Elevated dietary salt intake does not invariably increase arterial blood pressure (ABP) but does contribute to the development of hypertension or the severity of hypertension in salt-sensitive individuals and experimental models. Compelling data in several laboratories indicate that dietary salt acts centrally with other factors to increase sympathetic nerve activity (SNA) and peripheral resistance. Moreover, dietary salt potentiates the sympathetic and/or pressor responses to stress, hyperinsulinemia, and activation of somatic afferents. Collectively, these observations suggest that dietary salt may alter the gain of central sympathetic-regulatory networks. This hypothesis is supported by data from several laboratories that SNA and ABP responses to microinjection of various excitatory and inhibitory neurotransmitters into the rostral ventrolateral medulla (RVLM) are enhanced in animals chronically maintained on a high-salt diet.

Elevated dietary salt intake causes widespread changes in neurohormonal profiles, including suppression of the peripheral renin-angiotensin (Ang) system and increases in plasma sodium concentration or osmolality. One of the major sites where the central nervous system detects such changes in neurohormonal stimuli is the forebrain lamina terminalis (LT). The LT consists of several interconnected structures located along the rostral wall of the third ventricle, including the median preoptic nucleus, subfornical organ (SFO), and organum vasculosum of the LT (OVLT). The latter 2 structures lack a complete blood-brain barrier and are thereby responsive to a number of circulating factors. LT lesions severely disrupt physiological responses to a number of neurohormonal stimuli, including osmolality and circulating Ang II. Interestingly, lesions of the anteroventral third ventricle region (AV3V), which encompasses the LT, prevent the development or reverse hypertension in Dahl salt-sensitive rats. Collectively, these observations suggest that the responsiveness of LVLM sympathetic-regulatory neurons can be modulated by the forebrain LT. The purpose of the present study was to determine whether the forebrain LT mediated the ability of dietary salt to enhance sympathetic and cardiovascular responses from the RVLM.
Lesion of the LT

Rats were anesthetized with 3% isoflurane and placed into a stereotaxic frame with the skull level between lambda and bregma. After a small craniotomy, a Teflon-coated tungsten electrode (50- or 250-μm tip, 0.008 OD, AM Systems) angled 8° from the midsagittal plane was lowered into the ventral LT using coordinates in reference to bregma: 0.0- to 0.5-mm rostral, 1.0-mm lateral, and 8.0-mm ventral to dura. DC current (100 or 500 μA) was applied for 30 seconds. Electrode tip size and current intensity were varied to produce small (OVLT) versus large (ventral LT) lesions, respectively. Sham control rats consisted of 2 groups: identical procedures except no current was applied or lesions were placed lateral to the midsagittal plane. SFO lesions were produced by applying DC current (500 μA, 30 seconds) to a tungsten electrode (250-μm tip) angled 8° from the midsagittal plane at 2 different sites in reference to bregma: 0.8 versus 1.1 mm caudal, 0.7 versus 0.7 mm lateral, and 5.2 versus 4.9 mm ventral to dura. The craniotomy was filled with bone wax and the incision closed with suture. Rats were given ampicillin (100 mg/kg, IM), returned to home cages, and given access to deionized water for ≥7 days before experiments began.

Materials and Methods

Animals

All of the experimental procedures conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Kentucky Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (200 to 250 g, Charles River Laboratories) were housed in a temperature-controlled room (22 ± 1°C) with a 12:12-hour light-dark cycle (lights on 7 AM to 7 PM). Rats were fed standard rat chow containing 0.23% NaCl (Harlan Teklad Global Diet 2018) and given access to deionized water for ≥7 days before experiments began.

Circadian Analysis of Plasma Electrolytes, Osmolality, and Food and Fluid Intakes

Control and ventral LT-lesioned rats were fed normal chow and given access to water or 0.9% NaCl for 14 days. At 1 PM or 1 AM, rats were anesthetized with 3% isoflurane, and blood (0.5 mL) was collected by aortic puncture into heparinized tubes and analyzed for plasma electrolytes by an I-STAT1 analyzer and 6+ cartridges (Abbott). Plasma osmolality was determined in duplicate by freezing-point depression (Advanced Instruments). Food and fluid intake measurements were monitored daily except in a subset of animals where daytime and nighttime measurements were performed.

Statistical Analysis

All of the data are expressed as mean ± SE. Changes in integrated SNA were calculated by subtracting background noise after hexamethonium (30 mg/kg, IV). The 1-second peak SNA and ABP responses were compared with a 30-second baseline segment immediately before the injection. Renal SNA was only analyzed when injections were performed ipsilateral to the nerve recording. All of the data were analyzed by a 1- or 2-way ANOVA with repeated measures when appropriate (dose factor). All of the posthoc tests were performed with independent or paired t tests with a layered Bonferroni correction. A P < 0.05 was considered statistically significant.

Results

Ventral LT Lesion Prevents Salt-Induced Enhancement of RVLM Responses

A major goal of the present study was to determine whether LT neurons mediated the enhanced cardiovascular responses of RVLM neurons during increased dietary salt. Figure 1 illustrates histology for control and ventral LT-lesioned animals. Lesions of the ventral LT produced extensive damage to the OVLT and midline preoptic nuclei at the level of the anterior commissure. In the majority of cases, the ventral median preoptic nucleus at the commissural level was intact. Damage was not observed caudal to the median preoptic nucleus. As reported previously,9-11 RVLM injec-
to the ventral LT (data not shown). These responses were not different from control animals drinking 0.9% NaCl or water, respectively.

To examine whether LT neurons mediated the enhanced sympathoinhibitory responses evoked from the RVLM during increased dietary salt, GABA was microinjected into the contralateral RVLM. As reported previously, rats drinking 0.9% NaCl versus water displayed significantly greater depressor responses to every dose of L-glutamate or GABA in these animals were not different from control animals drinking 0.9% NaCl or water, respectively.

In a second group of animals, we examined whether chronic lesion of the ventral LT drinking 0.9% NaCl showed similar changes in renal SNA and ABP to RVLM injection of L-glutamate in rats with chronic lesion of the ventral LT. B, Individual examples of ABP, mean ABP, renal SNA, and raw renal SNA during injection of 1.0 nmol of L-glutamate. 

**Figure 2.** A, Peak change in mean ABP and renal SNA during RVLM injection of L-glutamate in rats with chronic lesion of the ventral LT. B, Individual examples of ABP, mean ABP, renal SNA, and raw renal SNA during injection of 1.0 nmol of L-glutamate. 

**OVLT Lesion Prevents Salt-Induced Enhancement of RVLM Responses**

A subset of animals had more focal lesions with damage restricted to the OVLT (Figure 5). Interestingly, chronic ingestion of 0.9% NaCl did not result in potentiated sympathoexcitatory responses to L-glutamate (Figure 5C) or GABA (Figure 5D). In fact, the changes in renal SNA or ABP evoked by injection of L-glutamate or GABA in these animals were not different from control and ventral LT-lesioned rats drinking water or ventral LT-lesioned rats drinking 0.9% NaCl (Figures 3 and 4).

**Enhanced RVLM Responses Are not Prevented by Acute Lesion of the Ventral LT or Chronic SFO Lesion**

Acute lesion of the ventral LT in rats drinking water or 0.9% NaCl produced a transient decrease in ABP (−2±4 mm Hg) and renal SNA (−31±10% versus −31±9%); however, both variables returned to baseline values within 30 minutes. Histology is illustrated in Figure S2. In marked contrast to chronic lesion of the ventral LT, RVLM injection of L-glutamate produced significantly greater renal SNA and ABP responses in rats with acute lesion of the ventral LT drinking 0.9% NaCl versus water.
Adams et al Salt Enhances RVLM Responses via Lamina Terminalis

Similarly, RVLM injection of L-glutamate produced significantly greater increases in renal SNA and ABP of SFO-lesioned rats drinking 0.9% NaCl versus water (Figure 6B). Histology is illustrated in Figure S3. Exaggerated sympathoinhibitory responses to RVLM injection of GABA were observed in both groups (data not shown). Moreover, the responses observed in acute LT- or chronic SFO-lesioned rats drinking 0.9% NaCl were not different from control rats drinking 0.9% NaCl.

Injection sites for all of the experiments were centered in the RVLM as defined previously9,10 (Figure S4).

Analysis of Plasma Electrolytes and Osmolality

There were no differences in plasma sodium concentration or osmolality during the day between control or lesioned rats drinking water or 0.9% NaCl (Table). However, control and lesioned rats drinking 0.9% NaCl displayed significant increases in plasma sodium concentration and osmolality at night. All of the groups ingested significantly more food and fluid during the dark versus light cycle, and rats drinking 0.9% NaCl ingested significantly more fluid and had higher daily sodium intakes (Tables S1 and S2). However, there were no differences in baseline mean ABP, heart rate, renal SNA, or plasma and/or blood volume (Tables S2 and S3).

Discussion

Increased dietary salt enhances sympathetic and cardiovascular responses evoked and/or mediated by RVLM sympathetic-regulatory neurons.4–7,9–11 However, the mechanism by which increased dietary salt is detected by the central nervous system and translates into functional differences in the regulation of RVLM sympathetic neurons was previously unknown. The present findings provide several new key observations: (1) chronic lesions of the ventral LT and OVLT prevent the enhanced cardiovascular responses to RVLM stimulation during increased dietary salt intake; (2) acute lesion of the ventral LT or chronic SFO lesion did not affect these responses; and (3) increased dietary salt intake elevated plasma sodium concentration and osmolality at night. Altogether, these findings suggest that ventral LT, and perhaps OVLT, neurons mediate the ability of increased dietary salt to enhance the responsiveness of RVLM sympathetic neurons.

The forebrain LT is a specialized group of structures that permit the central nervous system to detect changes in neurohumoral factors.16,17 Given the widespread neurohumoral changes associated with increased dietary salt intake, we hypothesized that forebrain LT neurons indirectly detect the changes in dietary salt to alter the responsiveness of RVLM neurons. Indeed, rats with chronic lesion of the ventral LT (and OVLT) and ingesting 0.9% NaCl had similar sympathoexcitatory and sympathoinhibitory responses versus those animals ingesting water. Chronic SFO lesions did not affect these responses. These findings cannot be explained by differences in salt intake, because rats with chronic lesions of the ventral LT or OVLT ingested similar amounts of 0.9% NaCl as control rats. Furthermore, the ability of chronic ventral LT and OVLT lesions to prevent the enhanced...

Figure 4. A, Peak change in mean ABP and renal SNA during RVLM injection of Ang II in rats with chronic lesion of the ventral LT. B, Individual examples of ABP, mean ABP, renal SNA, and raw renal SNA during injection of 6 pmol of Ang II. *P<0.05 control+water vs control+salt; †P<0.05 control+water vs control+salt versus lesion+salt.

Figure 5. A, Schematic drawings of OVLT lesions for rats drinking water (dashed) or 0.9% NaCl (black). Lines indicate the lesion boundary. B, Digital photomicrograph of OVLT lesion. Scale bar: 500 μm; arrow indicates lesion. C, Peak change in mean ABP and renal SNA of OVLT-lesioned and control rats during RVLM injection of (C)L-glutamate or (D) GABA. *P<0.05 control + water vs control + salt; †P<0.05 control + salt versus lesion + salt.
responsiveness of RVLM neurons is likely not attributed to some chronic adaptation as a result of the lesion per se, because the sympathoexcitatory and sympathoinhibitory responses were not different between control and lesioned rats drinking water. Therefore, these findings indicate that ventral LT or OVLT neurons mediate the ability of increased dietary salt to enhance the responsiveness of RVLM sympathetic neurons.

A critical question that arises from these studies is the nature of the neurohumoral factor(s) that activates LT neurons to alter the responsiveness of RVLM neurons. Indeed, neurons within these structures express receptors for a variety of circulating factors. Although AV3V lesions in rats clearly disrupt thirst and vasopressin secretion to a number of physiological stimuli,16 such lesions produce damage across the entire forebrain LT. However, discrete lesion of the SFO in rats20 and OVLT in dogs disrupts thirst and vasopressin secretion of circulating Ang II,18 whereas lesion of the SFO in rats20 and dogs21 blunts thirst stimulated by Ang II. Studies in sheep have demonstrated that neurons in the hypothalamic paraventricular nucleus.16,17 Previous studies have shown that osmosensory neurons in the hypothalamic paraventricular nucleus with descending projections are excited by hyperosmolality.30 Anatomic and functional data indicate that these neurons use Ang II as a neurotransmitter,31,32 and we recently reported a greater Ang II type 1 receptor activation in the RVLM of rats on a high-salt diet.10 Yet, available evidence suggests that the mechanism of the enhanced RVLM responsiveness is likely more complex. First, these enhanced responses are observed in rats drinking 0.9% NaCl after 14 or 21 days but not at 1 or 7 days.9 Second, rats drinking 0.9% NaCl for 14 days still exhibited enhanced responses when water was returned for 1 day.9 Third, acute lesion of the ventral LT in the present study did not reverse the enhanced responses evoked from the RVLM of rats drinking 0.9% NaCl for 14 days. Collectively, these observations indicate that dietary salt alters the responsiveness through a chronic change in neuronal function or some form of neuronal plasticity.

An interesting observation in the current study is that the ingestion of 0.9% NaCl significantly increased plasma sodium concentration at night but not during the day in both control and ventral LT-lesioned animals. Other studies have reported that dietary salt elevates plasma sodium concentration or osmolality in rats3,15 and humans.14 Small increases in osmolality of 1% to 2% stimulate drinking in mammals,33,34 thereby suggesting that osmosensory cells can detect discrete changes in osmolality or plasma sodium concentration. These observations, together with the present study, raise the possibility that increased dietary salt elevates plasma osmolality to activate osmosensory neurons in the ventral LT and to alter the responsiveness of RVLM neurons. In fact, an enhanced responsiveness of RVLM neurons has been reported in 48-hour water-deprived rats.35 Such a model would suggest that chronic changes in plasma osmolality produced by dietary salt intake must reach the threshold of osmosensory neurons. Currently, there are no available data to directly address this issue; however, the altered responsiveness of RVLM neurons has been observed over a range of different salt intakes.7 Clearly, additional evidence is needed to directly link dietary salt intake and the changes in the responsiveness of RVLM neurons with osmotic perturbations or other circulating factors.

### Table. Plasma Sodium Concentration and Osmolality of Control and Ventral LT-Lesioned Rats Drinking Water or 0.9% NaCl

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Sodium, mEq/L</th>
<th>Plasma Osmolality, mosmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Night</td>
</tr>
<tr>
<td>Control + water</td>
<td>135.2±0.4 (9)</td>
<td>136.3±0.6 (11)</td>
</tr>
<tr>
<td>Control + salt</td>
<td>134.5±0.5 (9)</td>
<td>138.4±0.6 (13)*</td>
</tr>
<tr>
<td>Lesion + water</td>
<td>135.0±1.0 (7)</td>
<td>136.0±1.0 (8)</td>
</tr>
<tr>
<td>Lesion + salt</td>
<td>136.1±0.9 (6)</td>
<td>139.8±0.8 (8)*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Parentheses indicate number of animals: mosmol, milliosmol.

*Significant difference between water and 0.9% NaCl within the control or lesion group (P<0.05).
In the present study, lesion of the ventral LT did not produce profound deficits in fluid ingestion or sodium balance, as reported previously in AV3V-lesioned rats. AV3V lesions damage numerous structures along the rostral wall of the third ventricle, including the median preoptic nucleus, fibers of passage from the SFO, and other periventricular nuclei. Ventral LT lesions of the present study did not damage the median preoptic nucleus or the SFO. Therefore, the lack of fluid and osmoregulatory deficits in the present study is likely attributed to the smaller lesions and the presence of other osmoregulatory nuclei in the central nervous system.

Perspectives
Increased dietary salt raises plasma (or cerebrospinal fluid) sodium concentration and contributes to neurogenic forms of salt-sensitive hypertension in 1 of 2 ways: a direct sodium-driven increase in SNA and ABP or a chronic increased gain of sympathetic-regulatory networks. The increased gain of RVLM sympathetic neurons has physiological significance, because increased dietary salt enhances sympathoexcitatory responses to insulin and stimulation of somatic afferents, responses that depend on RVLM neurotransmission. The ability of ventral LT lesions to prevent the enhanced responsiveness of RVLM neurons during increased dietary salt intake is reminiscent to the effect of AV3V lesions on various models of neurogenic hypertension. AV3V lesions prevent the development of or reverse hypertension in Dahl salt-sensitive, DOCA-salt, Grollman, and Goldblatt hypertensive rats. The available data suggest that these models show exaggerated responses to the injection of l-glutamate in the RVLM. In marked contrast, AV3V lesions do not affect hypertension in the spontaneously hypertensive rat, and spontaneously hypertensive rats do not display enhanced responses to l-glutamate injection in the RVLM. Altogether, these observations raise the possibility that AV3V lesions attenuate neurogenic hypertension, in part, by preventing an enhanced excitability of RVLM sympathetic neurons.

Sources of Funding
This research was supported by Great Rivers American Heart Association postdoctoral (J.M.A.) and predoctoral (M.E.B.) fellowships, American Heart Association Scientist Development Grant (S.D.S.), and a National Institutes of Health National Heart, Lung, and Blood Institute grant HL090826 (S.D.S.).

Disclosures
None.

References
8. Stocker SD, Madden CJ. Excess dietary salt selectively enhances the excitability of sympathetic neurons in the rostral ventrolateral medulla [abstract 958.9]. Presented at the 2009 Experimental Biology Meeting; April 18–22, 2009, New Orleans, LA.


36. Bardgett ME, Stocker SD. Glutamatergic receptor activation in the rostral ventrolateral medulla contributes to the sympathoexcitatory response to hyperinsulinemia [abstract 958.16]. Presented at the 2009 Experimental Biology Meeting; April 18–22, 2009, New Orleans, LA.


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Hypertension. 2009;54:308-314; originally published online June 8, 2009;
doi: 10.1161/HYPERTENSIONAHA.108.127803

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Ventral Lamina Terminalis Mediates Enhanced Cardiovascular Responses of RVLM Neurons During Increased Dietary Salt

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Department of Physiology, University of Kentucky
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Fax: 859-323-1070
Materials and Methods

RVLM microinjections and SNA recordings were performed as described previously in our laboratory\(^1\),\(^2\). Briefly, rats were anesthetized with isoflurane (2-3% in 100% O\(_2\)) and then replaced by a mixture of urethane (750 mg/kg, iv) and \(\alpha\)-chloralose (75 mg/kg, iv). Renal SNA recordings were performed using a bipolar stainless steel electrode, amplified (20,000 x), and filtered (low pass: 100 Hz, high pass: 3 kHz). Signals were digitized (5 kHz), rectified, and integrated (1s time constant) using a Micro1401 and Spike 2 software (Cambridge Electronic Design). Animals were artificially ventilated with oxygen-enriched room air and paralyzed with gallamine triethiodide (25 mg/kg/h, 25 \(\mu\)L/h, iv). End-tidal CO\(_2\) and body temperature was maintained at 4-4.5% and 37±1°C, respectively. An adequate depth of anesthesia was assessed by either the absence of a withdrawal reflex (before neuromuscular blockade) or a pressor response to foot pinch. Supplemental doses of anesthetic (10% initial dose) were given as necessary but rarely needed. Initially, L-glutamate (1 nmol) was injected into the RVLM at 3 different sites separated by 300\(\mu\)m in the rostral-caudal plane to identify the site that produced the largest increase in ABP; subsequent injections were performed at these coordinates. All injections (60 nL) were performed over 5 s by an experimenter blind to the salt and lesion condition. Injection sites were marked at the end of experiments with 0.2% rhodamine beads. At the end of experiments, animals were perfused transcardially with 4% paraformaldehyde (50 mL). Brains were harvested, post-fixed, sectioned at 50 \(\mu\)m, and counterstained with cresyl violet. RVLM injection sites and lesions were analyzed by an experimenter blind to the injection results, salt group, and lesion group.

References


Table S1.  Food and fluid intakes during the light and dark cycles of control and lesion rats.

<table>
<thead>
<tr>
<th>Group</th>
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<th></th>
<th>Dark Cycle</th>
<th></th>
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<td></td>
<td></td>
<td>Food (g)</td>
<td>Fluid (mL)</td>
<td>Food (g)</td>
<td>Fluid (mL)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>6</td>
<td>5±1</td>
<td>3±1</td>
<td>22±1</td>
<td>31±2</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>6</td>
<td>5±1</td>
<td>3±1</td>
<td>23±1</td>
<td>51±4*</td>
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<tr>
<td>Ventral LT Lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>8</td>
<td>5±1</td>
<td>5±1</td>
<td>25±2</td>
<td>36±2</td>
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<tr>
<td>0.9% NaCl</td>
<td>8</td>
<td>4±1</td>
<td>6±1</td>
<td>24±1</td>
<td>56±5*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Significant difference within group between water vs 0.9% NaCl (P<0.05)
Table S2. Characteristics of rats with various lesions and drinking either water or 0.9% NaCl.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Water</th>
<th>Control 0.9%</th>
<th>Chronic LT/OVLT Water</th>
<th>Chronic LT/OVLT 0.9%</th>
<th>Acute LT Water</th>
<th>Acute LT 0.9%</th>
<th>SFO-Lesion Water</th>
<th>SFO-Lesion 0.9%</th>
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<tr>
<td>n</td>
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<td>7</td>
<td>17</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Initial BWT (g)</td>
<td>334±8</td>
<td>333±11</td>
<td>356±8</td>
<td>347±16</td>
<td>223±13*</td>
<td>239±14*</td>
<td>273±11*</td>
<td>273±12*</td>
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<tr>
<td>Final BWT (g)</td>
<td>430±14</td>
<td>429±17</td>
<td>411±10</td>
<td>446±7</td>
<td>362±15*</td>
<td>384±8*</td>
<td>368±19*</td>
<td>380±19*</td>
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<tr>
<td>Food Intake (g/day)</td>
<td>28±1</td>
<td>31±1</td>
<td>30±1</td>
<td>29±1</td>
<td>31±4</td>
<td>29±1</td>
<td>29±2</td>
<td>33±3</td>
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<td>Fluid Intake (mL/day)</td>
<td>33±2</td>
<td>54±3†</td>
<td>37±2</td>
<td>59±6†</td>
<td>35±3</td>
<td>48±5†</td>
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<td>53±6†</td>
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<td>Na⁺ Intake (mEq/day)</td>
<td>1.1±0.1</td>
<td>9.6±0.5†</td>
<td>1.2±0.1</td>
<td>10.2±0.9†</td>
<td>1.2±0.2</td>
<td>8.4±0.8†</td>
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<td>9.5±1.0†</td>
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<tr>
<td>Baseline Mean ABP</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>121±5</td>
<td>125±3</td>
<td>124±2</td>
<td>119±4</td>
<td>112±6</td>
<td>117±3</td>
<td>124±3</td>
<td>125±1</td>
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<tr>
<td>Baseline HR (bpm)</td>
<td>389±16</td>
<td>366±9</td>
<td>370±11</td>
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<td>392±19</td>
<td>406±19</td>
<td>380±4</td>
<td>391±7</td>
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<tr>
<td>Renal SNA (µv)</td>
<td>147±35</td>
<td>131±13</td>
<td>137±19</td>
<td>156±29</td>
<td>134±14</td>
<td>152±13</td>
<td>130±21</td>
<td>160±24</td>
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</table>

Value are mean ± SEM. *P<0.05 versus water treatment in same lesion group, †P<0.01 versus water treatment in same group
Table S3. Characteristics of control or lesioned rats drinking water or 0.9% NaCl

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Group</th>
<th>Chronic Ventral LT</th>
<th>Chronic SFO</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Salt</td>
<td>Water</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45±1</td>
<td>43±1</td>
<td>43±1</td>
</tr>
<tr>
<td>P Protein (g/dl)</td>
<td>6.9±0.1</td>
<td>6.9±0.1</td>
<td>6.8±0.2</td>
</tr>
<tr>
<td>Plasma Na+ (mEq/L)</td>
<td>138±1</td>
<td>139±2</td>
<td>136±1</td>
</tr>
<tr>
<td>Plasma K+ (mEq/L)</td>
<td>4.3±0.2</td>
<td>4.7±0.2</td>
<td>4.6±0.2</td>
</tr>
<tr>
<td>Plasma Volume (mL)</td>
<td>10.3±0.2</td>
<td>11.0±0.6</td>
<td>11.5±0.4</td>
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<tr>
<td>Blood Volume (mL)</td>
<td>16.6±0.4</td>
<td>18.3±0.9</td>
<td>18.2±0.6</td>
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<tr>
<td>Blood Volume per 100 g body weight</td>
<td>4.1±0.2</td>
<td>3.5±0.6</td>
<td>4.3±0.1</td>
</tr>
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Values are mean ± SE. Plasma protein was determined by protein refractometry (Refractometer Veterinary ATC, VWR International), and plasma Na⁺ and K⁺ concentration by flame photometry (Model 2655-10, Cole Palmer Instrument Co.). In a subset of animals, plasma and blood volume were determined using Evan’s Blue Dye as described previously. Animals with ventral LT and OVLT lesions were combined.
Figure S1. Schematic drawings of VLT lesions for rats drinking (A) water or (B) 0.9% NaCl and receiving an injection of AngII into the RVLM. The lesion boundary is outlined in black; control animals receiving a misplaced lesion are in grey. Abbreviations: LV, lateral ventricle; DBB, diagonal band; AC, anterior commissure; OVLT, organum vasculosum of the lamina terminalis; MnPO, median preoptic nucleus; f, fornix; 3V, third ventricle; OC, optic chiasm.

Figure S2. Schematic drawings of acute ventral LT lesions for rats drinking (A) water or (B) 0.9% NaCl. The lesion boundary is outlined in black.
Figure S3. Schematic drawings of SFO lesions for rats drinking (A) water or (B) 0.9% NaCl. The lesion boundary is outlined in red. Abbreviations: LV, lateral ventricle; 3V, 3rd ventricle; SFO, subfornical organ; PVH, hypothalamic paraventricular nucleus; f, fornix; vhc, ventral hippocampal commissure; PVA, thalamic paraventricular nucleus; PT, paratenial thalamic nucleus; sm, stria medullaris of the thalamus; Re, reunions thalamic nucleus
Figure S4. Schematic drawings of RVLM injection sites in rats drinking water (open) or 0.9% NaCl (filled) in one of four groups: A) control, B) chronic ventral LT lesion, C) acute ventral LT lesion, or D) chronic SFO lesion. Injections sites L-glutamate and AngII are illustrated on the left side whereas those for GABA are illustrated on the right side. Abbreviations: ST, spinal trigeminal nucleus; NA, nucleus ambiguus; IO, inferior olive; p, pyramidal tracts.