

# NaCl Detection Thresholds: Comparison of Fischer 344 and Wistar rats

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## Abstract

Adult Fischer 344 (F344) rats fail to display any preference for NaCl solutions at concentrations typically preferred by other rat strains. To determine whether this behavior is due to a strain difference in NaCl detection threshold, a conditioned taste aversion (CTA) was first established to a suprathreshold concentration of NaCl (0.1 M). Then, a series of dilute NaCl solutions, ranging from 0.0 to 0.011 M NaCl, were presented to F344 ( $n = 16$ ) and Wistar ( $n = 16$ ) rats. The lowest concentration at which there was a reliable difference in the preference scores of conditioned and control rats was defined as the detection threshold. Results indicate that the detection threshold for NaCl lies between 0.001 and 0.002 M NaCl for both F344 and Wistar rats. The addition of the sodium channel blocker amiloride to the NaCl solutions raised the detection threshold 10-fold to 0.03–0.04 M NaCl for both strains of rats. These results suggest that the NaCl detection thresholds of F344 and Wistar rats are similar and that these strains do not differ in the degree to which amiloride raises this threshold.

## Introduction

Fischer 344 (F344) rats do not exhibit a preference for NaCl solutions over water and typically avoid concentrations of NaCl ( $>0.1$  M) preferred by other rat strains. Available evidence suggests that the NaCl avoidance displayed by F344 rats is mediated by taste rather than post-ingestive events (Midkiff *et al.*, 1985; Grill and Bernstein, 1988).

A number of findings point to gustatory signaling as a key factor in the NaCl avoidance of F344 rats. For one, transection of the chorda tympani (CT) nerve, the gustatory nerve conveying signals about NaCl stimulation from the anterior tongue, eliminates the NaCl avoidance of F344 rats (Sollars *et al.*, 1991; Sollars and Bernstein, 1994; Chappell *et al.*, 1998). Furthermore, electrophysiological recordings of CT whole nerve activity suggest differences between F344 (NaCl-avoidant) and Wistar rats (NaCl-preferring) in responsiveness to NaCl stimulation (Bernstein *et al.*, 1991). The relative response to above-threshold concentrations of NaCl is significantly higher in F344 than Wistar rats. Since this strain difference is eliminated by application of the sodium transport blocker amiloride, it has been suggested that the response of the F344 strain to NaCl solutions has an elevated amiloride-sensitive component.

Evidence of an amplified peripheral nerve response to NaCl in the F344 strain, along with the elimination of their NaCl aversion by peripheral nerve transection, suggests that the F344 NaCl aversion could be due to a distorted and/or exaggerated sensitivity to NaCl stimulation. However, with the exception of their avoidance of NaCl solutions, no behavioral evidence has been obtained in support of strain

differences in NaCl perception or sensitivity (Midkiff and Bernstein, 1987).

One behavioral index of sensitivity is the detection threshold for NaCl. If F344 rats are more sensitive to NaCl and this sensitivity is manifest over the full concentration range, then it might be predicted that they would display a lower detection threshold for NaCl in solution. The present study examines this hypothesis by determining detection thresholds for NaCl in F344 and Wistar rats. Thresholds were measured using a modified conditioned taste aversion (CTA) methodology that established a strong aversion to a suprathreshold (0.1 M NaCl) concentration through over-training. Conditioned and control subjects were then tested with a series of dilute NaCl concentrations. The detection threshold for NaCl was defined as the lowest concentration at which there was a reliable difference in the preference scores of conditioned and control rats. This represents a modification of prior studies assessing generalization of CTAs to different NaCl concentrations after a single conditioning trial (Tapper and Halpern, 1968; Nowlis, 1974).

## Materials and methods

### Subjects

Male F344 ( $n = 16$ ) and Wistar ( $n = 16$ ) rats (Charles River, CA) weighing 230–246 g at the start of the experiment were housed individually in stainless steel cages in a temperature- and humidity-controlled room, illuminated by a 12:12 h light/dark cycle (lights on at 6 a.m.). Behavioral tests took

place during the light phase. The rats had unrestricted access to pelleted rat chow (Teklad) and tap water unless otherwise indicated.

### Training

Rats were given 3 days to adapt to the colony room before a restricted water access schedule was implemented. On this schedule each rat received 30 min access to water in the morning (10:00–10:30 a.m.) and 60 min in the afternoon (1:30–2:30 p.m.). Body weight and water intake were monitored daily. Training continued for 8 days until water intake stabilized.

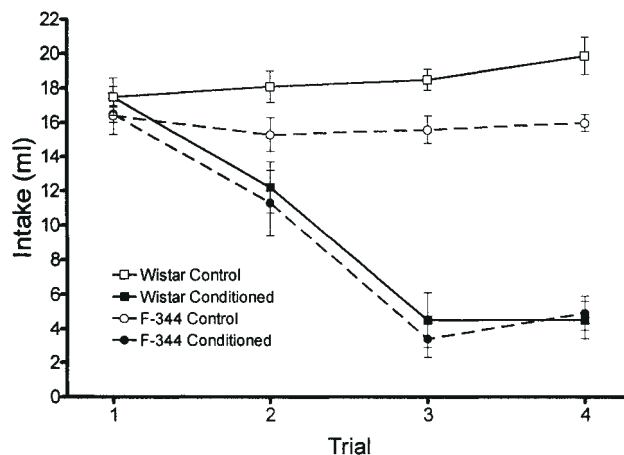
### Conditioning

Following training, rats of each strain were divided into conditioned or control (unconditioned) groups matched on body weight and water intake during training. Both groups received 30 min access to 0.1 M NaCl solution followed immediately by i.p. injections of 0.15 M LiCl (20 mg/kg) (conditioned group) or 0.15 M NaCl (saline control group). Distilled water continued to be available for 60 min in the afternoon. A rest day followed during which rats had the usual access to distilled water in the morning and afternoon but no drug injections were given. Three conditioning trials were conducted.

### Assessing NaCl detection threshold

Following conditioning, a series of 30 min one-bottle tests was administered, with NaCl solutions presented in ascending order by concentration: 0.000 (distilled water), 0.002, 0.001, 0.003, 0.005, 0.007, 0.009 and 0.011 M. However, based on a lack of systemic differences between conditioned and control animals, even at solution concentrations well above what we presumed to be the detection threshold (Carr, 1952; Slotnick, 1982; Spector *et al.*, 1990), we concluded that one-bottle tests in thirsty rats were too insensitive to measure the detection threshold. Therefore, after establishing that these animals still had strong conditioned taste aversions, we began a series of two-bottle tests (see Figure 1).

During the 30 min test rats were given access to two solutions: a NaCl solution and distilled water. A forced tasting method was adopted to ensure that each animal sampled both solutions at the beginning of the test. Thus, the first bottle was presented alone until the rat approached and commenced licking its spout. The rat was then allowed to lick for ~5 s, at which time the first bottle was removed and the second bottle was presented in the same manner. After the second bottle was removed both bottles were presented simultaneously for 30 min. With this procedure all of the subjects were observed to sample the contents of each bottle before data collection began. NaCl preference scores were derived by dividing total NaCl intake by total fluid intake (distilled water + NaCl intake). The position of each bottle on the home cage and the order of presentation were



**Figure 1** Mean intake of 0.1 M NaCl by F344 and Wistar rats during CTA acquisition (Trial 1) and expression (Trials 2–4). Trial 4 was conducted immediately prior to the two-bottle tests of detection threshold. Conditioned (LiCl-injected) rats (of each strain) demonstrated a robust decrease in the intake of the 0.1 M NaCl solution. Error bars, SEM.

counterbalanced on a daily basis to avoid presentation or side bias. The following NaCl solutions were used and administered in the order listed: 0.011, 0.009, 0.007, 0.005, 0.003, 0.001, 0.0009, 0.001 and 0.002 M. Distilled water was available in the afternoon for 60 min.

### NaCl and amiloride

In the rat, gustatory responses to NaCl are suppressed by the sodium transport blocker amiloride (Heck *et al.*, 1984). Thus, in the present study amiloride was used to evaluate the hypothesis that strain difference in amiloride sensitivity would lead to differential effects of amiloride exposure on NaCl detection thresholds of F344 and Wistar rats. Rats were presented with two solutions: distilled water and NaCl solutions mixed with amiloride hydrochloride. The following concentrations of NaCl with amiloride (100  $\mu$ M) were presented in the following order: 0.01, 0.02, 0.04 and 0.03 M. A two-bottle test with 0.01 M NaCl without amiloride and distilled water was conducted at the end of this series of testing to ensure that the CTA was still present.

### Data analysis

The data were analyzed using factorial analysis of variance (ANOVA). Paired comparisons were performed using the Tukey test. Statistical reliability was set at  $P < 0.05$ .

## Results

### Conditioning

As illustrated by Figure 1, rats in the conditioned groups of both strains demonstrated a significant reduction in intake of the 0.1 M NaCl solution relative to their respective controls. A repeated measures ANOVA revealed a significant interaction between the Drug and Conditioning Trial

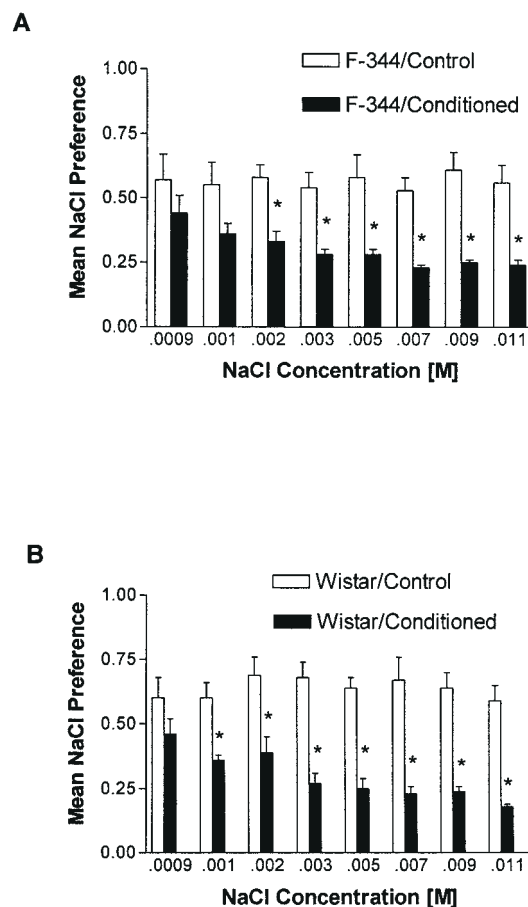
[ $F(1,26) = 120.2, P < 0.001$ ]. On the first conditioning trial there were no reliable differences in the intake of the conditioned and control groups of each strain. On the subsequent conditioning trials, however, the conditioned groups had a lower intake of the tastant than did their respective control groups ( $P_s < 0.05$ ). This CTA was still robust 1 week later when evaluated before the start of the two-bottle tests. Conditioned rats of either strain had equivalently lower intakes of the 0.1 M NaCl solution than did control rats [ $F(1,28) = 197.74, P < 0.0001$ ] (Figure 1, Trial 4).

#### NaCl detection threshold

Mean NaCl preference scores of conditioned and control F344 and Wistar rats are illustrated in Figure 2. First, while F344 rats typically display NaCl avoidance and Wistar rats display NaCl preference, in the present study there was no reliable strain difference with respect to NaCl preference for any of the concentrations that were presented. This finding was not unexpected in light of our observations that F344 NaCl avoidance is reduced or eliminated when rats are water deprived, as was the case in the present study (Bernstein, unpublished observations). Second, in the present study the detection threshold was defined as the lowest concentration at which there was a reliable difference in NaCl preference displayed by conditioned and control rats within each strain. Based on this definition our results indicate that the NaCl detection threshold for both F344 and Wistar rats lies between 0.001 and 0.002 M (Figure 2). A 2 (Drug)  $\times$  2 (Strain)  $\times$  8 (Concentration) factorial ANOVA revealed a significant main effect of Drug [ $F(1,224) = 191.9, P < 0.0001$ ], as well as a significant Concentration  $\times$  Drug interaction [ $F(7,224) = 2.07, P < 0.05$ ]. As depicted in Figure 2, the conditioned rats of the Wistar strain showed significant reductions in preference for dilute NaCl concentrations ranging from 0.011 to 0.001 M NaCl. Similarly, the conditioned rats of the F344 strain also showed significant reductions in preference for the NaCl concentrations ranging from 0.011 to 0.002 M NaCl. Notably, while there was no reliable difference in the preference scores of the conditioned and control F344 rats at the 0.001 M concentration, the degree of suppression was comparable between both strains [degree of suppression  $\{1 - (\text{preference scores of conditioned rats}/\text{preference scores of control rats}) \times 100\}$ : 19.9% for F344 rats versus 29.6% for Wistar rats,  $t(14) = 0.378, n.s.$ ]. In contrast, at the 0.0009 M NaCl concentration there was no longer a reliable difference in the preference scores of the conditioned and control rats of either strain. Collectively, these results identify a similarity in the NaCl detection thresholds of F344 and Wistar rats, falling between 0.001 and 0.002 M NaCl.

#### NaCl and amiloride

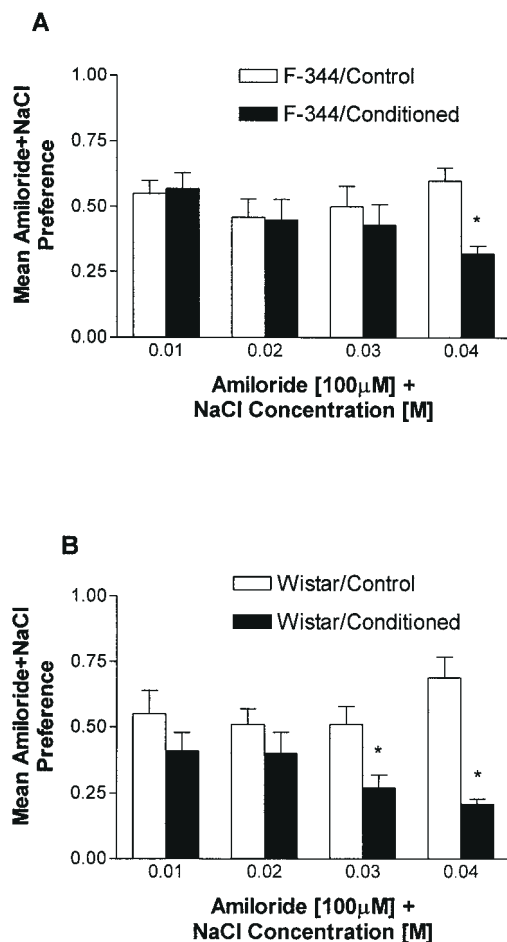
The addition of the sodium channel blocker amiloride to the NaCl solutions led to an almost 10-fold increase in the



**Figure 2** Mean NaCl preference displayed by conditioned or control F344 (A) or Wistar (B) rats to a series of dilute NaCl solutions ranging from 0.0009 to 0.011 M NaCl. \* $P < 0.05$ . Error bars, SEM.

NaCl detection thresholds of both strains of rats. As is evident in Figure 3 the Wistar rats exhibited a reliable difference in preference scores for the 0.03 M NaCl + amiloride and the 0.04 M NaCl + amiloride solutions. Similarly, the F344 rats exhibited a reliable difference in preference scores for the 0.04 M NaCl + amiloride solution. Notably, while there was no significant difference in preference scores for F344 rats for the 0.03 M NaCl + amiloride solution, there was also no difference in the suppression ratios of either strain at this concentration [ $t(14) = 2.03, n.s.$ ]. These results were supported by statistical analyses which revealed a significant main effect of Drug [ $F(1,111) = 21.73, P < 0.0001$ ], as well as a significant Concentration  $\times$  Drug interaction [ $F(3,111) = 4.64, P < 0.005$ ]. Thus, the addition of amiloride to the solutions required a higher concentration of NaCl in order to be detected by both strains of rats. Collectively, these results suggest that amiloride raised the NaCl detection thresholds of both strains ~10-fold, from 0.001–0.002 to 0.03–0.04 M. Even at this elevated threshold level, however, a strain difference was not evident.

At the end of this series of tests a final two-bottle test of 0.01 M NaCl without amiloride and distilled water was



**Figure 3** Mean preference displayed by conditioned or control F344 (A) or Wistar (B) rats to a series of amiloride (100 µM) + NaCl solutions. \* $P < 0.05$ . Error bars. SEM.

conducted. Preference scores were as follows: F344/control = 0.53 ( $\pm 0.06$ ), F344/conditioned = 0.32 ( $\pm 0.06$ ), Wistar/control = 0.62 ( $\pm 0.06$ ) and Wistar/conditioned = 0.29 ( $\pm 0.05$ ). Analyses indicated a significant main effect of Drug [ $F(1,28) = 23.4$ ,  $P < 0.001$ ], evidenced by conditioned animals of both strains having lower intakes of this 0.01 M NaCl solution relative to the controls. Both the main effect of Strain and the Strain  $\times$  Drug interaction were not significant, indicating that the magnitude of the CTA in both F344 and Wistar rats was equivalent.

## Discussion

Three key findings emerge from this study. First, there was no indication of differences in threshold sensitivity to NaCl between the salt-avoidant F344 and the salt-preferring Wistar strains. In this study, detection threshold was defined as the lowest concentration at which there was a reliable difference in the preference scores of conditioned and control rats. Results indicated that the detection threshold of both strains was similar, lying between 0.001 and

0.002 M NaCl. Therefore, these findings provide no support for the notion that unusual sensitivity to NaCl underlies the F344 strain's dislike of salt.

A second finding is that the CTA paradigm can be adapted to efficiently determine taste detection thresholds. Although single-bottle tests proved ineffective and insensitive for this purpose, two-bottle tests yielded threshold values which were clearly within the range of those obtained using other methods, such as operant training (for a review see McCaughey and Scott, 1998). For example, we found NaCl detection threshold concentrations to be  $\sim 0.001$ – $0.002$  M NaCl. This compares favorably with studies of other strains of rats using a variety of experimental paradigms, which report a range of NaCl detection thresholds of 0.0001–0.002 M NaCl (Carr, 1952; Slotnick, 1982; Spector *et al.*, 1990). This application of the CTA paradigm to estimate detection thresholds is not intended to minimize the importance of operant techniques in the careful assessment of psychophysical functions in animals. Since our technique is based on conditioning a response to a single stimulus concentration and measuring responses to other concentrations in extinction, it could strictly be classified as an 'intensity generalization threshold' rather than a 'detection threshold'. Strong aversions, achieved through overtraining, made it likely that detection of the taste of NaCl would lead to reductions in intake. To confirm this, however, it would be necessary to demonstrate that changing the conditioning concentration does not significantly alter the 'detection threshold'. Despite this limitation, the use of the CTA paradigm provides an alternative technique that has the distinct advantage of being more widely available to a range of investigators who may have questions regarding gustatory sensitivity of animals.

Third, although we found dramatic effects of amiloride on threshold sensitivity, we found no evidence of strain differences in the magnitude of this effect. This investigation was based on previous work indicating that lingual application of amiloride strongly attenuates the neural response provoked by NaCl stimulation (Heck *et al.*, 1984) and apparently alters the taste of NaCl so that rats are unable to discriminate it from KCl (Spector *et al.*, 1996). Recently it has been reported that amiloride significantly elevates detection thresholds for NaCl in an operant paradigm (Geran and Spector, 2000). Thus, we expected the addition of amiloride to NaCl to elevate detection threshold. We also anticipated that a stronger amiloride effect might be seen in the F344 strain based on prior electrophysiological studies that pointed to a greater amiloride-sensitive component to the F344 CT response to NaCl as compared with that of the Wistar strain (Bernstein *et al.*, 1991). The effect of amiloride on detection threshold could be based on the channel blocker's attenuation of the neural response and hence reduced sensitivity *per se*. Alternatively, while amiloride solutions by themselves do not appear to have a detectable taste to rats (Bernstein and Hennessey, 1987; Markison and



Spector, 1995), the amiloride-adulterated NaCl solution may be sufficiently unlike the conditioning solution that it is perceived as a novel tastant (Formaker and Hill, 1988). However, the similarity between our results and those of Geran and Spector (2000) with respect to the effect of amiloride on NaCl detection threshold favors the reduction of NaCl sensitivity as an explanation of both sets of data.

## Acknowledgement

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## References

- Bernstein, I.L.** and **Hennessey, C.J.** (1987) *Amiloride-sensitive sodium channels and expression of sodium appetite in rats*. *Am. J. Physiol.*, 253, R371–R374.
- Bernstein, I.L., Longley, A.** and **Taylor, E.M.** (1991) *Amiloride-sensitivity of the chorda tympani response to NaCl in Fischer-344 and Wistar rats*. *Am. J. Physiol.*, 261, R329–R333.
- Carr, W.J.** (1952) *The effect of adrenalectomy upon the NaCl taste threshold in rats*. *J. Comp. Physiol. Psychol.*, 45, 377–380.
- Chappell, J.P., St John, S.J.** and **Spector, A.C.** (1998) *Amiloride does not alter NaCl avoidance in Fischer-344 rats*. *Chem. Senses*, 23, 151–157.
- Formaker, B.** and **Hill, D.L.** (1988) *An analysis of residual NaCl response after amiloride*. *Am. J. Physiol.*, 255, R1002–R1007.
- Geran, L.C.** and **Spector, A.C.** (2000) *Amiloride increases sodium chloride taste detection threshold in rats*. *Behav. Neurosci.*, 114, 623–634.
- Grill, H.J.** and **Bernstein, I.L.** (1988) *Strain differences in taste reactivity to NaCl*. *Am. J. Physiol.*, 255, R424–R430.
- Heck, G.L., Mierson, S.** and **Desimone, J.A.** (1984) *Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway*. *Science*, 223, 403–405.
- Markison, S.** and **Spector, A.C.** (1995) *Amiloride is an ineffective conditioned stimulus in taste aversion learning*. *Chem. Senses*, 20, 559–563.
- McCaughey, S.A.** and **Scott, T.R.** (1998) *The taste of sodium*. *Neurosci. Biobehav. Rev.*, 22, 663–676.
- Midkiff, E.E.** and **Bernstein, I.L.** (1987) *Generalization of conditioned taste aversion to sodium chloride in Fischer-344 and Wistar rats*. *Ann. N.Y. Acad. Sci.*, 510, 498–500.
- Midkiff, E.E., Fitts, D.A., Simpson, J.B.** and **Bernstein, I.L.** (1985) *Absence of sodium chloride preference in Fischer-344 rats*. *Am. J. Physiol.*, 249, R438–R442.
- Nowlis, G.H.** (1974) *Conditioned stimulus intensity and acquired alimentary aversions in the rat*. *J. Comp. Physiol. Psychol.*, 86, 1173–1184.
- Slotnick, B.M.** (1982) *Sodium chloride detection threshold in the rat determined using a simple operant task*. *Physiol. Behav.*, 28, 707–710.
- Sollars, S.I.** and **Bernstein, I.L.** (1994) *Gustatory deafferentation and desalivation: effects on NaCl preference of Fischer 344 rats*. *Am. J. Physiol.*, 266, R510–R517.
- Sollars, S.I., Sollars, P.J.** and **Bernstein, I.L.** (1991) *Reversal of the sodium chloride aversion of Fischer 344 rats by chorda tympani nerve transection*. *Behav. Neurosci.*, 105, 603–605.
- Spector, A.C., Schwartz, G.J.** and **Grill, H.J.** (1990) *Chemospecific deficits in taste detection after selective gustatory deafferentation in rats*. *Am. J. Physiol.*, 27, R820–R826.
- Spector, A.C., Guagliardo, N.A.** and **St John, S.J.** (1996) *Amiloride disrupts NaCl versus KCl discrimination performance: implications for salt taste coding in rats*. *J. Neurosci.*, 16, 8115–8122.
- Tapper, D.N.** and **Halpern, B.P.** (1968) *Taste stimuli: a behavioral categorization*. *Science*, 161, 708–710.

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