

---

# Taste, Saliency, and Increased NaCl Ingestion after Repeated Sodium Depletions

---

David M. Dietz, Kathleen S. Curtis and Robert J. Contreras

Department of Psychology and Program in Neuroscience, The Florida State University, Tallahassee, FL 32306-1270, USA

Correspondence to be sent to: Kathleen S. Curtis, Department of Psychology and Program in Neuroscience, The Florida State University, Tallahassee, FL 32306-1270, USA. e-mail: [curtis@psy.fsu.edu](mailto:curtis@psy.fsu.edu)

---

## Abstract

Previous studies have shown that repeated sodium depletions using the natriuretic–diuretic furosemide induce progressive increases in NaCl ingestion. We investigated the role of taste in this behavioral sensitization in Sprague–Dawley rats using short-term lickometer testing along with 2-h stimulated intake tests. Our results show maximal licking across a range of NaCl concentrations after each of the three depletions, regardless of whether the solutions contained sucrose or were presented alone. Similarly, the presence of sucrose did not affect stimulated NaCl intake in long-term tests, although ingestion of NaCl solutions increased progressively with successive depletions. Finally, both licking and ingestion returned to baseline levels during need-free conditions. These results suggest that sodium imbalance acutely increases the saliency of sodium taste and thereby the likelihood of NaCl ingestion, which may, in turn, contribute to progressive increases in NaCl intake that occur with multiple furosemide-induced sodium depletions.

**Key words:** furosemide, lickometer, salt appetite, sensitization, Sprague–Dawley rats

## Introduction

The maintenance of appropriate body sodium levels is critical for survival and involves a concerted suite of physiological and behavioral mechanisms. Seeking and consuming salt in response to sodium need is an essential behavior for sodium homeostasis. Salt appetite has been suggested to be an innate response to a sodium deficit and independent of learned behavior (Epstein and Stellar, 1955). Consistent with this idea, rats that have been adrenalectomized at an early age consume large quantities of a novel hypertonic saline solution (Richter, 1936).

Adult rats also exhibit compensatory behavioral responses to challenges to body sodium balance. For example, rapid sodium and fluid depletion caused by the natriuretic furosemide elicits a strong and reliable stimulated NaCl intake. Interestingly, repeated depletions using furosemide have been reported to produce successive increases in the stimulated intake of concentrated NaCl solutions, as well as to enhance spontaneous or “need-free” ingestion of NaCl (Sakai *et al.*, 1987, 1989). Plasma levels of hormones associated with NaCl intake such as angiotensin II (AngII) and aldosterone (Aldo) increase after each furosemide treatment, but the increases are not enhanced with subsequent depletions (Sakai *et al.*, 1987). Furthermore, urinary sodium excretion remains constant over multiple depletions (Leshem *et al.*, 2004) and

returns to baseline levels between furosemide treatments. Thus, the biological basis for this behavioral sensitization, the progressive increase in need-free and stimulated NaCl intake, with subsequent furosemide treatments is not well understood. Given the role of taste in ingestive behavior in general, and in NaCl intake in particular, it seems possible that the progressive increase in NaCl intake after repeated furosemide treatments may involve sodium taste responses, as has been shown in other models of stimulated NaCl intake (Curtis *et al.*, 2001).

Clearly, the taste of sodium is not the sole determinant in the enhanced NaCl appetite after furosemide treatments, as rats that have undergone repeated sodium depletions followed by repletions that bypass the gustatory system nonetheless show progressive increases in stimulated and need-free NaCl intake (Sakai *et al.*, 1987, 1989). However, in the absence of postingestive consequences, ingestion is profoundly influenced by the taste of sodium (Mook, 1963; Frankmann *et al.*, 1996; Davis *et al.*, 2002). In addition, sodium state alters the sensory responses to sodium as electrophysiological activity in gustatory nerves is changed during dietary sodium deprivation (Contreras, 1977; Contreras and Frank, 1979) and after furosemide treatment (Bernstein and Taylor, 1992). Accordingly, we hypothesized that a shift in

the salience of sodium taste—the relevant feature to which the animal attends and which drives the behavior—may contribute to increased stimulated and need-free NaCl intake after multiple depletions. We used an established model to examine short-term behavioral taste responses (Curtis *et al.*, 2001, 2004), as well as long-term intake tests, to investigate the role of sodium taste in the behavioral sensitization of furosemide effects.

## General methods

Rats were housed individually in clear Plexiglas cages (19 × 10.5 × 8 inches) and habituated to the animal facility for 1 week. Temperature was held constant at 72 ± 2°F with humidity at 55%. The lights were maintained on a 12-h light cycle with lights on at 7:00 AM. All animals had *ad libitum* access to standard laboratory chow (Purina #5000) and water except where noted. All experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of Florida State University.

Rats were depleted using furosemide (Intervet Inc., Millsboro, DE; Furo, 10 mg/kg, subcutaneous, given in two 5-mg/kg injections separated by 90 min) at weekly intervals for a total of 3 weeks. Each week, cages were changed after the second furosemide injection, and normal rat chow was replaced with sodium-deficient diet chow (Harlan Teklad, Indianapolis, IN). Rats were tested 18–24 h after furosemide injections.

### Experiment 1

Bernstein and Taylor (1992) demonstrated that electrophysiological responses of the chorda tympani nerve, which innervates taste receptors on the anterior tongue, were altered after a single furosemide-induced sodium depletion. Moreover, in behavioral tests, rats increased licking across a range of NaCl concentrations after a single furosemide treatment (Breslin *et al.*, 1993; Brot *et al.*, 2000). We hypothesized that repeated furosemide depletions produce progressive and long-lasting changes in the salience of sodium taste. To assess taste responses to sodium, we used short-term (10 s) tests, which reduce the possibility of postingestive signals. In these tests, NaCl solutions were presented alone or mixed in a dilute sucrose solution. Using an NaCl–sucrose mixture enabled us to assess the salience of sodium taste via alterations in lick rates to concentrated NaCl solutions and also produced stable and reliable rates of baseline licking without the necessity for water deprivation (Curtis *et al.*, 2001, 2004).

### Methods

Male Sprague–Dawley (SD) rats ( $n = 8$ ), housed as described in General Methods, were trained to consume fluids during 10-s presentations of drinking tubes using the Davis MS80 Rig (Dilog Instruments and Systems, Tallahassee, FL), a programmable lickometer with a shutter that is opened or closed

electronically to give the rat access to one of eight drinking tubes that are mounted on a sliding platform. A microcomputer controls the shutter and the position of the platform, thus determining the order and duration of the tube presentation, as well as the interval between presentations. Contact with the spout of a drinking tube completes an electrical circuit generating <60 nA that is recorded by the microcomputer as a lick.

Training and testing followed procedures described previously (Curtis *et al.*, 2001, 2004). Briefly, rats were acclimated to the Davis MS80 Rig by being placed in the test chamber with access to one tube of 0.2 M sucrose for 15 min/day on 2 consecutive days. For the next 7 days, rats were given water for only 30 min/day in their home cages following 15-min access to 0.2 M sucrose in the Davis MS80 Rig. On the last 4 days, rats were given three 200-s presentations of 0.2 M sucrose to acclimate them to the sound and motion of the sliding platform. Rats were then given *ad libitum* access to water for the remainder of the training and testing, which was conducted at 2- to 5-day intervals. Presentations of 0.2 M sucrose were decreased to 10 s, and training continued for three to five sessions until rats reliably licked the tubes during each 10-s presentation.

As in our previous studies (Curtis *et al.*, 2001, 2004), we mixed NaCl in a dilute (0.05 M) sucrose solution (NaSuc) to elicit reliable, consistent licking in short-term tests without the use of water deprivation. This protocol allowed us to assess sodium taste responses while minimizing the confounding hormonal and physiological responses to the additional challenge to body fluid balance posed by water deprivation. Rats were given 10-s presentations of the following solutions: water, 0.05 M sucrose, 0.15 M NaCl, 0.28 M NaCl, 0.5 M NaCl, 0.15 M NaSuc, 0.28 M NaSuc, and 0.5 M NaSuc. Solutions were presented in semirandom order, and each solution was presented twice during one session. In these sessions, if a rat did not lick within 180 s, the shutter closed and the platform moved to present a different tube.

All rats were tested during three testing sessions before the first furosemide injection, and the average was used to establish baseline lick rates. Thus, for each solution, licks during baseline were the average of six 10-s trials (two trials on each of the 3 days). Rats were then injected with furosemide at weekly intervals for a total of 3 weeks as described and 24 h later were tested in the Davis MS80 Rig lickometer. For each solution, licks during each depletion are the average of two trials. Finally, licking rates were tested in the Davis Rig two times after each weekly depletion to examine taste responses during need-free conditions, and the average was used to determine lick rates in sodium-replete states. Thus, for each solution, licks during each need-free condition are the average of four trials (two trials on each of the 2 days).

### Statistical analyses

Data are shown as means ± SE. Data were analyzed using two-way (day, solution) repeated-measures analysis of

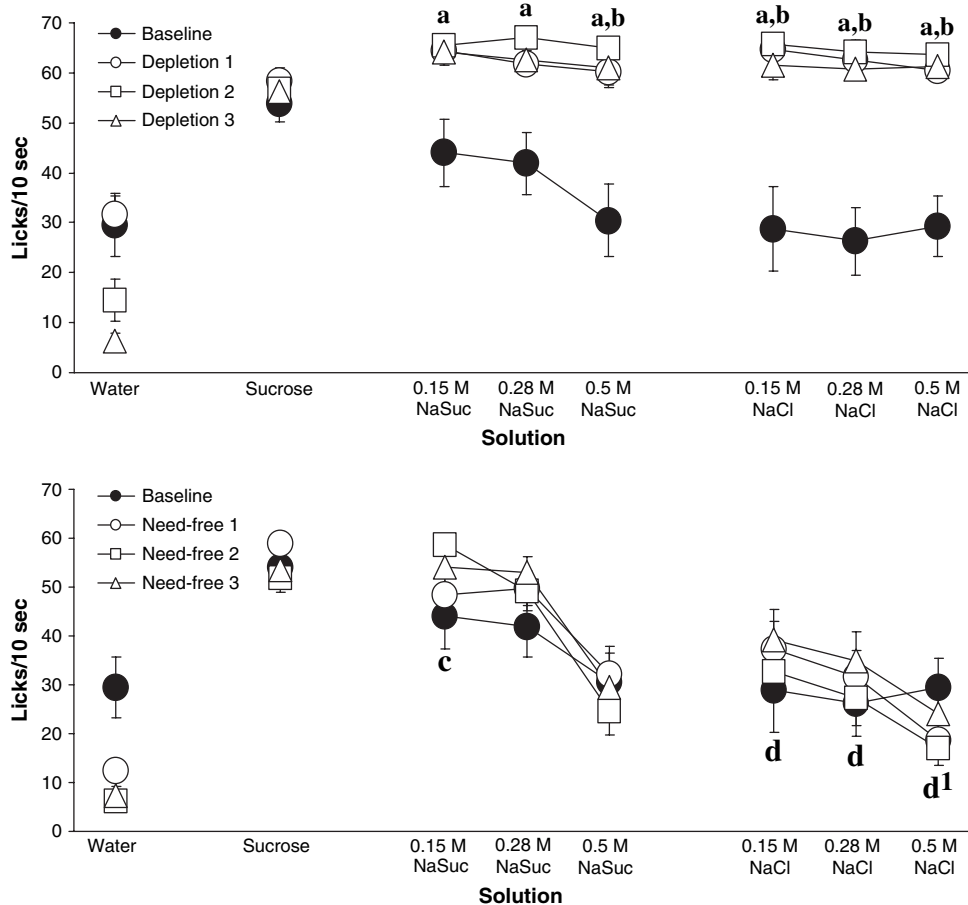
variance (ANOVA). Where appropriate, ANOVAs were followed by Newman–Keuls *post hoc* tests. The criterion for statistical significance was  $P < 0.05$ . All statistical analyses were performed using Statistica software (Statsoft Inc., Tulsa, OK).

### Results

Figure 1 shows the results from short-term behavioral taste tests. Licking was affected by day [ $F(6,42) = 19.136$ ;  $P < 0.001$ ], with overall licking rates on each furosemide-treatment day (Figure 1, top) significantly greater than those during both baseline and need-free conditions (Figure 1, bottom). Licking also was affected by solution [ $F(7,42) = 41.53$ ,  $P < 0.001$ ]. *Post hoc* comparisons revealed that, overall, rats licked at significantly lower rates to water compared to all other solutions and at consistently higher rates to 0.05 M sucrose compared to all solutions except 0.15 M NaSuc and 0.28 M NaSuc.

There also was a significant interaction between day and solution [ $F(42,294) = 10.15$ ,  $P < 0.001$ ]. Rats licked at consistently high rates to 0.05 M sucrose on all days and at similarly high rates to all NaCl solutions regardless of whether the solution contained sucrose on furosemide-treatment days. Thus, licking to 0.05 M sucrose, 0.15 M NaSuc, 0.28 M NaSuc, 0.5 M NaSuc, 0.15 M NaCl, 0.28 M NaCl, and 0.5 M NaCl did not differ from each other on furosemide-treatment days.

In regard to solutions containing NaCl alone, licking rates were significantly greater on each furosemide-treatment day (Figure 1, top) than those during both baseline and need-free conditions (Figure 1, bottom), whereas the licking rates during need-free conditions did not differ from those during baseline. In general, licking rates during baseline and need-free conditions were lowest to 0.5 M NaCl and highest to 0.15 M NaCl.



**Figure 1** Behavioral taste responses during baseline (filled circles), weekly furosemide-induced sodium depletions (top panel), or need-free conditions between depletions (bottom panel). Rats were given 10-s access to water; 0.05 M sucrose; 0.15, 0.28, and 0.5 M NaCl; and 0.15, 0.28, and 0.5 M NaCl mixed in 0.05 M sucrose (NaSuc). Open circle, depletion 1 or need-free 1; open square, depletion 2 or need-free 2; open triangle, depletion 3 or need-free 3. For a given solution, a = each depletion significantly greater than baseline, b = each depletion significantly greater than each need-free condition, and c = need-free 2 significantly greater than baseline. For a given concentration, d = all NaCl during baseline and need-free conditions significantly less than all NaSuc and d<sup>1</sup> = NaCl during need-free 1 significantly less than NaSuc during need-free 1.

Licking rates to all NaSuc solutions on each furosemide-treatment day also were significantly greater than those during baseline; however, only the rate of licking to 0.5 M NaSuc was significantly greater on furosemide-treatment days than during the need-free conditions. Licking rates to each NaSuc solution during need-free conditions did not differ from those during baseline except that licking to 0.15 M NaSuc during the second need-free condition was greater than that during baseline. In general, licking rates during baseline and need-free conditions were lowest to 0.5 M NaSuc, but rates to 0.15 M NaSuc and 0.28 M NaSuc were not different.

Finally, comparisons of licking to NaSuc and NaCl alone during baseline and need-free conditions showed that rats licked significantly more to 0.15 and 0.28 M NaSuc solutions than to the same concentrations of NaCl alone, both during baseline and during all need-free conditions. In contrast, licking rates to 0.5 M NaSuc differed from those to 0.5 M NaCl only during the first need-free condition.

### Discussion

The present results demonstrate that, in the absence of post-ingestive cues, a single sodium depletion using furosemide substantially increases licking to NaCl solutions. Others have reported that behavioral taste responses to NaCl solutions were altered after a single depletion (Breslin *et al.*, 1993; Brot *et al.*, 2000), although in those studies, the pattern of concentration-dependent licking tended to be more pronounced. In our shorter (10 s) tests using both NaCl and NaSuc solutions, we evaluated taste responses after multiple furosemide-induced sodium depletions and showed that, after each of the three depletions, rats licked at maximal rates to all NaCl-containing solutions, regardless of concentration or whether the solution was NaCl or NaSuc. Licking to both NaCl alone and NaSuc returned to baseline levels during need-free conditions. Thus, contrary to our hypothesis, and despite previous reports that prior episodes of sodium depletion enhance ingestion during need-free states (Sakai *et al.*, 1987, 1989; Leshem *et al.*, 2004), behavioral taste responses to sodium did not increase progressively.

Interestingly, the furosemide-induced increase in licking was much larger for the NaCl solutions than for the NaSuc solutions. In fact, rates of licking to all NaCl solutions were equivalent during depletions. In contrast, rates of licking to NaSuc solutions were greater than those to NaCl during both baseline and need-free conditions. We cannot discount the possibility that the maximal rate of licking in response to sodium depletion may have prevented the detection of subtle differences in licking to the two solutions. Nonetheless, these observations are suggestive of a depletion-induced change in behavioral taste responses that does not depend on the number of sodium depletions and is not influenced by the presence of sucrose. We propose that, regardless of depletion history, a change in sodium state alters sodium taste

responses and thereby permits NaCl intake above baseline levels. In other words, although the NaSuc solution is more palatable, the taste of sodium is more salient during depletion states.

### Experiment 2

During short-term taste tests in Experiment 1, rats licked maximally to all solutions containing NaCl regardless of the presence of sucrose. Interestingly, the increase from baseline was more dramatic for solutions of NaCl alone. Rats are able to distinguish different tastes in complex mixtures (Hsiao and Fan, 1993), and one component of these mixtures may be of greater physiological relevance than the others. Accordingly, we hypothesized that sodium is the salient taste during furosemide-induced sodium depletions and employed long-term intake tests using NaCl and NaSuc to evaluate this hypothesis.

### Methods

Twenty-nine male SD rats (Charles River, Wilmington, MA) weighing 300–325 g at the beginning of the experiment were used in this study. Rats were housed and habituated to the animal facility for 1 week. All rats were then given 3 days of free access to sipper tubes containing water and either 0.5 M NaCl ( $n = 14$ ) or 0.5 M NaSuc ( $n = 15$ ). Prior exposure to the NaCl or NaSuc solutions eliminated the possibility of novelty-induced consumption and allowed measurement of baseline intakes.

Animals were then sodium depleted using furosemide as described above or were given injections of the 0.15 M saline vehicle (control) at weekly intervals for a total of 3 weeks. After injections, sipper tubes containing NaCl solutions were removed, and the standard rat chow was replaced with sodium-deficient food. Twenty-four hours later, chow was removed and sipper tubes containing water and either NaCl or NaSuc were hung from cages. During the 2-h intake tests, the amount of either 0.5 M NaCl or NaSuc ingested was recorded at 15-min intervals. After the 2-h tests, standard rat chow was returned. Sipper tubes remained in place, and 24-h intakes were then recorded for 3 days beginning 48 h after the 2-h tests (need-free condition).

### Statistical analyses

NaCl or NaSuc intakes during the 2-h intake tests and the 24-h tests in need-free conditions were evaluated using three-way (treatment, solution, day) repeated-measures ANOVA. Two-way (depletion, time) repeated-measures ANOVA was used to further examine the 2-h intakes of the NaCl/NaSuc solutions by furosemide-treated rats over time. There were no differences in intakes between the NaCl and NaSuc solutions after furosemide; thus, intakes were collapsed across solutions, and comparisons were made of intakes during the first 15 min and the remaining 105 min of the 2-h tests. Where appropriate, ANOVAs were followed

by Newman–Keuls *post hoc* tests. The criterion for statistical significance was  $P < 0.05$ . All statistical analyses were performed using Statistica software (Statsoft Inc.).

### Results

Figure 2 shows stimulated NaCl intake in the 2-h tests after each furosemide-induced sodium depletion. Intakes were affected by treatment [ $F(1,25) = 86.7555$ ,  $P < 0.001$ ], with rats injected with furosemide ingesting significantly more NaCl during the 2-h intake tests compared to rats given control injections. Intakes also were affected by day [ $F(2,50) = 22.824$ ,  $P < 0.001$ ], with NaCl intake increasing over time. Intakes also were affected by the interaction between treatment and day [ $F(2,50) = 13.468$ ,  $P < 0.001$ ], and *post hoc* analysis revealed that, overall, NaCl intake in the 2-h tests after each furosemide treatment was greater than that after control injections. In addition, regardless of whether the solutions contained sucrose, NaCl intake increased progressively with each furosemide treatment. Intake after the second furosemide treatment was greater than that after the first treatment, and intake after the third furosemide treatment was greater than that after both the first and second treatments (all  $P$  values  $< 0.001$ ). In contrast, repeated control treatments had no effect on intake. Finally, whether or not the NaCl solutions contained sucrose had no effect on intake [ $F(1,25) = 0.515$ ,  $P > 0.5$ ].

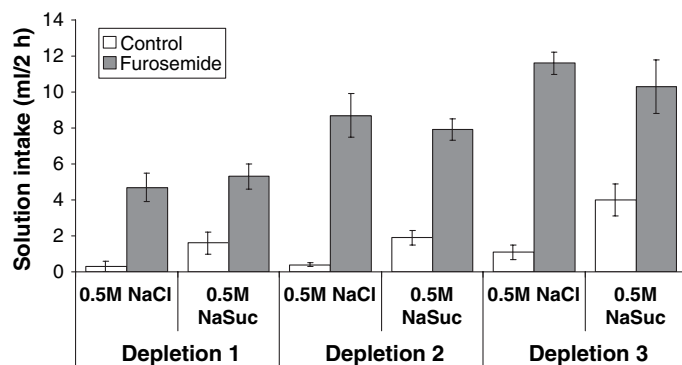
Figure 3 shows NaCl intake within the 2-h tests. The amount of NaCl solutions ingested after furosemide treatments depended on depletion [ $F(1,17) = 32.172$ ,  $P < 0.001$ ], with overall intake increasing with repeated depletions. Intake also depended on time [ $F(2,34) = 34.922$ ,  $P < 0.001$ ]. Regardless of the number of depletions, intake during the first 15 min was greater than that during the remainder of the 2-h test. There also was a significant interaction between depletion and time [ $F(2,34) = 14.738$ ,  $P < 0.001$ ]. *Post hoc* analyses revealed that intake during the first 15 min increased progressively after each furosemide

treatment. After the second depletion, the 15-min intake was greater than that after the first depletion, and the 15-min intake after the third furosemide treatment was greater than that after both the first and second depletions (all  $P$  values  $< 0.05$ ). Furthermore, whereas intake during the first 15 min was not different from that during the remaining time after the first depletion, intake during the first 15 min was significantly greater than that during the remaining time after both the second and third depletions. Finally, intakes during the last 105 min of the 2-h tests were not different after any of the depletions.

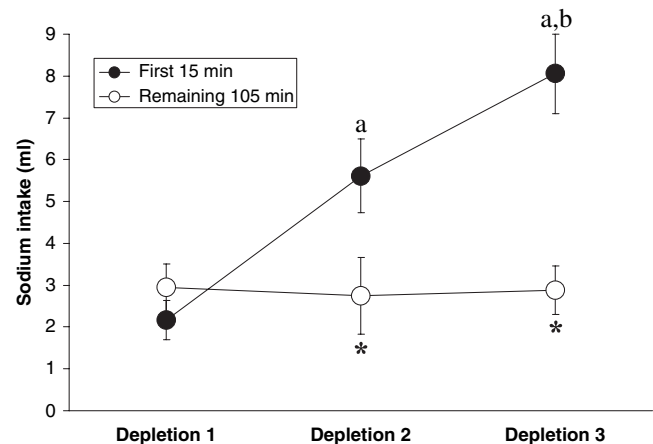
Unlike the 2-h stimulated intake tests, intake during need-free conditions (Figure 4) was not affected by treatment [ $F(1,25) = 0.86455$ ,  $P > 0.05$ ; Figure 3] but was significantly affected by solution [ $F(1,25) = 15.706$ ,  $P < 0.001$ ]. Regardless of day or treatment, rats given 24-h access to NaSuc ingested significantly more than those given NaCl alone. Intake during need-free conditions also depended on day [ $F(2,50) = 14.14$ ,  $P < 0.001$ ], indicating that, regardless of solution or whether rats had been treated with furosemide, intake increased from the first need-free condition to the third need-free condition. Finally, need-free intake depended on the interaction between day and solution [ $F(2,50) = 5.557$ ,  $P < 0.05$ ]. *Post hoc* analyses revealed that, regardless of furosemide treatment, rats given NaCl alone did not increase intake during need-free conditions. In contrast, rats given NaSuc significantly increased intake during the third need-free condition compared to both the first and second conditions ( $P$  values  $< 0.05$ ).

### Discussion

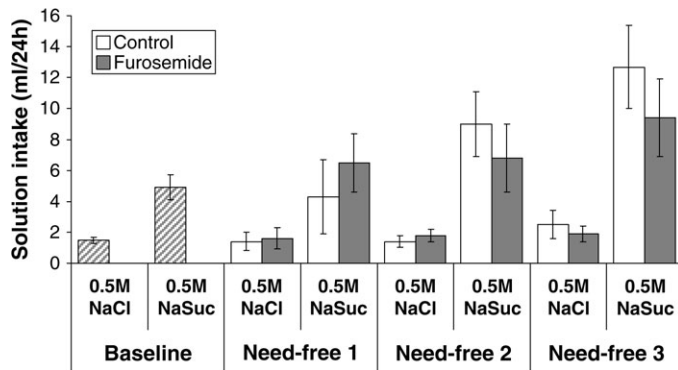
Our results show that SD rats progressively increased NaCl intake in the 2-h tests after repeated injections of furosemide.



**Figure 2** Two-hour intakes of 0.5 M NaCl or 0.5 M NaSuc by male rats after injection with the 0.15 M NaCl vehicle (control; open bars) or after furosemide-induced sodium depletion (filled bars). Rats were given control or furosemide injections at weekly intervals.



**Figure 3** Intakes of NaCl solutions during the first 15 min (filled symbols) and the remaining 105 min (open symbols) during the three 2-h tests conducted after furosemide-induced sodium depletions. Intakes of 0.5 M NaCl and 0.5 M NaSuc did not differ from each other in any of these 2-h tests and were collapsed. For a given time period, a = significantly greater than depletion 1, b = significantly greater than depletion 2, and \* = significantly less than 15 min for a given depletion.



**Figure 4** Twenty-four-hour intakes of 0.5 M NaCl or 0.5 M NaSuc by male rats during baseline (hatched bars) and need-free conditions between weekly control (open bars) or furosemide injections (filled bars). Intakes are the means of 3 days.

Whether or not the solution also contained sucrose had no effect on the amount consumed by furosemide-treated rats. These findings are consistent with the results from Experiment 1 and support our suggestion that the taste of sodium is the salient feature that drives intake during depletion states. In contrast, during need-free conditions, rats increased their consumption of NaSuc regardless of depletion history, while neither furosemide-treated rats nor rats given control injections increased NaCl intake. This observation suggests that the sucrose taste may have made the taste of concentrated NaCl less aversive and/or more palatable. Consistent with this idea, in Experiment 1, all rats licked more to the NaSuc solutions than to the NaCl solutions during baseline and need-free conditions. Thus, during need-free states, sucrose appears to be the driving taste in the ingestion of NaSuc solutions.

Our findings of a progressive increase in NaCl ingestion with repeated furosemide treatments are in agreement with previous reports of behavioral sensitization in stimulated NaCl intake tests after multiple furosemide-induced sodium depletions (Sakai *et al.*, 1987, 1989; Ruhf *et al.*, 2001; Roitman *et al.*, 2002; Leshem *et al.*, 2004). Previous studies also have shown that urinary sodium excretion does not change with multiple depletions (Sakai *et al.*, 1987; Leshem *et al.*, 2004). Thus, the increased consumption of highly concentrated NaCl solutions after repeated furosemide depletions is unlikely to be a compensatory response to greater sodium loss. However, similar to our short-term taste tests in which there was no increase in licking during need-free conditions, we did not see increased ingestion of NaCl solutions by furosemide-treated rats during need-free conditions compared to that by rats given control injections. Thus, unlike results from some previous studies (Sakai *et al.*, 1987, 1989; Leshem *et al.*, 2004), the increased NaCl ingestion seen during furosemide conditions did not persist during need-free conditions between furosemide treatments, although it is important to note that Leshem *et al.* (2004) also demon-

strated that increased need-free intake is both strain and laboratory dependent.

Taken together, these observations suggest that the progressive increase in ingestion of NaCl during depleted states may be separable from increased need-free intakes that have been reported to occur after multiple furosemide-induced depletions. In any event, the present results clearly indicate that the enhanced NaCl intake, whether presented alone or as part of a complex taste mixture, reflects the salience of sodium taste during states of sodium depletion.

## General discussion

Taste detection undoubtedly has survival value to all animals. Whether identifying food, which frequently is sweet tasting, or avoiding potentially poisonous substances, which often are bitter tasting, orosensory detection allows the “decision” to ingest to be made prior to postingestive consequences. The ability to identify the salient orosensory cue may be critical to an animal’s survival, as in the ability to reject a taste previously paired with a toxic dose of LiCl (Smith *et al.*, 2000; Frank *et al.*, 2003; Smith, 2004). In this regard, studies using knockout mice have demonstrated that the ability to form conditioned taste aversions depends on the salience of the taste stimulus paired with the toxin (Stafstrom-Davis *et al.*, 2001).

It has been proposed that seeking and consuming NaCl is a taste-guided behavior and that the magnitude of gustatory input signals the intensity of the NaCl taste (Contreras and Lundy, 2000). Thus, decreased input may reduce the perceived taste intensity, making normally avoided concentrated NaCl solutions less aversive/more palatable and, consequently, more likely to be consumed (Contreras and Lundy, 2000). Consistent with this idea, furosemide-induced sodium depletion decreases the responses of gustatory sensory nerves to sodium taste (Bernstein and Taylor, 1992). The present findings clearly show depletion-induced changes in behavioral taste responses (see also Breslin *et al.*, 1993; Brot *et al.*, 2000); however, neither decreased aversiveness nor increased palatability can fully account for these findings. If so, the combination of decreased sodium taste intensity and greater palatability attributable to the presence of sucrose (suggested by higher rates of licking to NaSuc during baseline and need-free conditions, as well as by increased consumption of NaSuc independent of furosemide treatments) would be expected to produce furosemide-induced intakes of NaSuc greater than those of NaCl alone. This expectation clearly was not met. Rather, in both long-term and short-term tests, the presence of sodium appeared to drive ingestion during depletion states, suggesting that sodium is the salient taste.

It is of interest that such depletion-related changes in the salience of sodium taste neither depended on the number of sodium depletions nor persisted after rats had consumed sufficient NaCl to correct the sodium imbalance. Rather than

increasing gradually with repeated sodium depletions, licking responses to all NaCl solutions, regardless of concentration, were maximal with the first furosemide-induced sodium depletion. In addition, during all need-free conditions between depletions, neither licking responses to NaCl in short-term taste tests nor NaCl ingestion in long-term tests differed from baseline. Together, these observations suggest that sodium imbalance acutely increases the saliency of sodium taste, which, in conjunction with altered gustatory sensory input, permits NaCl intake above baseline levels.

But does the increased saliency of sodium taste and/or NaCl consumption during conditions of sodium depletion contribute to the progressive increase in NaCl intake with repeated depletions? It is possible that, with multiple tests, rats learn to locate the NaCl more rapidly and thus ingest more NaCl in a shorter time. However, when greater amounts of NaCl are ingested and absorbed early in the test, the ensuing activation of visceral or central osmoreceptors (Johnson and Thunhorst, 1997) would be expected to provide greater inhibition of subsequent ingestion, ultimately decreasing NaCl intake during the remainder of the test. Our observations of NaCl intake over time during the 2-h tests after furosemide treatments (Figure 3) clearly show that, whereas NaCl intake increased progressively in the first 15 min, the amount of NaCl consumed in the remaining 105 min of each test did not change. Thus, rats were not simply locating the NaCl more quickly.

It has been suggested that satiation of salt appetite produced by sodium imbalance requires repletion of body sodium (Wolf *et al.*, 1984). Certainly, the volume of NaCl consumed after furosemide treatment meets or exceeds the amount necessary to replace that lost in urine (Sakai *et al.*, 1987). Therefore, an alternative explanation for the enhanced NaCl intake with repeated sodium depletions is that the taste of sodium that is consumed becomes associated with the subsequent repletion of body sodium. The association between taste and repletion may increase the motivation to consume NaCl upon additional depletions. As a result, the taste of sodium that is, perhaps, innately rewarding (McCaughy and Scott, 1998) acquires greater rewarding or reinforcing properties such that subsequent depletions produce even greater intakes (Lucas *et al.*, 2002).

This idea has been investigated in several studies focused on central reward pathways and, in particular, on the mesolimbic dopamine system (Pompei *et al.*, 1997; Lucas *et al.*, 1998, 1999, 2000; Roitman *et al.*, 2002), which has been widely implicated in processing the saliency of rewarding cues (Ungless, 2004). Dopamine release in the nucleus accumbens (NAc) increases in rats given access to NaCl after sodium depletion (Lucas *et al.*, 2002), and repeated episodes of furosemide-induced sodium depletion and repletion are associated with morphological changes in neurons in the shell of NAc (Roitman *et al.*, 2002). These alterations in the mesolimbic dopamine system induced by NaCl ingestion after depletion overlap with those ob-

served after the administration of drugs of abuse. In fact, rats with a history of sodium depletions demonstrate a cross-sensitization to the locomotor-activating effects of amphetamine (Roitman *et al.*, 2002). Thus, the neurobiological changes that underlie some aspects of behavioral sensitization to drugs of abuse leading to drug-seeking behaviors and compulsivity to consume drugs (Spanagel and Weiss, 1999) also may contribute to the behavioral sensitization, the progressive increase in NaCl intake, after furosemide. In both cases, the sensitization leads to behaviors that are, at best, physiologically inappropriate (NaCl intake in excess of the sodium loss) (Sakai *et al.*, 1989) and, at worst, pathological (drug abuse).

These observations suggest that alterations in the mesolimbic dopamine system underlie the progressive increase in NaCl intake after repeated furosemide depletions; however, the relative contributions of sodium taste and sodium repletion to this behavioral sensitization remain unclear. Sakai *et al.* (1989) reported that, regardless of whether rats were given NaCl solutions, were given intraperitoneal injections of NaCl but had no access to NaCl solutions, or had no access to NaCl and were not experimentally “repleted” after initial furosemide-induced sodium depletion, NaCl intakes after the second furosemide treatment were comparably elevated. On the surface, these results would seem to indicate that neither the taste of sodium nor sodium repletion is necessary for the progressive increase in NaCl intake; however, all rats were given their normal, sodium-rich chow in the weeks between the first and second furosemide treatments. Admittedly, prolonged maintenance on sodium-deficient chow in combination with repeated pharmacological sodium depletions may have deleterious consequences. Nonetheless, it is possible that an association was made between the taste of sodium in the chow and the subsequent repletion. To date, therefore, definitive experiments to determine the relative role of sodium taste and sodium repletion in the progressive increase in NaCl intake with repeated furosemide-induced sodium depletions—and, more specifically, in underlying alterations in the mesolimbic dopamine system—remain to be done.

Finally, circulating levels of AngII and Aldo increase after sodium depletion (Fitzsimons, 1998), suggesting that hormone signaling related to sodium state may be critical for changes in central reward pathways that underlie behavioral sensitization to furosemide. In this regard, Sakai *et al.* (1989) reported that combined pharmacological blockade of AngII and Aldo delays the progressive increase in furosemide-stimulated NaCl intake, whereas simultaneous pretreatment with exogenous AngII and Aldo facilitates it. Moreover, sodium depletion enhances the expression of the AngII type 1 (AT<sub>1</sub>) receptor subtype in brain areas including the median preoptic nucleus and the subfornical organ (Charron *et al.*, 2002), and lesions of these areas reduce furosemide-induced NaCl intake (Fitts *et al.*, 1990; Thunhorst *et al.*, 1990; Weisinger *et al.*, 1990). Thus, changes in AT<sub>1</sub> receptor

expression, in conjunction with elevated levels of AngII and Aldo, also may play a role in the behavioral sensitization to furosemide.

In summary, our results show that behavioral taste responses to sodium did not increase progressively with multiple furosemide-induced sodium depletions. In fact, rats licked maximally to all NaCl solutions after each of the three depletions, regardless of whether the solution contained sucrose. Similarly, the presence of sucrose did not affect the stimulated NaCl intake in long-term tests, although ingestion increased progressively after multiple sodium depletions. Finally, both licking and ingestion returned to baseline levels during need-free conditions. Together, these results suggest that sodium depletion acutely increases the salience of sodium taste. The role of taste in progressive increases in NaCl intake with repeated sodium depletions warrants further investigation. Nonetheless, in conjunction with previous studies, our results suggest that the taste of sodium and/or the repletion of body sodium contribute to long-term changes in central reward pathways, thereby redefining the salience of sodium taste during conditions of sodium need and ultimately resulting in behavioral sensitization to furosemide.

## Acknowledgements

This research was supported by National Institutes of Health grants from the National Institute on Deafness and Communication Disorders (DC04785, R.J.C.; DC06360, K.S.C.). Portions of these data were presented in preliminary form at the 33rd Annual Meeting of the Society for Neuroscience in New Orleans, LA. The authors thank Dr James Smith and Ms Karen Dietz for critical evaluation of the manuscript and Ms Deneice Johnson for assistance with behavioral testing.

## References

- Bernstein, I.L. and Taylor, E.M. (1992) *Amiloride sensitivity of the chorda tympani response to sodium chloride in sodium-depleted Wistar rats*. *Behav. Neurosci.*, 106, 722–725.
- Breslin, P.A., Kaplan, J.M., Spector, A.C., Zambito, C.M. and Grill, H.J. (1993) *Lick rate analysis of sodium taste-state combinations*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 264, R312–R318.
- Brot, M.D., Watson, C.H. and Bernstein, I.L. (2000) *Amiloride-sensitive signals and NaCl preference and appetite: a lick-rate analysis*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 279, R1403–R1411.
- Charron, G., LaForest, S., Gagnon, C., Drolet, G. and Mougnot, D. (2002) *Acute sodium deficit triggers plasticity of the brain angiotensin type 1 receptors*. *FASEB J.*, 16, 610–612.
- Contreras, R.J. (1977) *Changes in gustatory nerve discharges with sodium deficiency: a single unit analysis*. *Brain Res.*, 121, 373–378.
- Contreras, R.J. and Frank, M. (1979) *Sodium deprivation alters neural responses to gustatory stimuli*. *J. Gen. Physiol.*, 73, 569–594.
- Contreras, R.J. and Lundy, R.F. (2000) *Gustatory neuron types in the periphery: a functional perspective*. *Physiol. Behav.*, 69, 41–52.
- Curtis, K.S., Davis, L.M., Johnson, A.L., Therrien, K.L. and Contreras, R.J. (2004) *Sex differences in behavioral taste responses to and ingestion of sucrose and NaCl solutions by rats*. *Physiol. Behav.*, 80, 657–664.
- Curtis, K.S., Krause, E.G. and Contreras, R.J. (2001) *Altered NaCl taste responses precede increased NaCl ingestion during Na<sup>+</sup> deprivation*. *Physiol. Behav.*, 72, 743–749.
- Davis, J.D., Smith, G.P. and McCann, D.P. (2002) *The control of water and sodium chloride intake by postgestional and orosensory stimulation in water-deprived rats*. *Physiol. Behav.*, 75, 7–14.
- Epstein, A.N. and Stellar, E. (1955) *The control of salt preference in the adrenalectomized rat*. *J. Comp. Physiol. Psychol.*, 48, 167–172.
- Fitts, D.A., Tjepkes, D.S. and Bright, R.O. (1990) *Salt appetite and lesions of the ventral part of the ventral median preoptic nucleus*. *Behav. Neurosci.*, 104, 818–827.
- Fitzsimons, J.T. (1998) *Angiotensin, thirst, and sodium appetite*. *Physiol. Rev.*, 78, 583–686.
- Frank, M.E., Formaker, B.K. and Hettinger, T.P. (2003) *Taste responses to mixtures: analytic processing of quality*. *Behav. Neurosci.*, 117, 228–235.
- Frankmann, S.P., Sollars, S.I. and Bernstein, I.L. (1996) *Sodium appetite in the sham-drinking rat after chorda tympani nerve transection*. *Am. J. Physiol.*, 271, R339–R345.
- Hsiao, S. and Fan, R.J. (1993) *Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats*. *Behav. Neurosci.*, 107, 317–326.
- Johnson, A.K. and Thunhorst, R.L. (1997) *The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration*. *Front. Neuroendocrinol.*, 18, 292–353.
- Leshem, M., Kavushansky, A., Devys, J.-M. and Thornton, S. (2004) *Enhancement revisited: the effects of multiple depletions on sodium intake in rats vary with strain, substrain, and gender*. *Physiol. Behav.*, 82, 571–580.
- Lucas, L.R., Hajnal, A., Grillo, C.A., Celen, Z. and McEwen, B.S. (2002) *Appetite for salt induces dopamine release in the nucleus accumbens*. Program No. 774.15. 2002 Abstract Viewer/Itinerary Planner. Society for Neuroscience, Washington, DC.
- Lucas, L.R., Pompei, P. and McEwen, B.S. (1999) *Correlates of deoxycorticosterone-induced salt appetite behavior and basal ganglia neurochemistry*. *Ann. N. Y. Acad. Sci.*, 897, 423–428.
- Lucas, L.R., Pompei, P. and McEwen, B.S. (2000) *Salt appetite in salt-replete rats: involvement of mesolimbic structures in deoxycorticosterone-induced salt craving behavior*. *Neuroendocrinology*, 71, 386–395.
- Lucas, L.R., Pompei, P., Ono, J. and McEwen, B.S. (1998) *Effects of adrenal steroids on basal ganglia neuropeptide mRNA and tyrosine hydroxylase radioimmunoreactive levels in the adrenalectomized rat*. *J. Neurochem.*, 71, 833–843.
- McCaughey, S.A. and Scott, T.R. (1998) *The taste of sodium*. *Neurosci. Biobehav. Rev.*, 22, 663–676.
- Mook, D.G. (1963) *Oral and postgestional determinants of the intake of various solutions in rats with esophageal fistulas*. *J. Comp. Physiol. Psychol.*, 4, 645–659.
- Pompei, P., Lucas, L.R., Angeletti, S., Massi, M. and McEwen, B.S. (1997) *In situ hybridization analysis of preprotachykinin-A and -B mRNA levels in short-term sodium depletion*. *Brain Res. Mol. Brain Res.*, 49, 149–156.
- Richter, C.P. (1936) *Increased salt appetite in adrenalectomized rats*. *Am. J. Physiol.*, 115, 155–161.

- Roitman, M.F., Na, E., Anderson, G., Jones, T.A. and Bernstein, I.L.** (2002) *Induction of a salt appetite alters dendritic morphology in nucleus accumbens and sensitizes rats to amphetamine.* J. Neurosci., 22, RC225.
- Ruhf, A.A., Starbuck, E.M. and Fitts, D.A.** (2001) *Effects of SFO lesions on salt appetite during multiple sodium depletions.* Physiol. Behav., 74, 629–636.
- Sakai, R.R., Fine, W.B., Epstein, A.N. and Frankmann, S.P.** (1987) *Salt appetite is enhanced by one prior episode of sodium depletion in the rat.* Behav. Neurosci., 101, 724–731.
- Sakai, R.R., Frankmann, S.P., Fine, W.B. and Epstein, A.N.** (1989) *Prior episodes of sodium depletion increase the need-free sodium intake of the rat.* Behav. Neurosci., 103, 186–192.
- Smith, J.C.** (2004) *Gustation as a factor in the ingestion of sweet and fat emulsions by the rat.* Physiol. Behav., 82, 181–185.
- Smith, J.C., Fisher, E.M., Maleszewski, V. and McClain, B.** (2000) *Orosensory factors in the ingestion of corn oil/sucrose mixtures by the rat.* Physiol. Behav., 69, 135–146.
- Spanagel, R. and Weiss, F.** (1999) *The dopamine hypothesis of reward: past and current status.* Trends Neurosci., 22, 521–527.
- Stafstrom-Davis, C.A., Ouimet, C.C., Feng, J., Allen, P.B., Greengard, P. and Houpt, T.A.** (2001) *Impaired conditioned taste aversion learning in spinophilin knockout mice.* Learn. Mem., 8, 272–278.
- Thunhorst, R.L., Ehrlich, K.J. and Simpson, J.B.** (1990) *Subfornical organ participates in salt appetite.* Behav. Neurosci., 104, 637–642.
- Ungless, M.A.** (2004) *Dopamine: the salient issue.* Trends Neurosci., 27, 702–706.
- Weisinger, R.S., Denton, D.A., Di Nicolantonio, R., Hards, D.K., McKinley, M.J., Oldfield, B. and Osborne, P.G.** (1990) *Subfornical organ lesion decreases sodium appetite in the sodium-depleted rat.* Brain Res., 526, 23–30.
- Wolf, G., Schulkin, J. and Simson, P.E.** (1984) *Multiple factors in the satiation of salt appetite.* Behav. Neurosci., 98, 661–673.

Accepted November 1, 2005