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Variable effects of parabrachial nucleus lesions on salt appetite in rats depending upon experimental paradigm and saline concentration

Edward M. Stricker¹, Patricia S. Grigson², and Ralph Norgren²

¹Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

²Department of Neural and Behavioral Sciences, Pennsylvania State College of Medicine, Hershey, PA 17033

Abstract

Previous studies have demonstrated that bilateral lesions of the gustatory (medial) zone of the parabrachial nucleus (PBN) in the pons eliminate the salt appetite induced in rats by treatment with the diuretic drug, furosemide. The present studies re-examined NaCl intake of rats with PBN lesions induced by ibotenic acid, using multiple models of salt appetite. The impairment of a conditioned taste aversion, an established consequence of PBN damage, was used as an initial screen with which to assess the effectiveness of the lesions. Rats with PBN lesions did not drink either 0.3 M NaCl or 0.5 M NaCl in response to daily treatment with desoxycorticosterone acetate. These findings suggest that the excitatory stimulus of salt appetite mediated by mineralocorticoids is abolished by PBN lesions. In contrast, rats with PBN lesions drank some 0.5 M NaCl, and more 0.3 M NaCl, in addition to water in response to hypovolemia induced by subcutaneous injection of 30% polyethylene glycol solution. Those findings suggest that an excitatory stimulus of salt appetite, presumably mediated by angiotensin II, is not abolished by PBN lesions. These and other observations indicate that lesions of the gustatory PBN in rats may or may not eliminate salt appetite, depending on which model is used and which concentration of NaCl solution is available.

Keywords

conditioned taste aversion; DOCA; hypovolemia; NaCl; thirst

Salt appetite is a strong motivation to consume salty tasting fluids and foods, usually based on a bodily need for Na⁺. The taste of salt appears to be crucial to the mediation of salt appetite (Kriekhaus & Wolf, 1968; Smith, Holman, & Fortune, 1968). Salt appetite is impaired but not eliminated by lesions of the first central taste relay in the anterior half of the nucleus tractus solitarius (NTS) in the medulla (Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Shimura, & Norgren, 1997a). Nevertheless, similar damage to the second central relay in the pons, the parabrachial nuclei (PBN) (Norgren & Leonard 1973), is reported to

Address editorial correspondence to: Dr. Edward M. Stricker, Department of Neuroscience, 210 Langley Hall, University of Pittsburgh, Pittsburgh, PA 15260, 412-624-4569 (phone), 421-624-9198 (fax), edstrick@pitt.edu.

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eliminate salt appetite induced by the diuretic drug furosemide (Flynn et al. 1991; Scalera, Spector, & Norgren, 1995).

Furosemide induces a negative Na^+ balance by causing the rapid excretion of Na^+ in urine (Jalowiec 1974). However, sodium deficiency is only one of three popular models of salt appetite in rats (Stricker & Verbalis, 1990). Another model, subcutaneous injection of polyethylene glycol (PEG) solution, does not produce body Na^+ deficits but instead leaches fluid from the intravascular compartment and thereby causes progressive decreases in plasma volume (Stricker & Jalowiec, 1970). A third model, elevated levels of mineralocorticoid hormone, normally occurs in intact rats during Na^+ deficiency or hypovolemia (Gross, Brunner, & Ziegler, 1965; Stricker, Vagnucci, McDonald, & Leenen, 1979) but it can be produced in the absence of a physiological need for Na^+ by daily injections of the mineralocorticoid drug desoxycortico-sterone acetate (DOCA) (Rice & Richter 1943). In contrast to the 2-hr drinking test usually employed after furosemide treatment, PEG-treated rats can be studied for many hours because the treatment effects are so long-lasting, and DOCA-treated rats can be studied for multiple days because the treatment can be administered daily. In other words, the other models of salt appetite allow relatively long time periods in which to observe the ingestive behavior of brain-damaged rats.

The experimental question we addressed was whether gustatory (medial) PBN lesions impair the expression of salt appetite in all three models. Because the Na^+ need induced by PEG treatment is larger than that induced by furosemide treatment, and the salt appetite elicited by DOCA treatment is even larger, the apparent elimination of diuretic-induced NaCl intake after PBN lesions may hinge on the fact that this is a relatively weak appetite; hence, our interest in these other models of salt appetite. In addition, 0.5 M NaCl was the available source of Na^+ in previous tests, so the apparent absence of salt appetite in rats with PBN lesions may reflect their relatively low preference for that concentrated saline solution; hence, the present work used both 0.3 M and 0.5 M NaCl solutions to examine this variable.

Method

Subjects

The subjects were 46 naive, male, Sprague-Dawley rats weighing 340 – 520 g at the start of the experiment. They were individually housed in stainless steel cages in a vivarium maintained at 21° C with a 12:12 h light:dark cycle (lights on at 7 a.m.). Unless otherwise noted, food, water, and NaCl solution were provided *ad libitum*. Inverted graduated cylinders (± 0.5 ml) with stainless steel spouts, affixed to the front of the cage, were used to measure fluid intake.

General Experimental Protocols

Experimental protocols were approved by the Institutional Animal Care and Use Committees of Pennsylvania State University College of Medicine.

The goal of these experiments was to evaluate the effects of gustatory PBN lesions on NaCl intake induced by multiple models of salt appetite. In Experiment 1 appetite was assessed

using a series of two-bottle tests with water and 0.5 M NaCl available, whereas in Experiment 2 appetite was assessed using a series of two-bottle tests with water and 0.3 M NaCl available. Each series additionally included a one-bottle test, as described below. All rats were initially screened to assess the effectiveness of the lesions by determining whether they could form a LiCl-induced conditioned taste aversion (CTA) to a novel saccharin solution, because medial PBN damage is known to eliminate such taste aversion learning (Flynn et al. 1991; Scalera et al. 1995; Spector, Norgren, & Grill, 1992). Similarly, salt appetite induced by furosemide treatment was evaluated prior to Experiment 1 in order to confirm that the appetite was, in fact, eliminated by PBN lesions (Flynn et al. 1991; Scalera et al., 1995).

Surgery—Twenty-five rats ($n = 11$ in Experiment 1, $n = 14$ in Experiment 2) received bilateral electrophysiologically-guided ibotenic acid lesions of the PBN. The ibotenic acid (Sigma, St. Louis, MO) was dissolved in phosphate buffered saline (pH = 7.4) at a concentration of 20 $\mu\text{g}/\mu\text{l}$. Eleven control rats were not given any surgical treatment ($n = 6$ in Experiment 1 and 5 in Experiment 2) and 10 other control animals received bilateral electrophysiologically-guided injections of phosphate buffered saline into the taste-responsive area of the PBN ($n = 6$ in Experiment 1, $n = 4$ in Experiment 2). The surgical procedures were identical to those described elsewhere (Grigson et al., 1997a).

Fifteen min prior to surgery each rat was weighed and given an intraperitoneal (ip) injection of atropine sulphate (0.1 mg). Each rat then was anesthetized with Nembutal (50 mg/kg ip), which was supplemented as needed to maintain a surgical level of anesthesia. Once anesthetized, the rat was injected with Gentamicin (6 mg, ip) and then mounted in a modified Kopf stereotaxic instrument using blunt ear bars. The cranial sutures were exposed by a midline incision and a flat skull position was achieved when the incisor bar was lowered 3.3 below horizontal zero. Two holes (2.0 mm diameter) were drilled in the intraparietal bone, centered 12.0 mm posterior to bregma and 1.8 mm on either side of the midline. Physiological saline was used to prevent the dura and skin from drying during surgery. On completion of the surgery, the holes were filled with Gelfoam and the incision was closed with wound clips.

Gustatory neurons in the PBN were located bilaterally by recording multiunit activity through a glass-insulated tungsten search electrode ($Z = 0.5 - 2.0$ Mohms at 1000 Hz) while stimulating the anterior tongue with 0.3 M NaCl and rinsing with distilled water. The initial coordinates were 12.0 mm posterior to bregma, 1.8 mm lateral to the midsagittal suture, and 5.4 mm ventral to skull surface. For all penetrations, the electrode holder was oriented 20° off vertical in the A/P plane with the tip pointed rostrally. Sensory testing began after the electrode penetrated into the pons. Following bilateral location of pontine taste responses, the recording electrode was replaced with a micropipette/electrode (O.D. = 50 – 60 μm ; $Z = 0.5 - 1.0$ Mohms) one lumen of which was glued directly to the needle of a Hamilton microsyringe (1- or 2- μl). The micropipette was relocated electrophysiologically and ibotenic acid (4 μg in 0.2 μl solution) was injected by pressure over a 10-min period. To minimize the spread of fluid up the track, the micropipette remained in place for an additional 10 min. Identical surgical procedures were employed for the vehicle-treated

control rats except that phosphate buffered saline (PBS, pH = 7.4) rather than ibotenic acid was injected into the PBN.

CTA—The rats were adapted to a schedule in which they had access to water for 15 min each morning and 60 min each afternoon. Once intake stabilized, rats were given 0.15% saccharin instead of water one morning. Thirty min later, they were injected with 0.15 M LiCl (1.33 ml/100 g bwt, ip). There were three taste-illness pairings followed by one test trial in which LiCl was not administered, with the four tests spaced three days apart; on each test day, water was available for 60 min in the afternoon to allow rehydration. The schedule of restricted water access was maintained on the intervening days. Intakes that diminished from >9 ml on the first trial to <3 ml on subsequent trials was taken as evidence that a CTA had been acquired.

The control rats in each experiment evidenced a CTA following a single saccharin-LiCl pairing. In contrast, none of the rats with PBN lesions in Experiment 1 and Experiment 2 evidenced a CTA following a single saccharin-LiCl pairing ($\chi^2 = 17.12, 16.16$, respectively; $P < 0.001, < 0.001$).

Furosemide—One morning, food and fluids were removed from the cages and rats in Experiment 1 were injected subcutaneously (sc) with furosemide (7 mg/rat), twice, 2 hr apart. Sodium-free diet (ICN Biomedicals; Irvine, CA) and drinking water then were provided but 0.5 M NaCl was not available. Twenty-four hr after the first injection, the rats were given access to 0.5 M NaCl and water but not to food. The left/right location of the fluids on the cage was counterbalanced across animals. Intakes of water and the concentrated saline solution were measured at 15 and 30 min, and at 1, 2, and 24 hr. One week later, this procedure was repeated but all rats were injected with 0.15 M NaCl rather than furosemide, and a subsistence level of NaCl (0.05%) was added to the Na⁺-free diet.

The control rats drank 0.5 M NaCl promptly when tested 24 hr after treatment with furosemide, consuming 6.8 ± 0.9 ml of saline in 15 min and an additional 3.3 ± 0.6 ml during the balance of the 2-hr test. In contrast, 8 of the 9 rats with PBN lesions failed to drink saline at all ($\chi^2 = 13.67, P < 0.001$); the remaining rat, which appeared to have complete damage of the gustatory area bilaterally, unaccountably drank 9 ml in 15 min but nothing afterwards ($t = 7.50, P < 0.001$, including this animal). Similarly, the cumulative saline intake of control rats by 24 hr was 14.8 ± 1.4 ml whereas the 8 brain-damaged rats consumed only 1.9 ± 0.5 ml ($\chi^2 = 13.67, P < 0.001$; including the outlier: $t = 7.18, P < 0.001$). Baseline values of saline intake were very small both in rats with PBN lesions and in control animals at 2 hr (0.1 ± 0.1 ml, 0.3 ± 0.2 ml, respectively) and at 24 hr (0.4 ± 0.4 ml, 2.8 ± 0.9 ml, respectively).

Experiment 1

This series of studies consisted of four tests given in the following sequence: DOCA, angiotensin II (AngII), PEG, and water deprivation. Treatments were separated by 1–2 wk of *ad libitum* access to food and drinking fluids to allow baseline intakes to restabilize. All animals received each treatment. The intake tests were considered to have begun when the

animals commenced drinking (usually within 30 min after they were given access to NaCl solution).

DOCA—The day before the first injection, rats were acclimated to 0.5 M NaCl for 7 hr daily beginning at 10 a.m. On the morning of the first test day, food was removed and 1 hr later all rats were injected with DOCA (3 mg in 0.4 ml peanut oil, sc). Thirty min later, they were given access to 0.5 M NaCl and water but not to food, and fluid intakes were measured after 7 hr. After the drinking test, the concentrated saline solution was removed from the cage and food was returned. This regimen was repeated for six days followed by three additional days of assessment during which rats were not injected with DOCA.

AngII—On the first test day chow was removed from the cage and, 60 min later, the rats were injected with 0.15 M NaCl (1 ml/kg, sc). Four days later, the procedure was repeated except this time rats were given an injection of AngII (3 mg/ml/kg, prepared in 0.15 M NaCl, sc). In both cases intakes of water and 0.5 M NaCl were measured at 5, 10, 15, 30, 60, 90, and 180 min.

30% PEG—All rats received an injection of 30% PEG (wt/wt; Carbowax, Compound 20-M; 5.5 ml, prepared in 0.15 M NaCl, sc), after which no food, water, or concentrated saline solution were available for 24 hr. Then the rats were given access to water and 0.5 M NaCl but not to food. Intakes were measured at 5, 10, 15, 30, and 45 min, and at 1, 2, 3, 4, and 5 hr.

Water Deprivation—After monitoring 24-hr intakes of 0.5 M NaCl and water for 3 days, the rats had one day with access to water only followed by one day with access to 0.5 M NaCl only. Food was available continuously. This one-bottle test was conducted to determine whether rats with PBN lesions would consume 0.5 M NaCl when it was the only fluid available.

Histology—After data collection was completed, the 23 rats (i.e., 11 with PBN lesions, 6 surgical controls, and 6 unoperated controls) were anesthetized with an overdose of pentobarbital sodium (100 mg/kg) and perfused through the heart, first with isotonic saline and then with buffered 10% formalin. The brains were removed, initially stored in 10% formalin, and then transferred to 10% formalin/30% sucrose for 24 hr. Then the brains were blocked and sectioned at 50 μ m on a freezing microtome. Alternate series of sections were mounted and stained with the cresyl Lecht violet or the Weil method.

The lesions were evaluated primarily from the cresyl violet series by an observer (RN) who was naïve to the data from individual animals. On each side of the brain, the lesion was judged to be “complete” (i.e., all of the gustatory PBN was destroyed on that side of the brain), “partially complete” (75% of the area was damaged), or “non-existent” (no damage was evident at any level) depending on the degree of cell loss in the areas of the PBN from which gustatory responses can be recorded (Norgren & Pfaffmann 1975). The evaluations included the PBN from the point at which the inferior colliculus joins the pons to the posterior border of the nucleus, a distance of 1.0 – 1.2 mm (Paxinos & Watson, 2005). Note

that two other categories were needed in Experiment 2: “partial” (50% of the PBN taste area was damaged) and “missed” (damage was present but not in the PBN taste area).

Experiment 2

This series of studies included two tests conducted in Experiment 1 (DOCA, PEG). Similar procedures were employed except 0.3 M NaCl was available instead of 0.5 M NaCl, and the DOCA treatment was given for 7 days instead of 6 days. A third test involved injection of 2 M NaCl (2 ml, ip), after which rats were given access to water but not food or saline, and intake was measured every 5 min for 60 min. This one-bottle test was conducted to determine whether PBN lesions generally impair the drinking response to fluid need. The three tests were given in the following sequence: DOCA, hypertonic saline, and PEG. All animals received each treatment.

The order of the tests was not counterbalanced in either series. This strategy reduced both the number of animals needed and the complexity of the subsequent analyses. Based on prior experience with many similar experiments, we assumed that the sequence in which the treatments were given had no significant bearing on the outcome (Flynn et al., 1991; Grigson et al., 1998).

Histology—In order to allow for staining of neuron specific protein (NeuN), when the experiments ended the 14 rats with PBN lesions and two surgical control animals were deeply anesthetized (pentobarbital, 100 mg/kg, ip) and perfused transcardially with cold heparinized saline (1.5:1000, 5 min) followed by ice-cold 4% paraformaldehyde in 0.1 M PBS. The brains post-fixed for an hour *in situ* before being removed from the skull. Subsequently, they were placed in 20% sucrose solution in 4% paraformaldehyde for another 2–3 hr and then were cryoprotected in 20% sucrose/0.1 M PBS overnight at 4°C. Then the brains were sectioned coronally at 50 µm on a freezing microtome and the sections were collected in three series. The first series was processed immunohistochemically for NeuN (Jongen-Rêlo & Feldon, 2002; Mullen, Buck, & Smith, 1992). The second series was stained with cresyl Lecht violet, and the third was stored in a cryoprotectant solution for a replication if the immunohistochemistry in the first series was inadequate. Sections from control brains were processed in tandem with experimental brains to minimize immunohistological variability.

The immunohistochemical procedures have been documented elsewhere (Mungarndee, Lundy, & Norgren, 2006). Briefly, free-floating sections were rinsed in 0.1 M PBS, treated with 0.5% H₂O₂ for endogenous peroxidase, rinsed again, and then placed in a blocking solution for 1 hr. The sections were then transferred to mouse NeuN antibody (1:5,000; Chemicon International, Temecula, CA) for 24 hr. After further rinses, the tissue was incubated with 2% biotinylated horse anti-mouse IgG (Sigma Chemical, St. Louis, MO) and 0.3% Triton X-100/0.1 M PBS (1:1000) for 2 h. After more PBS rinses, the sections were processed using an avidin-biotin kit (ABC, Vectastain Elite®, Vector Laboratories, Burlingame, CA) followed by 0.05% diaminobenzidine and 0.01% H₂O₂ in 0.175 M sodium acetate for 5 min.

Lesion evaluation was done using the NeuN series and procedures identical to those for Experiment 1.

Statistical Analyses

All data are presented as means $\pm$ SE. Statistical reliability of observed differences was determined using *t*-tests (two-tailed, unless indicated otherwise) or chi-square analyses with Yates correction. Regression equations were calculated by the method of least squares and significance was determined using Pearson's correlation coefficients. $P < 0.05$ was considered to be statistically significant. The behavior of the surgical and non-surgical controls did not differ significantly (all P s > 0.05) and, consequently, the data from the two groups were combined in each experiment.

Results

Histology

Of the 11 rats with PBN lesions in Experiment 1, 9 had confirmed lesion placement and their behavioral data were included in the analysis. The sections through the PBN were inadvertently lost in one rat while another rat died prior to fixation and the resulting sections were of too poor quality to judge the extent of the lesions. The data from these two rats were not included in this report although they behaved on each test like most of the other nine rats with PBN lesions. Six of the 9 rats with PBN lesions had complete damage of the gustatory area bilaterally (Fig. 1A, 1B). In three of them tissue was spared ventral to the brachium conjunctivum (BC) but rostral to the anterior edge of the taste area, whereas two other animals had damage that extended into the supratrigeminal area ventral to PBN and, in one of them, it also included the locus coeruleus medial to PBN but only unilaterally. In the other three rats with PBN lesions the damage was partially complete, two unilaterally and one bilaterally. In two of them the sparing was ventral to the BC rostrally but included a small amount of the taste area, whereas in the third animal the PBN was spared around the medial tip of the BC (an area that includes taste neurons).

Of the 14 rats with PBN lesions in Experiment 2, one had no evidence of neuronal loss in the dorsal pons (Fig. 1D) and two others had only partial lesions bilaterally (Fig. 1F), with considerable amounts of the gustatory area preserved on at least one side. The data from these three rats were not included in this report. Not surprisingly, all three animals learned a CTA and drank 0.3 M NaCl and water like control rats did in response to the three other tests. Five of the remaining 11 rats with PBN lesions had bilaterally complete lesions. The damage extended beyond the PBN into the supratrigeminal area and the locus coeruleus, at least unilaterally, in two of them. The lesions were confined to the medial and lateral PBN in the other three animals, often with some sparing below the BC rostrally (Fig. 1C). The other six rats had partially complete damage on one side and a complete PBN lesion on the other (Fig. 1E). Typically the sparing was in the lateral half of the medial PBN and thus included some gustatory neurons. All 11 rats contributed data to the analyses.

DOCA

Control rats in Experiment 1 drank increasing amounts of 0.5 M NaCl during the 6 days of DOCA treatment, with a peak daily intake of 17.0 ± 1.2 ml in 7 hr on the last day of treatment and the first day afterwards. In contrast, the 9 rats with PBN lesions drank negligible amounts of saline on each of the test days ($t = 7.53$ on Day 6 of treatment, $P < 0.001$; Fig. 2A).

Similarly, control rats in Experiment 2 drank increasing amounts of 0.3 M NaCl during the 7 days of DOCA treatment, with a peak daily average of 54.1 ± 3.6 ml on the last two days of treatment and the first day afterwards. In contrast, 10 rats with PBN lesions drank negligible amounts of saline on each of the test days ($\chi^2 = 12.93$, $P < 0.001$; Fig. 2B). The other rat with PBN lesions unaccountably ingested large amounts of saline in the baseline period as well as after DOCA treatment (here and elsewhere, the data from a single outlier is not included in the figure or in the statistical analyses: $t = 14.72$, $P < 0.001$).

PEG

When water and 0.5 M NaCl were made available 24 hr after 30% PEG treatment in Experiment 1, control rats began drinking both fluids within 5 min, drank steadily throughout the 5-hr test (Fig. 3A), and consumed a total of 34.2 ± 2.4 ml of water and 6.9 ± 1.1 ml of saline. The intakes of water and saline varied considerably across subjects but were significantly correlated with one another ($r = 0.69$, $P < 0.01$).

In contrast, although 8 of the 9 rats with PBN lesions ingested some of the 0.5 M NaCl solution after PEG treatment, their drinking response was much less consistent than that of control animals. Specifically, 8 PEG-treated rats with PBN lesions drank an average of 47.4 ± 5.2 ml of water and only 2.3 ± 0.8 ml of 0.5 M NaCl. As shown in Fig. 3A, these rats consumed relatively large amounts of water and little saline until 120 min, but thereafter they drank little of either fluid. One rat with PBN lesions was an outlier; it consumed 34 ml of saline and 4 ml of water in the first 30 min of the test, and during the balance of the test it drank only 1 ml of saline and 89 ml of water. (This animal was the only one with large PBN lesions that nevertheless spared the medial tip of the nuclei, an area that contains gustatory neurons.) In comparing the 5-hr intakes of the two groups, the rats with PBN lesions drank significantly less saline after PEG treatment than control rats did (including the outlier: $\chi^2 = 5.55$, $P < 0.02$; excluding the outlier: $t = 3.48$, $P < 0.01$) but they drank significantly more water (including the outlier: $\chi^2 = 5.47$, $P < 0.02$; excluding the outlier: $t = 2.31$, $P < 0.05$).

When water and 0.3 M NaCl were made available 24 hr after 30% PEG treatment in Experiment 2, control rats began drinking both fluids within 5 min and drank steadily throughout the test. They consumed predominantly water during the first 30 min but thereafter they drank water and 0.3 M NaCl in roughly equal amounts (Fig. 3B), and ingested a total of 29.9 ± 2.2 ml of water and 11.6 ± 2.5 ml of saline in 5 hr. These amounts (3.5 mEq Na^+ , 41.5 ml total volume) were almost identical to the amounts consumed by control rats after PEG treatment in Experiment 1 (3.5 mEq Na^+ , 40.9 ml total volume), as were their Na^+ intakes (in mEq) at each time point during the tests (Fig. 4; $r = 0.99$, $P < 0.001$).

In contrast to their impaired drinking response on Day 7 of DOCA treatment, all 11 rats with PBN lesions drank 0.3 M NaCl after PEG treatment. Indeed, during the first 2 hr of the test their Na⁺ intakes were comparable to those of control rats (Fig. 4). Similarly, their cumulative 5-hr intakes, 31.8 ± 2.2 ml of water and 6.7 ± 1.0 ml of saline, were not significantly different from the volumes ingested by control animals.

AngII

Control animals in Experiment 1 drank 14.1 ± 1.5 ml of water and 0.5 ± 0.3 ml of 0.5 M NaCl in 30 min after sc injection of AngII. These amounts were similar to those consumed by 8 of the 9 rats with PBN lesions: 12.3 ± 4.0 ml, 0.1 ± 0.1 ml, respectively. However, the control rats drank relatively little water but significant amounts of saline during the balance of the test (3.1 ± 1.1 ml and 5.0 ± 0.6 ml, respectively) whereas the 8 rats with PBN lesions consumed more water but no saline (15.0 ± 2.0 ml and 0.0 ± 0.0 ml, respectively). Thus, in comparison to the rats with PBN lesions, the control rats cumulatively drank less water (excluding the outlier: $\chi^2 = 5.55$, $P < 0.02$; $t = 1.86$, $P < 0.05$, one-tailed) and more saline (excluding the outlier: $\chi^2 = 8.05$, $P < 0.01$; $t = 6.86$, $P < 0.001$) during the 3-hr test. In contrast, neither group averaged more than 1.0 ml of water or saline when injected with the 0.15 M NaCl vehicle solution.

The outlier rat with PBN lesions ingested 15 ml of water and 17 ml of saline in 30 min, and a total of 37 ml and 19 ml, respectively, in 3 hr.

Hypertonic Saline

In experiment 2, rats in the two groups drank similar amounts of water at similar rates throughout the 1-hr test after sc injection of 2 M NaCl. Control animals consumed 13.4 ± 1.1 ml of water in 60 min, whereas rats with PBN lesions drank 14.8 ± 2.4 ml.

Water Deprivation

In response to the 24-hr period of water deprivation, control rats consumed 20.7 ± 5.8 ml of 0.5 M NaCl, which was significantly less than the volume of water, 32.5 ± 2.4 ml, they had ingested during baseline testing ($t = 1.90$, $P < 0.05$, one-tailed). In contrast, rats with PBN lesions consumed 70.4 ± 13.8 ml of saline, which was much more than their baseline water intake of 21.4 ± 2.4 ml ($t = 3.49$, $P < 0.01$) and also much more than the saline intake of control rats ($t = 3.32$, $P < 0.01$). Evaluated another way, 9 of 12 control rats drank lesser amounts of 0.5 M NaCl in these one-bottle tests than their baseline water intake, whereas 8 of 9 rats with PBN lesions drank more saline than water (Fig. 5; $\chi^2 = 6.05$, $P < 0.02$).

Discussion

Consistent with published findings, bilateral lesions of the gustatory zone of the PBN in rats eliminated a CTA and salt appetite induced by furosemide. These lesions also abolished salt appetite induced by treatment with DOCA. In contrast, rats with PBN lesions drank 0.3 M NaCl in comparable amounts as control animals when injected sc with PEG solution, although they consumed much less NaCl when 0.5 M NaCl was instead available. Thus, PBN lesions may or may not eliminate salt appetite in rats, depending on the treatment given

and the concentration of NaCl solution used as a test stimulus (see Table 1). These results suggest the importance of employing multiple models of salt appetite when considering the effects of brain lesions or other experimental treatments, as has become commonplace in studies of thirst.

In the present experiments, lesions of the gustatory PBN eliminated salt appetite induced by DOCA treatment in 19 of the 20 rats tested. Because DOCA treatment stimulates salt appetite both directly, as an excitatory stimulus (Geerling, Engeland, Kawata, & Loewy, 2006; Geerling & Loewy, 2006), and indirectly, by inhibiting the inhibition of salt appetite mediated by central oxytocin neurons (Stricker & Verbalis, 1986), these results suggest that rats with PBN lesions can no longer respond to one or both of those changes in stimulation.

PEG treatment induces plasma volume deficits in rats, which stimulate renin secretion (Stricker et al., 1979), and the consequent formation of AngII provides a stimulus of NaCl appetite (Fluharty & Epstein, 1983; Stricker, 1983). PEG treatment also stimulates aldosterone secretion (Stricker et al., 1979), which also stimulates salt appetite in rats (Geerling et al., 2006). In addition, it stimulates thirst (Stricker, 1968), and renal retention of the ingested water reduces plasma osmolality (Stricker & Jalowiec, 1970), which lowers central oxytocin secretion and thereby removes its inhibition of salt appetite (Stricker & Verbalis, 1987). The amount of Na⁺ that is retained in rats given 30% PEG treatment is ~5 meq (Stricker & Jalowiec, 1970), which is contained in 10 ml of 0.5 M NaCl or 16.7 ml of 0.3 M NaCl. Few of the control rats ingested that much in the present 5-hr tests and none of the rats with PBN lesions did so, which suggests that hypovolemia was not completely repaired by the fluid consumed in the tests. Because 19 of 20 rats with PBN lesions consumed some saline after PEG treatment in Experiments 1 and 2, yet only one of these animals responded to DOCA treatment, we presume that the NaCl intake induced in the brain-damaged rats during hypovolemia was stimulated by AngII rather than by mineralocorticoids. If so, then the finding that the PEG-induced intake of 0.3 M NaCl by rats with PBN lesions was comparable to that of control animals for 1 hr but then slowed considerably (Fig. 4) probably reflects a substantial reduction in the contribution of AngII, as might be caused by the partial repair of plasma volume deficits (Stricker et al., 1979). Note that this speculation assumes that the PEG treatments had comparable physiological effects in the two groups of rats.

Salt appetite in response to furosemide treatment is believed to reflect the influence of both AngII and aldosterone in part because central blockade of the effects of either hormone alone decreases the induced NaCl intake whereas blockade of the effects of both hormones eliminates it (Sakai, Nicolaïdis, & Epstein, 1986; Weiss, Moe, & Epstein, 1986; see also Rowland & Morian, 1999). Thus, the apparent failure of rats with PBN lesions to increase salt appetite after furosemide treatment, seen in this report and previously (Flynn et al. 1991; Scalera et al. 1995), may indicate that the lesions interfere with the excitatory stimuli mediated by both AngII and aldosterone. However, that possibility seems unlikely given the positive response of the rats to PEG treatment. An alternative hypothesis is that the salt appetite induced by furosemide is not very strong. In this regard, the urinary Na⁺ losses produced by the diuretic are known to be relatively small (~2 meq Na⁺), as are the plasma volume deficits (Jalowiec, 1974), and consequently much less renin and aldosterone are

secreted than after PEG treatment (Rowland & Morrian, 1999; Stricker et al., 1979). If it takes more motivation for rats to consume a highly concentrated saline solution like 0.5 M NaCl than a less concentrated solution like 0.3 M NaCl, then there may not have been enough excitation for furosemide to stimulate NaCl intake in rats with PBN lesions under these testing conditions. It would be of interest, therefore, to determine whether salt appetite after furosemide treatment is evident in such rats when 0.3 M NaCl is available in a two-bottle test.

The basis of the salt appetite in control rats that was elicited by systemic injection of AngII in Experiment 1 is uncertain. It is not likely that the administered AngII acts in the brain to stimulate salt appetite. Instead, it may stimulate salt appetite indirectly by inducing a mild natriuresis, much as it does when the peptide is administered centrally (Fluharty & Manaker, 1983). If so it would mimic the effects of furosemide in this respect, and the failure of rats with PBN lesions to increase ingestion of 0.5 M NaCl in response to systemic AngII would therefore resemble the absence of salt appetite in response to furosemide in these animals.

Collectively, these results indicate that lesions of the gustatory PBN do not invariably eliminate salt appetite induced by experimental treatment in rats. The results also indicate that the impairment in salt appetite, when it occurs, does not reflect a general dysfunction in the drinking response to a need for body fluids. In these experiments, there evidently was no impairment in the water drinking response of rats with PBN lesions to an increase in plasma osmolality (produced by injection of hypertonic saline), a decrease in plasma volume (produced by PEG treatment), or sc injection of AngII, the three known stimuli of thirst (Stricker, 2004). It is not clear, however, why PBN lesions interfere with the expression of salt appetite. The PBN receives afferent input from the NTS regarding both taste and visceral information. Since DOCA apparently stimulates salt appetite by acting in the medial NTS (Geerling et al., 2006; Geerling & Loewy, 2006), which projects to the PBN (Geerling & Loewy, 2007), that excitatory pathway might plausibly have been disrupted by PBN lesions. In contrast, AngII seems to work more remotely, perhaps in the subfornical organ (Thunhorst, Beltz, & Johnson, 1999) or elsewhere along the lamina terminalis (Fitts & Masson, 1990), and thus PBN lesions may not interfere with its stimulation of salt appetite. It is not clear where in the brain the inhibitory effects of central oxytocin are mediated.

Some of the observations in this study resemble similar findings in rats with area postrema lesions; in particular, impaired taste aversion learning (Berger, Wise, & Stein, 1973; Ritter, McGlone, & Kelly, 1980) and notably large intakes of water after treatment with sc PEG (Curtis, Verbalis, & Stricker, 1996) and of 0.5 M NaCl in a one-bottle test when water-deprived (Stricker, unpublished observations, 1999). Comparably large water intakes after sc PEG, and enhanced intakes of 0.4 M NaCl after DOCA treatment, also have been observed in rats with capsaicin-induced destruction of peripheral sensory nerves (Curtis & Stricker, 1977). Furthermore, very large intakes of 0.3 M NaCl by rats have been observed after bilateral injection of various drugs into the lateral PBN in combination with systemic injection of hypertonic saline and other dipsogens (Kimura et al., 2008; Menani, De Luca, Thunhorst, & Johnson, 2000), as if the normal inhibition of salt appetite had been removed (see review by Stricker, 2004). Similarly, rats with ibotenic acid-induced lesions of the LPBN consumed relatively large amounts of water in one-bottle tests when they were

injected with AngII but not with hypertonic saline (Edwards & Johnson, 1991; see also Ohman & Johnson, 1986). Presumably the early osmotic effects of ingesting water or concentrated saline are detected by visceral osmoreceptors (Stricker & Hoffmann, 2007) and communicated neurally to the PBN via the NTS and area postrema (Geerling & Loewy, 2007; Sequeira, Geerling, & Loewy, 2006), and therefore destruction of the PBN, the area postrema, or the efferent nerves from the viscera eliminate the neurally-mediated, early inhibitory effects of fluid consumption. Note that those early effects of water consumption would not be as significant when thirst is stimulated by injection of hypertonic saline because ingested water reduces systemic plasma osmolality so rapidly (Stricker, 2004).

Distinct from the impaired ability of rats with PBN lesions to develop a salt appetite is their severely impaired ability to acquire a CTA. This effect, which was used as an initial screen for the effectiveness of the lesions, has been examined extensively elsewhere (for reviews, see Lundy & Norgren, 2004; Reilly, 1999; Spector, 1995). Thus, our further comments will focus on one interpretation of the impaired acquisition of a CTA, namely, that PBN lesions prevent rats from readily inhibiting fluid ingestion by interfering with their ability to associate the taste of fluid with postingestive visceral effects when those biological consequences are aversive or, if not actually harmful, not helpful. It is not that the animals can no longer detect tastes or make use of such adaptive associations because rats with PBN lesions that cannot learn a CTA may still be able to use gustatory cues in a threshold detection task (Spector, Scalera, Grill, & Norgren, 1995), and they will avoid a taste that had been associated with nausea before the lesions were made (Grigson, Shimura, & Norgren, 1997b). Instead, we support the suggestion, made previously (Grigson, Reilly, Shimura, & Norgren, 1998; Reilly, Grigson, & Norgren, 1993; Spector et al., 1992), that rats with PBN lesions cannot make new associations of this sort as readily as intact control rats can, if at all.

If rats with PBN lesions do in fact have this dysfunction, then it should not be manifest only in tests of taste aversion learning. For example, it may contribute to the very large intakes of 0.5 M NaCl by rats with PBN lesions when water-deprived (in Experiment 1), despite the induced osmotic concentration of body fluids. Similarly, the single PEG-treated rat with PBN lesions in Experiment 1 that drank a very large volume of 0.5 M NaCl in the first 30 min of the 5-hr drinking test behaved as if it did not get inhibitory feedback from the high osmolality of the fluid it was consuming (whereas the very large volume of water it consumed in 5 hr was appropriate in that the total amounts ingested compute to a mixed solution approaching isotonicity). Finally, the large cumulative water intakes by rats with PBN lesions in response to a prolonged hypovolemic signal for thirst after PEG treatment (Fig. 3A) may indicate an additional impairment in inhibiting fluid intake when its consequences do not service an animal's needs. Water does not repair the plasma volume deficits of these animals (Stricker, 1968), and pronounced osmotic dilution should have inhibited their thirst (Stricker, 1969).

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Figure 1.

Photomicrographs of 50 μm coronal sections from the dorsolateral pons of six rats in these experiments. Each section is at approximately the same rostrocaudal level, midway through the gustatory area of the parabrachial nucleus (PBN). Medial is to the right in panels A, C, and E, and to the left in panels B, D, and F. Dorsal is up. Panels A and B depict a “Complete” PBN lesion and a control, respectively (cresyl violet, Experiment 1). In panel B note the abundant neurons (small dark stippling) on either side of the brachium conjunctivum (BC) compared with the collapsed, acellular zones in the same relative positions in panel A. Panels C and D show similar Complete PBN damage and a control from Experiment 2 (NeuN stain). The oval in panel D indicates the approximate extent of the gustatory area. Panels E and F compare a “Partial-Complete” lesion with “Partial” damage, respectively. Although it had a few normal neurons at the lateral edges of both the medial and lateral PBN, the rat whose brain histology is shown in panel E failed to learn a conditioned taste aversion (CTA) or to display a NaCl appetite. The rat whose brain histology is shown in panel F had substantial sparing of neurons in the medial PBN

(ventromedial to BC). It learned a CTA in one trial and exhibited normal NaCl appetite in response to each challenge in Experiment 2. The horizontal line in panel E represents approximately 400 μm . [Note that the NeuN procedure shrinks the sections about 20% more than does cresyl violet.] Abbreviations: BC – brachium conjunctivum, LC – locus coeruleus, MV –trigeminal motor nucleus, MesV – mesencephalic trigeminal nucleus, STA – supratrigeminal area.

Fig 2A

Fig 2B

Figure 2. Mean intake (ml/7 hr) of NaCl solution by rats given DOCA treatments daily, as a function of days. Rats drank either (A) 0.5 M NaCl (Experiment 1) or (B) 0.3 M NaCl (Experiment

2). Control rats consumed increasingly more saline solution during the 6- or 7-day treatment period (D1–D6 or D1–D7), peaking in the last day or two of the treatment period and during the subsequent day of baseline testing (B2). In contrast, rats with PBN lesions did not have a stimulated salt appetite in either experiment (but for one rat in Experiment 2, not included, which unaccountably drank substantial amounts of saline; see text for explanation). Note the scales of the y-axes in the two figures are not the same.

Fig 3A

Fig 3B

Figure 3.

Cumulative intake of water by PEG-treated rats, plotted as a function of cumulative intake of either (A) 0.5 M NaCl (Experiment 1) or (B) 0.3 M NaCl (Experiment 2). Shown are mean intakes (ml) at 5, 10, 15, 30, and 45 min, and at 1, 2, 3, 4, and 5 hr in a test begun 24 hr after the injection. Control rats drank progressively more of each fluid in both experiments. Rats with PBN lesions in Experiment 2 drank similarly, whereas in Experiment 1 they consumed much more water but little NaCl after 15 min. Note the scales of the axes in the two figures are not the same. (Not included were the intakes of one outlier with PBN lesions in Experiment 1; see text for explanation.)

Figure 4.

Cumulative intakes of Na⁺ (mEq) by PEG-treated rats in Experiments 1 and 2. Shown are mean intakes at 5, 10, 15, 30, and 45 min, and at 1, 2, 3, 4, and 5 hr. Control rats drank comparable amounts of Na⁺ whether they consumed 0.3 M NaCl (Experiment 1) or 0.5 M NaCl (Experiment 2). Rats with PBN lesions drank comparable amounts of saline as control rats did after 0.5 hr (Experiment 1) or 1 hr (Experiment 2), but much less saline thereafter.

Figure 5.

Cumulative intakes of water (WI, left column) and 0.5 M NaCl solution (SI, right column) in 1-bottle tests by control rats and rats with PBN lesions in Experiment 1. Each symbol represents datum from one animal. Control rats usually drank less saline in these one-bottle tests than their baseline water intake, whereas rats with PBN lesions usually drank more saline than water.

Table 1

Summary of results in Experiments 1 and 2.

	Control		PBN lesions	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
DOCA	Na(+)	Na(+)	Na(-)	Na(-)
PEG	W(+)/Na(+)	W(+)/Na(+)	W(++)/Na(-)	W(+)/Na(+)
AngII	W(+)/Na(+)		W(++)/Na(-)	
Water depriv	Na(-)		Na(+)	
2 M NaCl		W(+)		W(+)

Abbreviations: W = water intake, Na = saline intake, + = positive response, ++ = enhanced response, - = deficient response. Rats drank 0.5 M NaCl in Experiment 1 and 0.3 M NaCl in Experiment 2.