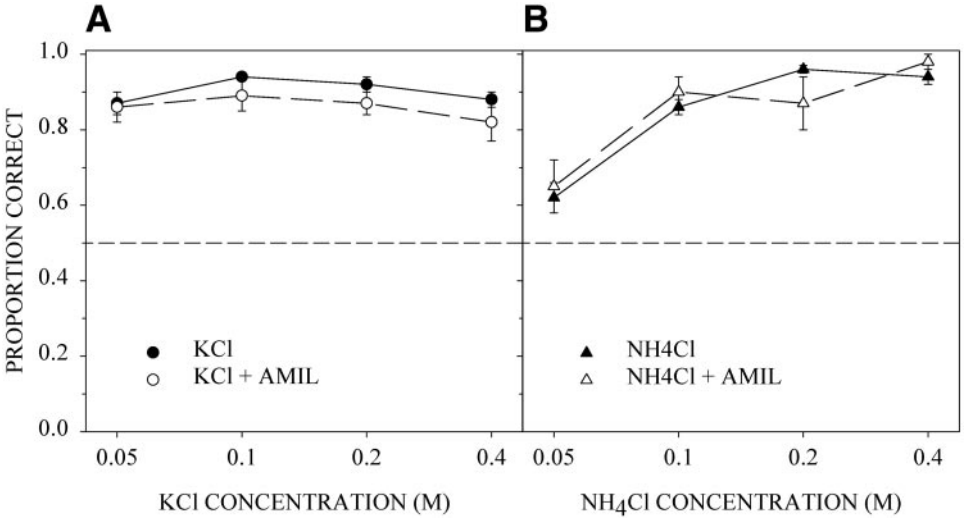


Fig. 3. Mean (±SE) proportion correct during sessions with water as the solvent (closed symbols) and sessions with 100 μM amilroide as the solvent (open symbols) for all rats trained on the KCl vs. $NH_4Cl$ discrimination. Performance on KCl trials is shown in *A* and $NH_4Cl$ trials in *B*. Both panels use molar concentration as the abscissa and a dotted line at 0.5 to represent chance performance.

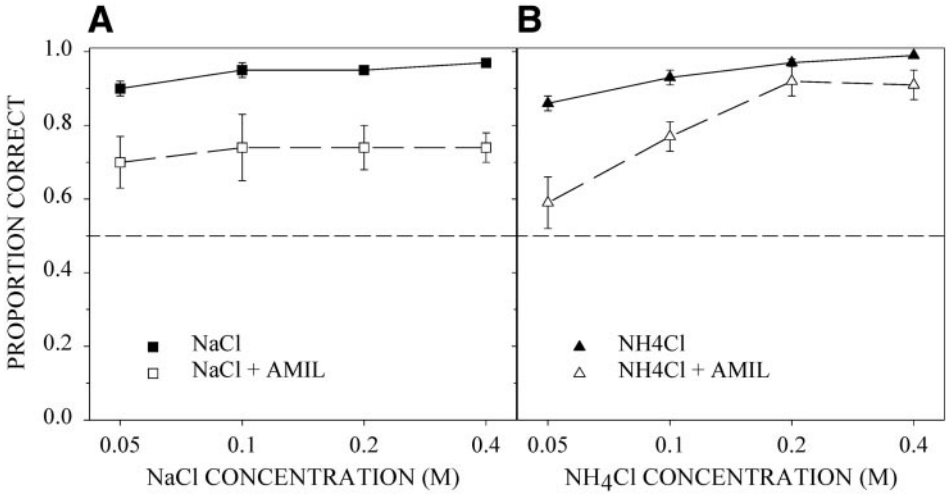


Fig. 4. Mean (±SE) proportion correct during sessions with water as the solvent (closed symbols) and sessions with 100 μM amilroide as the solvent (open symbols) for all rats trained on the NaCl vs. $NH_4Cl$ discrimination. Performance on NaCl trials is shown in *A* and $NH_4Cl$ trials in *B*. Both panels use molar concentration as the abscissa and a dotted line at 0.5 to represent chance performance.

of *t*-tests performed. This interpretation is supported by the fact that we failed to find significance for this rat when a Bonferroni adjustment was performed ($z = 1.73$, $P > 0.62$).

*Experience testing.* Nerve-transected animals were killed immediately after the water control test, and sham-transected rats were put back on the original discrimination for a period of 4 days. Mean performance over this 4-day period was not different from postsurgical testing ($P > 0.4$ for both KCl and NaCl-trained rats using a paired *t*-test). Sham-transected rats were then placed on a discrimination task with a novel stimulus to test the effects of training (i.e., KCl or NaCl experience) on test performance. Rats in the KCl vs. $NH_4Cl$ group were tested on a NaCl vs. $NH_4Cl$ discrimination task with all taste stimuli dissolved in amiloride and rats trained to discriminate NaCl from $NH_4Cl$ were now tested on the KCl vs. $NH_4Cl$ discrimination. Animals were tested for 4 days.

Rats trained to discriminate KCl from $NH_4Cl$ and tested on NaCl vs. $NH_4Cl$ dissolved in amiloride showed no change in mean performance ($P > 0.6$; Fig. 5). Likewise, rats initially trained on the NaCl vs. $NH_4Cl$ task showed no difference between performance on the KCl vs. $NH_4Cl$ task and NaCl vs. $NH_4Cl$ discrimination with amiloride ($P > 0.96$; Fig. 5).

*Histology.* Histological analysis of the palate and anterior tongue confirmed the transection of the CT and GSP nerves. Sham-transected animals had a significantly greater number of taste buds in the soft palate (192.4 ± 7.7 vs. 13.3 ± 4.9) and incisive papilla (96.6 ± 6.3 vs. 3.6 ± 1.9) than animals that underwent

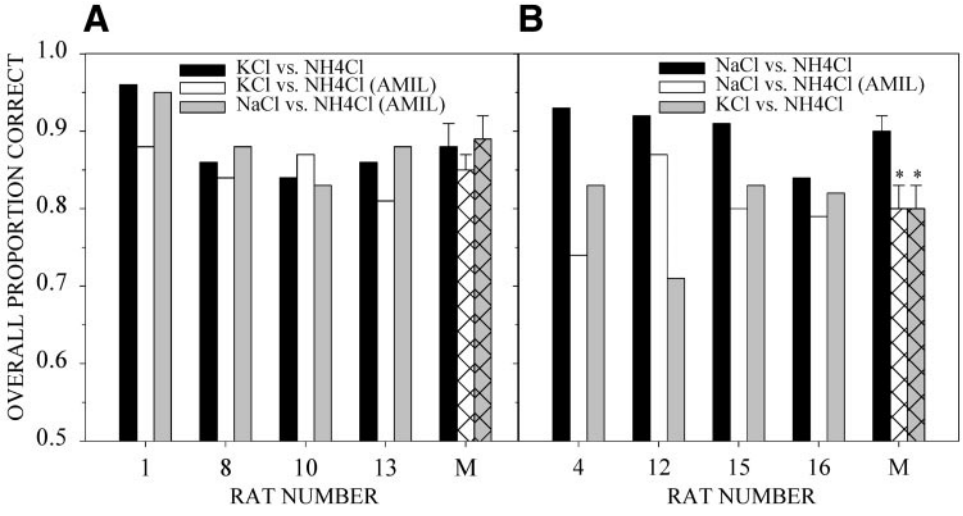


Fig. 5. Overall proportion correct for sham-operated rats only. This graph incorporates data from amiloride testing (previously shown in Fig. 1) with individual data from experience testing and the 4-day mean before experience testing during which each rat received training on the original discrimination (i.e., baseline). Baseline, black; amiloride testing, white; and experience testing, shaded bars. Rats trained on the KCl vs. $NH_4Cl$ task are shown in *A* and rats trained on the NaCl vs. $NH_4Cl$ task are shown in *B*. The mean (±SE) performance for each test phase and discrimination group is shown at *right* of each panel using crosshatched bars. Note that the performance of rats trained on the KCl vs. $NH_4Cl$ discrimination did not change significantly when NaCl was substituted for KCl and all the stimuli were dissolved in amiloride (i.e., experience testing). This suggests that KCl and NaCl + amiloride taste similar to the rat even compared with a $NH_4Cl$ standard.

the CTX + GSPX surgery ($P < 0.001$ for both). The mean number of fungiform papillae was also found to be greater for the sham-transected rats ($157.5 \pm 4.5$ vs. $132.9 \pm 4.8$, $P < 0.004$), as was the number of papillae with visible taste pores ($150.1 \pm 3.8$ vs. $11.9 \pm 3.0$, $P < 0.001$).

## DISCUSSION

Intact rats were clearly able to discriminate $NH_4Cl$ from either KCl or NaCl at the concentrations tested. The results of the water control test confirm that the rats were responding to the chemical properties of the stimulus and not to extraneous cues. The fact that combined transection of the CT and GSP dropped discrimination performance virtually to chance regardless of salt pair demonstrates that trigeminal and/or olfactory cues were not sufficient to guide the behavior and, furthermore, that gustatory input from the seventh cranial nerve was necessary for these salt discriminations under these testing conditions.

Given that lingual stimulation with $NH_4Cl$ produces a greater response in the gustatory nerves of the rat than similar concentrations of KCl (12, 15, 18, 19, 21, 30), any corresponding difference in the perceived intensity of the two salts could potentially guide the discrimination. Although we cannot rule out this possibility, it is unlikely that intensity was the sole basis of the discrimination. First of all, stimulus concentrations ranging one order of magnitude were used to increase the probability that there would be substantial intensity overlap between the salts and, second, the concentration-performance functions do not support this hypothesis. If the rats were discriminating purely on the basis of intensity, as KCl concentration increased, performance on KCl trials would decrease because the intensity of the KCl stimulus would approach that of the $NH_4Cl$. This did not occur. As for the NaCl vs. $NH_4Cl$ discrimination, although rats performed significantly better at the higher salt concentrations, the concentration-performance curves were relatively flat, suggesting only a minor effect of concentration on NaCl vs. $NH_4Cl$ discrimination. Thus when the present results are added to our prior body of work (20, 40, 41, 44), the resultant profile suggests that rats can clearly distinguish among the taste qualities of $NH_4Cl$, KCl, and NaCl at mid to high concentrations.

Although never before tested, it was not particularly surprising that rats could discriminate NaCl from $NH_4Cl$. There is substantial evidence that NaCl activates a sodium-specific transduction pathway (2, 7, 31, 49) and produces a distinct taste quality in the rat not shared with $NH_4Cl$ (3, 11, 13, 17, 27, 28). It was more remarkable to us that rats could discriminate between KCl and $NH_4Cl$ given that rats conditioned to avoid $NH_4Cl$ either with LiCl pairings (17) or shock (11) readily generalized the response to KCl. Furthermore, different groups of rats trained to discriminate NaCl from either quinine, HCl, or sucrose show the same pattern of responding to KCl and $NH_4Cl$ when these two salts are used as stimuli in generalization tests (27). Nevertheless, as the present results affirm, just because two chemical stimuli appear to possess qualitatively similar tastes does not necessarily mean that they cannot be discriminated.

*Amiloride did not affect $NH_4Cl$ vs. KCl discrimination.* The proposed presence of an amiloride-sensitive pathway activated by $NH_4^+$ does not appear to explain the results obtained on the $NH_4Cl$ vs. KCl discrimination task. No decrease in KCl vs. $NH_4Cl$ performance was observed during amiloride testing, suggesting that if amiloride affects $NH_4Cl$ taste quality within the range of concentrations tested, it does not cause $NH_4Cl$ to taste more like KCl. Thus the results from our amiloride manipulation suggest that the apical transduction pathway for $NH_4^+$ hypothesized by Kloub et al. (19) is not the basis for KCl vs. $NH_4Cl$ discrimination at the concentrations tested. It is possible, however, that because amiloride does not completely suppress $NH_4^+$ transduction through this pathway, the residual activity could be enough to support $NH_4Cl$ vs. KCl discrimination. However, the fact that amiloride fails to reduce either $NH_4Cl$ or KCl responding in the geniculate ganglion supports our interpretation (22).

*Taste quality coding of nonsodium salts.* In the periphery, fibers or their somata that respond well to KCl generally respond even more vigorously to $NH_4Cl$, regardless of the receptor field stimulated (12, 14, 15, 18, 19, 21, 22, 30). This suggests that rats in the present experiment were not discriminating between stimuli based on differences in the region of the oral cavity stimulated by the two salts. In their comprehensive study of the geniculate ganglion, Lundy and Contreras (21) reported that KCl and $NH_4Cl$ produced different patterns of responding across separate classes of neurons at this level, suggesting that perceptual discrimination of these salts might be possible. Even in that study, however, neurons that responded to KCl also responded to $NH_4Cl$.

A hierarchical cluster analysis of third-order neurons in the parabrachial nucleus (PBN) revealed a group maximally responsive to citric acid or quinine that was also highly responsive to 0.1 M $NH_4Cl$ but not 0.1 M KCl (32). However, the majority of neurons that respond to $NH_4Cl$ at this level also respond to KCl (32). This response to $NH_4Cl$ in acid- and bitter-"sensitive" cells might enable rats to discriminate between $NH_4Cl$ and other nonsodium chloride salts. It is important to keep in mind, however, that we do not know how this response might change with stimulus concentration or whether this neural activity reflects the taste quality of the stimulus or some other feature such as its hedonic value. As of yet there is no evidence for KCl vs. $NH_4Cl$ discrimination in the NTS, as the across-fiber patterns of responding for these salts are reported to be quite similar (11, 29).

In summary, the available electrophysiological data collected from first-, second-, and third-order neurons in the gustatory system do not reveal any striking difference in the patterns of activity generated by stimulation with KCl and $NH_4Cl$ other than the fact that the latter is a more effective stimulus. Nevertheless, as

noted above, there are some hints that differences in the respective neural patterns associated with the salts may exist and arguably must exist if rats can qualitatively distinguish between the taste qualities associated with KCl and $NH_4Cl$. The same logic suggests that the two salts are stimulating nonidentical but perhaps overlapping populations of taste receptor cells. Recent whole cell recordings from taste receptor cells lend credence to this hypothesis. Gilbertson et al. (16) reported that, although a large proportion of taste receptor cells responsive to 0.1 M KCl or 0.1 M $NH_4Cl$ responded to both stimuli, a few of these cells responded to only one of these stimuli.

*Comparisons with other salt taste discriminations.* Not surprisingly, discrimination performance in the NaCl vs. $NH_4Cl$ group was impaired by amiloride. This finding is consistent with the well-documented effects of the ENaC blocker on NaCl taste (see Refs. 1, 17, 20, 41). There are some interesting features to these results that are worth noting, however. First, unlike the results of the KCl vs. $NH_4Cl$ task, amiloride did impair performance on $NH_4Cl$ trials in the NaCl vs. $NH_4Cl$ task (Fig. 4). The fact that this disruption was concentration dependent, with the weakest concentration affected most, suggests that the proposed amiloride-sensitive apical ammonium channel (19) may contribute to the discriminability of the $NH_4Cl$ signal relative to NaCl at low $NH_4Cl$ concentrations. Alternatively, it is possible that this decrease in $NH_4Cl$ performance with amiloride could simply be due to the effect of the ENaC blocker on NaCl taste quality. Note that the $NH_4Cl$ results from the KCl vs. $NH_4Cl$ discrimination task (Fig. 3) show the same concentration-dependent pattern as the NaCl vs. $NH_4Cl$ results with amiloride. In the KCl vs. $NH_4Cl$ task, this pattern suggests that these lower $NH_4Cl$ concentrations were perceived as more similar to KCl than the higher concentrations. Second, although the disruptive effects of amiloride on performance in the NaCl vs. $NH_4Cl$ task are in some ways similar to those previously reported for a NaCl vs. KCl discrimination, there are some important differences as well. In the previous work, when the stimuli were mixed with 100 μM amiloride, rats trained to discriminate NaCl from KCl, in a procedure similar to the one used here, responded to NaCl as if it was KCl (20, 41). That is, their overall performance on NaCl trials dropped below chance, indicating that they were pressing the KCl-associated lever significantly more often compared with the NaCl-associated lever. Responding on KCl trials was only slightly disrupted and remained well above chance. In contrast, in the present experiment, the performance of rats tested on the NaCl vs. $NH_4Cl$ task never dropped significantly below chance on trials with either salt even when amiloride was the solvent. This indicates that although amiloride adulteration made the discrimination more difficult, it was not necessarily because the taste quality of one salt became more similar to the other. Thus amiloride significantly disrupts both a pretrained NaCl vs. KCl and a pretrained NaCl vs. $NH_4Cl$ taste discrimination but in noticeably different ways. This indirectly lends further behavioral support to the hypothesis that the peripheral taste signals representing $NH_4Cl$ and KCl are dissociable.

*Necessity of facial nerve input.* Although the mechanism by which rats are able to discriminate KCl from $NH_4Cl$ remains a puzzle, it is clear that input from the facial nerve is crucial for this discrimination. Our results indicate that the facial nerve is as important for nonsodium salt discriminations as it is for discriminations between sodium and nonsodium salts (e.g., NaCl vs. KCl discrimination; 3, 20, 40, 44, 45). In our experiment, bilateral transection of the gustatory branches of the facial nerve dropped discrimination performance to chance in 13 of 15 animals. The discrimination performance of the remaining two rats, although better than chance, decreased significantly after transection. These data clearly show that gustatory input from the facial nerve is necessary for both KCl vs. $NH_4Cl$ and NaCl vs. $NH_4Cl$ discrimination. It is unclear, however, if input from both the CT and GSP is necessary or if only one of these nerves is required for normal discrimination. It is important to note that input from the GL, vagus, trigeminal, or olfactory nerves may also be necessary, although insufficient for these discriminations. It would be worthwhile to test the ability of the facial nerve branches to support taste discrimination between nonsodium salts by transecting the GL nerve. Although this would not strictly test the sufficiency of the CT and GSP nerves because the superior laryngeal branch (SLN) of the vagus nerve would be left intact, researchers consider the SLN to be important for airway protection rather than qualitative taste perception (9, 37, 42, 43, 46). Likewise, the trigeminal and olfactory nerves would also remain intact in such a preparation, but transection of the GL could test what effect, if any, denervation of ~60% of the taste buds of the oral cavity might have on KCl vs. $NH_4Cl$ discrimination. This manipulation would complement the current experiment in assessing the hypothesis that the facial nerve is more important for taste recognition and discrimination than the GL (see Ref. 46).

It is likely that the rats performed poorly after CT + GSP transection because they were unable to discriminate between the taste qualities of the salt stimuli, but we cannot be certain. It is possible that taste quality, per se, was unaffected but that the rats were no longer able to use this information to make the correct response after the gustatory branches of the facial nerve were transected. Alternatively, because the detection thresholds for these salts after combined CT and GSP transection are unknown, it is possible that CTX + GSPX rats might have experienced a large shift in intensity rather than a change in taste quality. The fact that the GL is responsive to both KCl and $NH_4Cl$ at the concentrations tested, however, suggests that these stimuli are capable of producing a response in the periphery even though this response might not result in detection (14, 18).

*Experience testing.* Interestingly, rats initially trained on the KCl vs. $NH_4Cl$ task performed significantly better than rats trained to discriminate NaCl from

$NH_4Cl$ performed in the presence of amiloride. This was surprising, as previous studies suggested that the taste of KCl is perceptually similar to that of NaCl + amiloride (17, 20, 41). Thus rats should have been able to discriminate $NH_4Cl$ from NaCl + amiloride as easily as they could discriminate $NH_4Cl$ from KCl. During experience testing, we attempted to determine whether this difference in performance between the two groups was the result of differential experience during training (i.e., with NaCl or KCl) or to some difference in the taste qualities of NaCl + amiloride and KCl compared with the $NH_4Cl$ standard. To accomplish this, rats trained to discriminate KCl from $NH_4Cl$ were placed on the NaCl vs. $NH_4Cl$ task with amiloride and rats trained on the NaCl vs. $NH_4Cl$ discrimination were tested on the KCl vs. $NH_4Cl$ task. There was no difference between performance during experience testing and prior performance for either discrimination group, suggesting that between-group differences were the result of training expectations rather than an underlying difference in the perception of NaCl + amiloride and KCl.

In conclusion, it is clear that despite a reportedly shared amiloride-insensitive route of transduction and similarities in taste quality, $NH_4Cl$ and KCl are nonetheless discriminable to the rat at the concentrations tested. Furthermore, this discrimination does not appear to be due to the activation of an amiloride-sensitive transduction pathway for $NH_4^+$, but is dependent on input from the gustatory branches of the facial nerve. The neural basis of this discrimination remains to be completely understood, but may involve nonidentical, likely overlapping, populations of receptor cells innervated by the facial nerve.

### *Perspectives*

The gustatory system clearly plays a role in helping the rat identify sodium in the environment. It has been shown in several experiments that rats can discriminate sodium from nonsodium salts on the basis of taste. The general relationship between this behavioral capacity and what is known about salt taste transduction as well as the response properties of neurons in the gustatory system, as depicted in the paragraphs above, is conceptually satisfying, if not entirely complete.

In contrast, the neurobiological basis of the rat's ability to discriminate among nonsodium salts such as KCl and $NH_4Cl$ promises to be more elusive to understand. In fact, the collective findings from a variety of previously conducted electrophysiological and behavioral studies would lead one to predict that rats would be unable to distinguish KCl from $NH_4Cl$ on the basis of taste, but as shown here, rats can make this discrimination. Accordingly, any neural coding theory explaining how the characteristics of taste stimuli are represented in the nervous system (e.g., labeled line, population codes, temporal codes) will have to account for these behavioral results.

The authors thank S. Palii and E. Rodgers for aid in data collection.

This research was supported by Grants R01-DC01628 and F31-DC05107 from the National Institute on Deafness and Other Communication Disorders

Portions of this work were presented at the annual meeting of the Society for the Study of Ingestive Behavior in Dublin, Ireland, July 2000.

## REFERENCES

1. **Bernstein IL and Hennessy CJ.** Amiloride-sensitive sodium channels and expression of sodium appetite in rats. *Am J Physiol Regul Integr Comp Physiol* 253: R371–R374, 1987.
2. **Brand JG, Teeter JH, and Silver WL.** Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Res* 334: 207–214, 1985.
3. **Breslin PA, Spector AC, and Grill HJ.** Chorda tympani section decreases the cation specificity of depletion-induced sodium appetite in rats. *Am J Physiol Regul Integr Comp Physiol* 264: R319–R323, 1993.
4. **Brown BW and Hollander M.** *Statistics: A Biomedical Introduction*. New York: Wiley, 1977.
5. **Contreras RJ and Studley JL.** Amiloride alters lick rate responses to NaCl and KCl in rats. *Chem Senses* 19: 219–229, 1994.
6. **Denton DA.** *The Hunger for Salt*. Berlin: Springer-Verlag, 1982.
7. **DeSimone JA and Ferrell F.** Analysis of amiloride inhibition of chorda tympani taste response of rat to NaCl. *Am J Physiol Regul Integr Comp Physiol* 249: R52–R61, 1985.
8. **DeSimone JA, Lyall V, Heck GL, Phan TT, Alam RI, Feldman GM, and Buch RM.** A novel pharmacological probe links the amiloride-insensitive NaCl, KCl and $NH_4Cl$ chorda tympani taste responses. *J Neurophysiol* 86: 2638–2641, 2001.
9. **Dickman JD and Smith DV.** Response properties of fibers in the hamster superior laryngeal nerve. *Brain Res* 450: 25–38, 1988.
10. **Elliot EJ and Simon SA.** The anion in salt taste: a possible role for paracellular pathways. *Brain Res* 535: 9–17, 1990.
11. **Erickson RP.** Sensory neural patterns in gustation. In: *Olfaction and Taste*, edited by Zotterman Y. Oxford, UK: Pergamon, 1963, p. 205–213.
12. **Erickson RP, Covey E, and Doetsch GS.** Neuron and stimulus typologies in the rat gustatory system. *Brain Res* 196: 513–519, 1980.
13. **Formaker BK and Hill DL.** An analysis of residual NaCl taste response after amiloride. *Am J Physiol Regul Integr Comp Physiol* 255: R1002–R1007, 1988.
14. **Frank ME.** Taste-responsive neurons of the glossopharyngeal nerve of the rat. *J Neurophysiol* 65: 1452–1463, 1991.
15. **Frank ME, Contreras RJ, and Hettinger TP.** Nerve fibers sensitive to ionic taste stimuli in the chorda tympani of the rat. *J Neurophysiol* 50: 941–960, 1983.
16. **Gilbertson TA, Boughter JD Jr, Zhang H, and Smith DV.** Distribution of gustatory sensitivities in rat taste cells: whole-cell responses to apical chemical stimulation. *J Neurosci* 21: 4931–4941, 2001.
17. **Hill DL, Formaker BK, and White KS.** Perceptual characteristics of the amiloride-suppressed sodium chloride taste response in the rat. *Behav Neurosci* 104: 734–741, 1990.
18. **Kitada Y, Mitoh Y, and Hill DL.** Salt taste responses of the IXth nerve in Sprague-Dawley rats: lack of sensitivity to amiloride. *Physiol Behav* 63: 945–949, 1998.
19. **Kloub MA, Heck GL, and DeSimone JA.** Chorda tympani responses under lingual voltage clamp: implications for $NH_4$ salt taste transduction. *J Neurophysiol* 77: 1393–1406, 1997.
20. **Kopka SL, Geran LC, and Spector AC.** The effects of time versus regeneration on the recovery of salt discrimination function following chorda tympani transection. *Am J Physiol Regul Integr Comp Physiol* 278: R720–R731, 2000.
21. **Lundy RF Jr and Contreras RJ.** Temperature and amiloride alter taste nerve responses to $Na^+$, $K^+$ and $NH_4^+$ salts in rats. *Brain Res* 744: 309–317, 1997.
22. **Lundy RF Jr and Contreras RJ.** Gustatory neuron types in rat geniculate ganglion. *J Neurophysiol* 82: 2970–2988, 1999.
23. **Lundy RF Jr, Pittman DW, and Contreras RJ.** Role for passive $Na^+$ channels and putative $Na^+/H^+$ exchange in salt

taste transduction in rats. *Am J Physiol Regul Integr Comp Physiol* 273: R1923–R1931, 1997.
24. **Markison S and Spector AC.** Amiloride is an ineffective conditioned stimulus in taste aversion learning. *Chem Senses* 20: 559–563, 1995.
25. **Miller IJ.** Gustatory receptors of the palate. In: *Food Intake and Chemical Senses*, edited by Katsuki Y, Sato M, Takagi S, and Oomura Y. Tokyo: University of Tokyo Press, 1977, p. 173–186.
26. **Minear MM, Hammack SE, Lundy RF Jr, and Contreras RJ.** Amiloride inhibits taste nerve responses to NaCl and KCl in Sprague-Dawley and Fischer 344 rats. *Physiol Behav* 60: 507–516, 1996.
27. **Morrison GR.** Behavioural response patterns to salt stimuli in the rat. *Can J Psychiatry* 21: 141–152, 1967.
28. **Nachman M.** Learned aversion to the taste of lithium chloride and generalization to other salts. *J Comp Physiol Psychol* 56: 343–349, 1963.
29. **Nakamura K and Norgren R.** Taste responses of neurons in the nucleus of the solitary tract of awake rats: an extended stimulus array. *J Neurophysiol* 70: 879–891, 1993.
30. **Nejad MS.** The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. *Chem Senses* 11: 283–293, 1986.
31. **Ninomiya Y and Funakoshi M.** Amiloride inhibition of responses of rat single chorda tympani fibers to chemical and electrical tongue stimulation. *Brain Res* 451: 319–325, 1988.
32. **Nishijo H and Norgren R.** Parabrachial neural coding of taste stimuli in awake rats. *J Neurophysiol* 78: 2254–2268, 1997.
33. **Parks JD and Whitehead MC.** Scanning electron microscopy of denervated taste buds in hamster: morphology of fungiform taste pores. *Anat Rec* 251: 230–239, 1998.
34. **Richter CP.** Salt appetite of mammals: its dependence on instinct and metabolism. In: *Feeding Instincts in Animals and Humans*. Paris: Masson, 1956, p. 578–629.
35. **Schulkin J.** *Sodium Hunger: The Search for a Salty Taste*. Cambridge, UK: Cambridge University Press, 1991, p. 6–30.
36. **Simon SA.** Influence of tight junctions on the interaction of salts with lingual epithelia: responses of chorda tympani and lingual nerves. *Mol Cell Biochem* 114: 43–48, 1992.
37. **Smith DV and Hanamori T.** Organization of gustatory sensitivities in hamster superior laryngeal nerve fibers. *J Neurophysiol* 65: 1098–1113, 1991.
38. **Sollars SI and Hill DL.** Taste responses in the GSP: substantial sodium salt and amiloride sensitivities demonstrated in two rat strains. *Behav Neurosci* 112: 991–1000, 1998.
39. **Spector AC, Andrews-Labinski J, and Letterio FC.** A new gustometer for psychophysical taste testing in the rat. *Physiol Behav* 47: 795–803, 1990.
40. **Spector AC and Grill HJ.** Salt taste discrimination after bilateral section of the chorda tympani or glossopharyngeal nerves. *Am J Physiol Regul Integr Comp Physiol* 263: R169–R176, 1992.
41. **Spector AC, Guagliardo NA, and St. John SJ.** Amiloride disrupts NaCl versus KCl discrimination performance: implications for salt taste coding in rats. *J Neurosci* 16: 8115–8122, 1996.
42. **Spector AC, Redman R, and Garcea M.** The consequences of gustatory nerve transection on taste-guided licking of sucrose and maltose in the rat. *Behav Neurosci* 110: 1096–1099, 1996.
43. **St. John SJ, Garcea M, and Spector AC.** Combined but not single gustatory nerve transection substantially alters taste-guided licking behavior to quinine in rats. *Behav Neurosci* 108: 131–140, 1994.
44. **St. John SJ, Markison S, Guagliardo NA, Hackenberg TD, and Spector AC.** Chorda tympani nerve transection and selective desalivation differentially disrupt two-lever salt discrimination performance in rats. *Behav Neurosci* 111: 450–459, 1997.
45. **St. John SJ, Markison S, and Spector AC.** Salt discriminability is related to number of regenerated taste buds after chorda tympani nerve transection in rats. *Am J Physiol Regul Integr Comp Physiol* 269: R141–R153, 1995.
46. **St. John SJ and Spector AC.** Behavioral discrimination between quinine and KCl is dependent on input from the seventh cranial nerve: implications for the functional roles of the gustatory nerves in rats. *J Neurosci* 18: 4353–4362, 1998.
47. **Wolf G.** Innate mechanisms for regulation of sodium intake. In: *Olfaction and Taste. Proceedings of the Third International Symposium*, edited by Pfaffmann C. New York: Rockefeller University Press, 1969, p. 548–553.
48. **Ye Q, Heck GL, and DeSimone JA.** The anion paradox in sodium taste reception: resolution by voltage clamp studies. *Science* 254: 724–726, 1991.
49. **Ye Q, Heck GL, and DeSimone JA.** Voltage dependence of the rat chorda tympani response to $Na^+$ salts: implications for the functional organization of taste receptor cells. *J Neurophysiol* 70: 167–178, 1993.
50. **Ye Q, Heck GL, and DeSimone JA.** Effects of voltage perturbation of the lingual receptive field on chorda tympani nerve responses to $Na^+$ and $K^+$ salts in the rat: implications for gustatory transduction. *J Gen Physiol* 104: 885–907, 1994.