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

Julye M. Adams, Megan E. Bardgett, and Sean D. Stocker
HYPERTENSION/2008/127803 [R2]

You might find this additional information useful.

**Topic Collections**
Articles on similar topics can be found in the following collections:
http://hyper.ahajournals.org/cgi/collection

**Reviews**
You can submit your review by logging in at http://submit-hyper.ahajournals.org and entering the Reviewer Area

Information about *Hypertension* can be found at:
http://hyper.ahajournals.org

To subscribe to *Hypertension*, please go to http://hyper.ahajournals.org/subscriptions/

Disclaimer: The manuscript and its contents are confidential, intended for journal review purposes only, and not to be further disclosed.
Author Disclosures

Julye M. Adams: No disclosures

Megan E. Bardgett: No disclosures

Sean D. Stocker:
Research Grant: American Heart Association Research Grant, Amount: $10,000
Ventral Lamina Terminalis Mediates Enhanced Cardiovascular Responses of RVLM Neurons During Increased Dietary Salt

Julye M. Adams, Megan E. Bardgett and Sean D. Stocker

Department of Physiology, University of Kentucky

Running Head: Salt Enhances RVLM Responses Via Lamina Terminalis

Manuscript Word Count: 6120 = 224 abstract + 4546 text + 1350 (1 Table + 6 Figures)

Abstract Word Count: 224

Number of Figures: 6

Address correspondence to:
Sean D. Stocker, Ph.D.
Assistant Professor
Department of Physiology, University of Kentucky
800 Rose St. MS-508
Lexington, KY 40536-0298
Email: sean.stocker@uky.edu
Phone: 859-323-4344
Fax: 859-323-1070
Abstract

Increased dietary salt enhances sympathoexcitatory and sympathoinhibitory responses evoked from the rostral ventrolateral medulla (RVLM). The purpose of the present study was to determine whether neurons of the forebrain lamina terminalis (LT) mediated these changes in the RVLM. Male Sprague-Dawley rats with and without LT lesions were fed normal chow and given access to water or 0.9% NaCl for 14-15 days. Unilateral injection of L-glutamate into the RVLM produced significantly larger increases in renal sympathetic nerve activity (SNA) and arterial blood pressure (ABP) of sham rats ingesting 0.9% NaCl versus water. However, these differences were not observed between ventral LT-lesioned rats drinking 0.9% NaCl versus water. Similar findings were observed when angiotensin II or GABA were injected into the RVLM. Interestingly, a subset of animals drinking 0.9% but with damage restricted to the organum vasculosum of the lamina terminalis did not show enhanced responses to L-glutamate or GABA. In marked contrast, RVLM injection of L-glutamate or GABA produced exaggerated SNA and ABP responses in animals drinking 0.9% NaCl versus water after an acute ventral LT lesion or chronic lesion of the subfornical organ. Additional experiments demonstrate plasma sodium concentration and osmolality were increased at night in rats ingesting 0.9% NaCl. These findings suggest that neurons of the ventral LT mediate the ability of increased dietary salt to enhance the responsiveness of RVLM sympathetic neurons.

Keywords: brain, sodium, hypertension, blood pressure, sympathetic nerve activity
Introduction

Elevated dietary salt intake does not invariably increase arterial blood pressure (ABP) but does contribute to the development of hypertension or severity of hypertension in salt-sensitive individuals and experimental models. Compelling data in several models indicates that dietary salt acts centrally with other factors to increase sympathetic nerve activity (SNA) and peripheral resistance \(^{1-3}\). Moreover, dietary salt potentiates the sympathetic and/or pressor responses to stress \(^{4,5}\), hyperinsulinemia \(^{6}\), and activation of somatic afferents \(^{7,8}\). 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 \(^{7,9-11}\).

Elevated dietary salt intake causes widespread changes in neurohumoral profiles including suppression of the peripheral renin-angiotensin system \(^{12}\) and increases in plasma sodium concentration or osmolality \(^{13-15}\). One of the major sites where the central nervous system detects such changes in neurohumoral stimuli is the forebrain lamina terminalis (LT) \(^{16,17}\). 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 lamina terminalis (OVLT). The latter two structures lack a complete blood-brain barrier and thereby are responsive to a number of circulating factors. LT lesions severely disrupt physiological responses to a number of neurohumoral stimuli including osmolality and circulating Ang II \(^{16,18-21}\). Interestingly, lesions of the anteroventral third ventricle region (AV3V) which
encompasses the LT prevent the development or reverse hypertension in Dahl salt-sensitive 22, DOCA-salt 23, Grollman 24,25, and Goldblatt 25,26 hypertensive rats. Although limited data exists, these models show exaggerated cardiovascular responses to injection of L-glutamate in the RVLM 27,28. Collectively, these observations suggest the responsiveness of RVLM sympathetic-regulatory neurons can be modulated by the forebrain lamina terminalis. 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.
**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 (200-250g, Charles River Laboratories) were housed in a temperature-controlled room (22±1°C) with a 12:12 h light-dark cycle (lights on 7AM-7PM). 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.

**Lesion of the Lamina Terminalis.** 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 in OD, AM Systems) angled 8° from the midsagittal plane was lowered into the ventral LT using coordinates in reference to Bregma: 0.0-0.5 mm rostral, 1.0 mm lateral, 8.0 mm ventral to dura. DC current (100 or 500 µA) was applied for 30 s. Electrode tip size and current intensity was varied to produce small (OVLT) versus large (ventral LT) lesions, respectively. Sham control rats consisted of two groups: 1) identical procedures except no current was applied, or 2) lesions were placed lateral to the ventral LT by applying DC current (500 µA, 30 s) using identical coordinates except the electrode was parallel to the midsagittal plane. SFO lesions were produced by applying DC current (500 µA, 30 s) to a tungsten electrode (250 µm tip) angled 8° from the midsagittal plane at 2 different sites in reference to Bregma: 0.8 vs 1.1 mm caudal, 0.7 vs 0.7 mm lateral, 5.2 vs 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 10% sucrose solution until water and food intakes returned to pre-lesion
levels (~2-5 days).

**Experimental Design.** Rats were fed normal chow and water ≥7 days and then
randomly assigned to drink water or 0.9% NaCl solution for 14-15 days. Food and fluid
intake were monitored daily. Then, animals were anesthetized with a mixture of
urethane/chloralose and prepared for renal SNA recordings and RVLM microinjections
as described elsewhere 9, 10 (see http://hyper.ahajournal.org). Experiment 1 – rats with
and without chronic ventral LT lesion received a unilateral injection of L-glutamate (0.1,
1, and 3 nmol) into the RVLM in a randomized manner with >5 min between injections.
GABA (0.03, 0.1, and 10 nmol) was injected on the contralateral side. Experiment 2 –
rats with and without chronic ventral LT lesion received a unilateral injection of AngII
(0.6 and 6 pmol) into the RVLM. One dose was tested per side, and 6 pmol was injected
ipsilateral to the SNA recording. Control groups in Experiments 1 and 2 were either
sham lesioned or received lesions placed lateral to the midline. Experiment 3 – rats
drinking water or 0.9% NaCl for 14-15 days received an acute ventral LT lesion ~60 min
before microinjections of L-glutamate and GABA. Experiment 4 – rats with and without
chronic SFO lesions received injections of L-glutamate and GABA. For experiments 1,
2, and 4, RVLM injections were performed at 24-26 days after the initial lesion. Plasma
electrolytes, hematocrit, plasma protein, and blood volume were measured in a subset
of animals as described previously 9.
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 1PM or 1AM, 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, East Windsor, NJ). Plasma osmolality was determined in duplicate by freezing point depression (Advanced Instruments, Norwood, MA). Food and fluid intake measurements were monitored daily except in a subset of animals where daytime and nighttime measurements were performed.

Statistical Analysis. All 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 response was compared to a 30-second baseline segment immediately before the injection. Renal SNA was only analyzed when injections were performed ipsilateral to the nerve recording. All data were analyzed by a 1- or 2-way ANOVA with repeated measures when appropriate (dose factor). All post hoc tests were performed with independent or paired t-tests with a layered Bonferonni 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 to 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 previously reported 9-11, RVLM injection of L-glutamate produced significantly greater renal SNA and ABP responses in control rats drinking 0.9% NaCl versus water (Figure 2). In marked contrast, these differences were completely absent in rats with ventral LT lesions. In fact, the sympathoexcitatory responses at every dose of L-glutamate were not different between ventral LT-lesioned rats drinking 0.9% NaCl versus control rats drinking water. As consequence of the experiments, an additional set of animals had lesions that missed the ventral LT (rostral or lateral), and injection of L-glutamate into the RVLM still produced significantly greater increases in renal SNA (1 nmol: 150±9 vs 105±7%, P<0.05) and mean ABP (1 nmol: 47±2 vs 27±3 mmHg, P<0.05) of rats ingesting 0.9% NaCl (n=4) versus water (n=3), respectively (data not shown for 0.1 and 3 nmol). 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 previously reported\textsuperscript{9}, rats drinking 0.9% NaCl versus water displayed significantly greater depressor responses to every dose of GABA. Again, these differences were absent in rats with lesions restricted to the ventral LT (Figure 3) but present in rats with lesions that missed the ventral LT (data not shown).

In a second group of animals, we examined whether chronic lesion of the ventral LT prevented the enhanced sympathoexcitatory responses to AngII in the RVLM during increased dietary salt. RVLM injection of AngII produced significantly greater increases in renal SNA and mean ABP of control rats drinking 0.9% NaCl versus water (Figure 4). In marked contrast, rats with lesion of the ventral LT and drinking 0.9% NaCl showed similar changes in renal SNA and ABP to RVLM injection of AngII versus control or ventral LT lesioned-rats drinking water. Again, injection of AngII (6 pmol) into the RVLM of rats drinking 0.9% NaCl but with lesions that missed the ventral LT (n=4) still produced enhanced renal SNA (66±8%) and mean ABP (19±2mmHg). Histology for ventral LT lesions is illustrated in Figure S1 (see http://hyper.ahajournals.org).

**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 vs -5±2 mmHg) and renal SNA (-31±10 vs -31±9%); however, both variables returned to baseline values within 30 min. Histology is illustrated in Figure S2 (see http://hyper.ahajournals.org). 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 (Figure 6A). 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 (see http://hyper.ahajournals.org). 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 experiments were centered in the RVLM as defined previously 9,10 (Figure S4, see http://hyper.ahajournals.org).

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 1). However, control and lesioned rats drinking 0.9% NaCl displayed significant increases in plasma sodium concentration and osmolality at night. All 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, see http://hyper.ahajournals.org). However, there were no differences in baseline mean ABP, heart rate, renal SNA, or plasma and/or blood volume (Tables S2 and S3, see http://hyper.ahajournals.org).
Discussion

Increased dietary salt enhances sympathetic and cardiovascular responses evoked and/or mediated by RVLM sympathetic-regulatory neurons. 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 unknown previously. The present findings provide several new key observations: 1) chronic lesion of the ventral LT and OVLT prevents the enhanced cardiovascular responses to RVLM stimulation during increased dietary salt, 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. Given the widespread neurohumoral changes associated with increased dietary salt intake, we hypothesized that forebrain LT neurons indirectly detected 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 lesion did not affect these responses. These findings cannot be explained by differences in salt intake as 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 responsiveness of RVLM neurons is likely not attributed to some chronic adaptation as a result of the lesion per se since the sympathoexcitatory and sympathoinhibitory responses were not different between control versus 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, such lesions produce damage across the entire forebrain LT. However, discrete lesion of the OVLT in dogs disrupts thirst and vasopressin secretion stimulated by elevated plasma sodium concentration and circulating AngII whereas lesion of the SFO in rats and dogs blunts thirst stimulated by AngII. Studies in sheep indicate that combined ablation of several LT structures are needed to attenuate such responses. Therefore, the factor(s) by which dietary salt activates ventral LT neurons to alter the responsiveness of RVLM neurons remains unclear.

The downstream pathways and cellular mechanisms that mediate the enhanced responsiveness of RVLM neurons during increased dietary salt is not known. The forebrain LT densely innervates many hypothalamic nuclei including the hypothalamic paraventricular nucleus. Previous studies have demonstrated that neurons in the hypothalamic paraventricular nucleus with descending projections are excited by
hyperosmolality\textsuperscript{30}. Anatomical and functional data indicate these neurons utilize AngII as a neurotransmitter\textsuperscript{31,32}, and we recently reported a greater AT1 receptor activation in the RVLM of rats on a high salt diet\textsuperscript{10}. Yet, available evidence suggests 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\textsuperscript{9}. Second, rats drinking 0.9% NaCl for 14 days still exhibit enhanced responses when water was returned for 1 day\textsuperscript{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 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 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 dietary salt elevates plasma sodium concentration or osmolality in rats\textsuperscript{13,15} and humans\textsuperscript{14}. Small increases in osmolality of 1-2% stimulate drinking in mammals\textsuperscript{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 alter the responsiveness of RVLM neurons. In fact, an enhanced responsiveness of RVLM neurons has been reported in 48-h water-deprived rats\textsuperscript{35}. Such a model would suggest chronic changes in plasma osmolality produced by dietary salt intake must reach the threshold of osmosensory neurons. Currently, there is 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 to osmotic perturbations or other circulating factors.

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 \(^{16}\). 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 \(^{16}\). 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 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 one of two ways: 1) a direct sodium-driven increase in SNA and ABP \(^1,^2\), or 2) a chronic increased gain of sympathetic-regulatory networks \(^7,^{9-11}\). The increased gain of RVLM sympathetic neurons has physiological significance as increased dietary salt enhances the sympathoexcitatory responses to insulin \(^6\) and stimulation of somatic afferents \(^7,^8\) – responses that depend on RVLM neurotransmission \(^36,^37\). 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\textsuperscript{22}, DOCA-salt\textsuperscript{23}, Grollman\textsuperscript{24,25}, and Goldblatt\textsuperscript{25,26} hypertensive rats. The available data suggests that these models show exaggerated responses to injection of L-glutamate in the RVLM\textsuperscript{27,28}. In marked contrast, AV3V lesions do not affect hypertension in the SHR\textsuperscript{38}, and SHR do not display enhanced responses to L-glutamate injection in the RVLM\textsuperscript{39}. Altogether, these observations raise the possibility that AV3V lesions attenuate neurogenic hypertension, in part, by preventing an enhanced excitability of RVLM sympathetic neurons.
Acknowledgements

None.

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 Heart Lung and Blood Institute Grant HL090826 (S.D.S.).

Disclosures

None.


Figure Legends

Figure 1. Schematic drawings of ventral LT lesions for rats drinking (A) water or (B) 0.9% NaCl. The lesion boundary is outlined in black; control animals had no lesion or received a directed misplaced lesion (grey). (C) Digital photomicrographs of two rostral-caudal levels of the lamina terminalis for control (i,ii) and ventral LT lesioned (iii, iv) rats. Scale bar = 500 µm. Coordinates are in reference to Bregma. 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 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 L-glutamate. *P<0.05 Control+water vs Control+Salt, †P<0.05 Control+Salt vs Lesion+Salt

Figure 3. Peak change in mean ABP during RVLM injection of GABA in rats with chronic lesion of the ventral LT. (B) Individual examples of ABP and mean ABP during injection of 0.1 nmol GABA. *P<0.05 Control+water vs Control+Salt, †P<0.05 Control+Salt vs Lesion+Salt

Figure 4. (A) Peak change in mean ABP and renal SNA during RVLM injection of AngII 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 AngII. *P<0.05 Control+water vs Control+Salt, †P<0.05 Control+Salt vs 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 vs Lesion+Salt

**Figure 6.** Peak change in mean ABP and renal SNA during RVLM injection of L-glutamate in rats drinking water or 0.9% NaCl for 14 days that received (A) an acute ventral LT lesion or (B) chronic SFO lesion. *P<0.05 water vs salt for both groups. There were no differences between control vs lesion group within same diet
Table 1. 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 (mOsm/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</td>
<td>136.3±0.6</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(11)</td>
</tr>
<tr>
<td>Control+Salt</td>
<td>134.5±0.5</td>
<td>138.4±0.6</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(13)</td>
</tr>
<tr>
<td>Lesion+Water</td>
<td>135.0±1.0</td>
<td>136.0±1.0</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(8)</td>
</tr>
<tr>
<td>Lesion+Salt</td>
<td>136.1±0.9</td>
<td>139.8±0.8</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(8)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ( ) indicates number of animals. *Significant difference between water vs 0.9% NaCl within control or lesion group (P<0.05)
Figure 2

A. Δ Mean ABP (mmHg) and Δ Renal SNA (%) for different treatments: Water (n=8), Salt (n=7), Lesion+Water (n=10), and Lesion+Salt (n=6). 

B. Graphs showing Mean ABP (mmHg) and Renal SNA (%) for Control and Lesion conditions with Water and Salt treatments.
Figure 3

A  ○ Water (n=8) ▼ Lesion+Water (n=10)
    ● Salt (n=7) ▼ Lesion+Salt (n=6)

Mean ABP (mmHg)

Δ Mean ABP (mmHg)

0          0.03  0.1  10

-40        -20

ABP (mmHg)

175

100

50

0

B

Control

Water

Salt

Lesion

Water

Salt

20 s

Mean ABP (mmHg)

175

100

150

100

Water

Salt

Lesion

Water

Salt

20 s
Figure 4

A  

Δ Mean ABP (mmHg)

<table>
<thead>
<tr>
<th>AngII (pmol)</th>
<th>Water (n=8)</th>
<th>Lesion+Water (n=4)</th>
<th>Salt (n=8)</th>
<th>Lesion+Salt (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>*</td>
<td>†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>*</td>
<td>†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Δ Renal SNA (%)

<table>
<thead>
<tr>
<th>AngII (pmol)</th>
<th>Renal SNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

B

Control

<table>
<thead>
<tr>
<th>Water</th>
<th>Salt</th>
<th>Water</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>100</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>Mean ABP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>∫ Renal SNA (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal SNA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

20 s
Figure 5

A

B

C

D

Δ Mean ABP (mmHg)

Δ SNA (%)

Δ Mean ABP (mmHg)

L-Glutamate (nmol)

GABA (nmol)

○ Water (n=8)  ● Salt (n=8)  ▽ Lesion+Water (n=3)  ▼ Lesion+Salt (n=3)

LV

DBB

OVLT

MnPO

AC

3V

OC

Water (n=8) Lesion+Water (n=3) Salt (n=8) Lesion+Salt (n=3)
Figure 6

A

○ Water (n=8)  ● Salt (n=8)

B

▽ Lesion+Water (n=3)  ▼ Lesion+Salt (n=3)

Δ Renal SNA (mmHg)

L-Glutamate (nmol)

Δ Renal SNA (%)

L-Glutamate (nmol)

* Significant difference

** Highly significant difference

*** Extremely highly significant difference

Water (n=8)  Salt (n=8)  Lesion+Water (n=3)  Lesion+Salt (n=3)