**Brain regions influenced by the lateral parabrachial nucleus
or angiotensin II-induced water intake**

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LIST OF ABBREVIATIONS

AngII – angiotensin II;
BST – bed nucleus of the lamina terminalis;
CeAm – central nucleus of the amygdala;
CVO – circumventricular organ
GABA – γ -aminobutyric acid;
i.p. – intraperitoneal;
LPBN – lateral parabrachial nucleus;
MnPO – median preoptic nucleus;
NTS – nucleus of the solitary tract;
OVLT – vascular organ of the lamina terminalis;
PVN – paraventricular nucleus of the hypothalamus;
SC – subcutaneous;
SFO – subfornical organ;
SON – supraoptic nucleus;

ABSTRACT

This study examined which brain regions are influenced by an inhibitory lateral parabrachial nucleus (LPBN) mechanism that affects water intake. Controls and rats with bilateral LPBN-lesions were administered AngII (0.5 mg/kg SC), drinking responses measured, and brains processed for Fos-immunohistochemistry. A separate group of LPBN-lesioned and non-lesioned animals were denied water for 90 min prior to perfusion to remove any confounding factor of water intake. LPBN-lesioned rats drank a cumulative volume of 9 mL compared with < 4 mL by controls ($p < 0.01$). Compared with sham-lesioned animals, Fos expression was attenuated in overdrinking LPBN-lesioned rats in the median preoptic nucleus (MnPO), paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus (SON) ($p < 0.001$), bed nucleus of the stria terminalis and central nucleus of the amygdala ($p < 0.01$). In LPBN-lesioned rats that did not drink, greater numbers of activated neurons were detected in the PVN ($p < 0.001$), SON ($p < 0.01$), MnPO, NTS and area postrema ($p < 0.05$) in response to SC AngII, compared with non-lesioned rats. These data suggest that the direct effects of LPBN lesions caused an increase in AngII-induced water intake and in rats that did not drink an increase in Fos expression, while indirect secondary effects of LPBN lesions caused a reduction in Fos expression possibly related to excessive ingestion of water. An inhibitory mechanism, likely related to arterial baroreceptor stimulation, relayed by neurons located in the LPBN influences the responses of the MnPO, PVN and SON to increases in peripheral AngII.

Key words: parabrachial nucleus, water intake, Fos-immunohistochemistry, angiotensin II.

1 The lateral parabrachial nucleus (LPBN) exerts an inhibitory influence on water
2 ingestion (Ohman and Johnson, 1986, Edwards and Johnson, 1991, Menani et al., 1995)
3 (Ohman and Johnson, 1989). Rats with bilateral LPBN lesions show excessive water
4 drinking in response to a number of dipsogenic treatments that include peripheral and
5 central administration of angiotensin II (AngII) (Ohman and Johnson, 1986, Ohman and
6 Johnson, 1989, Edwards and Johnson, 1991, Menani and Johnson, 1995) and
7 subcutaneous (SC) injection of the β -adrenergic agonist, isoproterenol (Ohman and
8 Johnson, 1986). In contrast, water intake following 24 h water deprivation was
9 unchanged in rats with bilateral LPBN lesions compared with sham-lesion control rats
10 (Menani et al., 1995) as was the drinking response to SC administered hypertonic saline
11 (Ohman and Johnson, 1986, Menani and Johnson, 1995), polyethylene glycol (Ohman
12 and Johnson, 1986), or the cholinergic receptor agonist, carbachol injected
13 intracerebroventricularly (Ohman and Johnson, 1989). These data suggest that neurons
14 within the LPBN exhibit a selective inhibitory influence on water intake associated with
15 increased activity of the renin-angiotensin system.

16 Neuroanatomical studies have identified a major projection arising from the
17 nucleus of the solitary tract (NTS) that innervates the LPBN to relay neural information
18 to other brain regions (Fulwiler and Saper, 1984, Jhamandas and Harris, 1992, Krukoff
19 et al., 1993, Jia et al., 1994). Furthermore, the NTS and the LPBN are thought to play a
20 crucial role in the inhibition of water intake following increases in arterial blood
21 pressure and extracellular volume, and conversely, stimulation of water intake in
22 response to hypovolemia and hypotension (Ohman and Johnson, 1986, Ohman and
23 Johnson, 1989, Edwards and Johnson, 1991, Menani and Johnson, 1995). Additionally,
24 several neuroanatomical studies have reported neural connections between the LPBN
25 and regions that respond to changes in body fluid balance including the area postrema in
26 the hindbrain, and median preoptic nucleus (MnPO), subfornical organ (SFO) and
27 hypothalamic paraventricular nucleus (PVN) in the forebrain (Herbert et al., 1990,
28 Moga et al., 1990). These forebrain regions play important roles in processing humoral
29 as well as neural signals that indicate changes in hydration status. The SFO, a
30 circumventricular organ (CVO) located at the dorsal end of the lamina terminalis, is of
31 particular relevance to AngII induced thirst because it is there that circulating AngII acts
32 on AT₁ receptors to initiate water drinking in the rat (Simpson et al. 1978). The SFO, as
33 well as the other CVO within the lamina terminalis, the vascular organ of the lamina

terminalis (OVLT), has a deficient blood brain barrier that facilitates blood-borne AngII having actions on neurons located therein. The OVLT is also of relevance to fluid intake because osmoreceptors that mediate thirst and vasopressin release are located there (McKinley et al. 1983; Thrasher et al. 1983; Oldfield et al. 1994; Bourque et al.). Therefore, neural connections from the LPBN that relay signals that ascend from the area postrema and NTS to the lamina terminalis may have a crucial role in regulating fluid intake.

There is considerable evidence to support the proposal that increased arterial pressure, which occurs with systemic administration of AngII, has an inhibitory influence on the dipsogenic action of this peptide (Robinson and Evered 1987; Evered 1992). In other words, the pressor effect of AngII may be “putting the brake” on its dipsogenic effect and it is possible that this inhibitory influence of arterial pressure may be relayed to the forebrain via the LPBN. In view of these considerations, our aims were to: (i) confirm an inhibitory influence for the LPBN in AngII-dependent fluid intake, and (ii) determine, by means of Fos immunohistochemistry, which brain regions are influenced by a putative inhibitory pathway arising from or relayed through the LPBN, and which therefore, are likely to play a role in limiting water intake in response to peripherally administered AngII. Because LPBN ablation causes a larger drinking response to SC AngII, we also aimed to: (iii) determine whether or not changes in Fos expression were due primarily to the LPBN lesion or secondary to influences of increased water intake. Therefore, we also examined whether there were differences in Fos expression in response to SC AngII in several brain regions of rats with bilateral LPBN lesions that were allowed to drink water compared with those not given access to water.

EXPERIMENTAL PROCEDURES

Surgery and animal preparation

The experimental procedures and protocols were approved by the Animal Ethics committee of the Howard Florey Institute which adheres to the Australian National Health and Medical Research Council’s Code of Practice for the Care and Use of Animals. Experiments were performed in 24 male Sprague Dawley rats weighing between 250 g and 300 g. The rats were housed in individual cages with *ad libitum* access to both food (at all times) and water (unless otherwise stated). Prior to

1 experiments, rats were surgically prepared with lesions in the LPBN. They were
2 anaesthetised with injections of equithesin (3 ml/kg i.p.), the head was placed in a
3 stereotaxic apparatus and a deep incision exposed the skull. The angle of the head was
4 adjusted until lambda and bregma aligned in a horizontal plane. Lesion and sham-lesion
5 coordinates for the LPBN were calculated from the Rat Stereotaxic Atlas (Paxinos and
6 Watson, 1982): 9.3 mm caudal from bregma, 2.0 mm medial and lateral of the
7 midsagittal sinus and 6.5 mm ventral to the dura mater. Bilateral electrolytic lesions
8 were made by passing a 1.0 mA direct current for 20 sec through an electrode that was
9 insulated except at the tip. In contrast, sham-lesions were made by lowering the same
10 electrode to the same lesion coordinates but no current was passed. The electrode was
11 then withdrawn from the brain and the skin sutured. Animals were allowed to recover
12 for approximately 1 week.

13 14 **Experimental Protocol**

15 Two different groups of rats were investigated: rats that were allowed to drink
16 water following SC injection of AngII (*Protocol 1*) and rats in which water was
17 withheld also following SC injection of AngII (*Protocol 2*). Daily water intakes were
18 monitored for 4 days prior to initiating treatment to ensure normal hydration and for 90
19 min prior to the experimental phase.

20 21 **Protocol 1**

22 In the first group (LPBN-lesioned, sham-lesioned, missed lesions and control
23 rats), animals were provided with water in graduated cylinders (accurate to 0.1 ml) that
24 allowed water intake to be measured throughout the experimental phase. Rats were
25 injected with AngII (0.5 mg/kg SC) and water intakes were measured every 15 min for
26 90 min. At this time, animals were deeply anaesthetised with sodium pentobarbitone
27 (100 mg/kg i.p) and perfused transcardially with 50-100 ml of 0.9% saline and 250-300
28 ml of 4% paraformaldehyde dissolved in 0.1 M phosphate buffer, pH 7.2 (PB). Brains
29 were removed and post-fixed for 1 h, placed in 20% sucrose in PB and refrigerated
30 overnight at 4°C before being processed for the immunohistochemical detection of Fos.

31 32 **Protocol 2**

In the second group of LPBN-lesioned rats, water drinking in response to AngII was tested 1 week prior to the investigation of Fos expression. Then, during the experimental phase, these lesioned-rats (and sham non-lesioned rats for a control group) were again administered AngII (0.5 mg/kg SC) but not given access to water and 90 min later deeply anaesthetised and brains perfused as described above.

Histology

All brains were processed for Fos-immunohistochemistry using standard methods. Briefly, coronal sections (40 μ m) were cut on a freezing microtome and placed in PB. Free-floating sections were incubated in 10% normal horse serum at room temperature for 1 h. Sections were incubated in primary antibody, rabbit anti-c-Fos (Ab-5, Oncogene, Cambridge, MA, USA) diluted 1:5000 in a solution of 2% normal horse serum and 0.3% Triton X-100 (Sigma) in PB at room temperature overnight. Sections were washed in PB prior to incubation in the secondary antiserum, biotinylated goat anti-rabbit immunoglobins (1:200, Vector), in PB containing 2% normal horse serum for 1 h. Thereafter, the sections were washed and incubated in avidin-biotin peroxidase complex (1:100, Vector) in PB also for 1 h. Following washes in 0.05 M Tris buffer (pH 7.6), sections were incubated in a solution of 40 mg nickel ammonium sulphate and 50 mg 3-3' diaminobenzidine hydrochloride per 100 ml Tris buffer for 10 min, 15 μ l of 30% hydrogen peroxide was added for a further 6 min. Following final washes, sections were mounted on gelatin coated microscope slides.

Analysis

Rats that were allowed to drink water following AngII infusion included LPBN-lesioned rats (n = 4) and control groups with access to water included: animals with brain lesions identified as outside the LPBN region (missed-lesion) (n = 4), animals with bilateral sham-lesions into the LPBN (n = 4) and non-lesioned rats (n = 4). A two-way analysis of variance with repeated measures on one variable (cumulative water intake) and independent measures on one variable (treatment/control) were used for comparisons in water intake with corrected Fisher's least significant difference test. Rats in which water was withheld following AngII infusion included a separate group of LPBN-lesioned rats (n = 3) and non-lesioned rats (n = 5) all denied access to water during the 90 min experimental phase only.

Immunohistochemically processed brains were used to identify rats with bilateral LPBN lesions. LPBN-lesioned rats were designated as those rats with electrolytic ablation of brain tissue that included the central, dorsal and external LPB subnuclei at a level mid to mid-caudal through the LPBN (Fig. 1) as described previously by others (Ohman and Johnson, 1986, Edwards and Johnson, 1991). Bright field illumination was used to assess sections that exhibited Fos-immunoreactivity as detected by black stained nuclei. Brain sites examined were the OVLT, MnPO, SFO, bed nucleus of the stria terminalis (BST), PVN, supraoptic nucleus (SON), central nucleus of the amygdala (CeAm) in the forebrain and the NTS and area postrema in the hindbrain. Counts were made of Fos-labelled cells in four sections per animal for each brain site. The counts were made by an observer who was not aware of which group the sections came from (blinded analysis). Statistical evaluation of Fos counts was performed by one-way analysis of variance and Bonferroni corrected post-hoc t-tests. Results are expressed as mean $\pm$ standard error of the mean and with the exception of midline structures represent a unilateral brain region. Values were considered significant when $p < 0.05$.

RESULTS

Effect of bilateral LPBN lesions on water intake induced by SC injection of AngII

Control groups including non-lesioned rats, rats with lesions identified as outside the LPBN (missed-lesion) and sham-lesioned control rats, with access to water, drank approximately 3 ml of water by 30 min after administration of SC AngII (Fig. 2). These control groups drank a cumulative volume of less than 4 mL by 45 min after SC AngII. Rats with bilateral ablation of the LPBN consumed approximately 5 ml of water, within 15 min after SC injection of AngII, significantly more than control groups ($p < 0.05$) (Fig. 2). The LPBN-lesion group consumed a cumulative volume of more than 7 mL by 30 min after treatment ($p < 0.01$ compared with control groups) and water intake continued to increase to a cumulative volume of approximately 9 mL at 60 min (Fig. 2). This volume remained unchanged for a further 30 min, at which point in time, 90 min after treatment, rats were killed and brains perfused with fixative in preparation for immunohistochemistry.

Effect of bilateral LPBN lesions on Fos-immunoreactivity in response to SC injection of AngII in rats with access to water

In LPBN-lesioned rats that overdrank, neuronal activation was significantly attenuated following SC injection of AngII in the MnPO ($F_{1,9}=58$), PVN ($F_{1,10}=53$) and SON ($F_{1,11}=27$) (Fig. 3; $p < 0.001$), BST ($F_{1,10}=21$) and CeAm ($F_{1,10}=16$) ($p < 0.01$) compared with sham-lesioned animals administered SC AngII only. By contrast, no significant difference was detected in Fos expression in the OVLT, SFO, NTS or area postrema between these two groups (Fig. 4). Similar results were observed between LPBN-lesioned rats and other control groups all with access to water following SC AngII. Specifically, Fos expression was attenuated in the MnPO ($F_{1,9}=37$), PVN ($F_{1,10}=53$), SON ($F_{1,11}=24$) ($p < 0.001$), BST ($F_{1,10}=15$) ($p < 0.01$) and CeAm ($F_{1,10}=7$) ($p < 0.05$) in the LPBN-lesion group compared with non-lesion control rats (Fig. 4). Less neuronal activation was also noted in the MnPO ($F_{1,9}=37$) (PVN ($F_{1,10}=43$), $p < 0.001$), SON ($F_{1,11}=19$), BST ($F_{1,10}=14$) and CeAm ($F_{1,10}=15$) ($p < 0.01$) in rats with bilateral LPBN lesions compared with animals with lesions identified as outside the LPBN region (missed-lesions). Again, there was no significant difference in Fos labelled cells in the OVLT, SFO, NTS and area postrema between rats with bilateral LPBN lesions and any of the control groups with access to water (Fig. 4).

Effect of bilateral LPBN lesions on Fos expression in response to SC AngII in rats not allowed drinking water

When the drinking response to AngII was examined in these LPBN-lesioned animals one week prior to undergoing the experimental phase when these animals were not given access to water for drinking, SC AngII caused rats to consume 5.9 ± 1.5 mL of water after 15 min that was comparable with water consumed by LPBN-lesioned rats in protocol 1. Cumulative water intake was more than 7 mL by 30 min after AngII was administered and continued to increase to 10.1 ± 0.8 mL at 60 min that was also comparable with approximately 9 mL of water consumed by LPBN-lesioned rats in protocol 1. These results suggest that the bilateral lesions of the LPBN were successful.

A week later these rats were again administered AngII, however during this experimental phase; they were not allowed to drink any water. Greater numbers of Fos-immunoreactive neurons were detected in LPBN-lesioned rats in the PVN ($F_{1,11}=51$) ($p < 0.001$), SON ($F_{1,12}=17$) ($p < 0.01$), MnPO ($F_{1,12}=5$) (Fig. 5), NTS ($F_{1,12}=7$) and area

1 postrema ($F_{1,10}=5$) ($p < 0.05$) compared with non-lesioned rats following injection of SC
2 AngII when rats did not drink. By contrast, no significant difference was detected in Fos
3 expression in the OVLT, SFO, BST and CeAm between these two groups (Fig. 6).

DISCUSSION

4
5
6 Rats with bilateral ablation of the LPBN ingested larger volumes of water in
7 response to peripherally administered AngII in comparison with non-lesioned and sham
8 lesioned animals. This result confirms several earlier reports that ablation or disabling
9 of the LPBN causes increased water intake and supports the conclusion that the LPBN
10 plays an inhibitory role in water ingestion (Ohman and Johnson, 1986, Edwards and
11 Johnson, 1991, Menani et al., 1995). Therefore, investigation of Fos expression in
12 LPBN-lesioned rats may provide insights into which regions in the brain may be
13 influenced by LPBN inputs to limit the drinking response to AngII.

14 We allowed 90 min for the development of Fos expression, (an indicator of
15 neuronal activation), in response to SC injections of AngII. During this 90 min, the
16 LPBN-lesioned rats ingested significant volumes of water, and this drinking response
17 may influence the expression of Fos as well as the action of circulating AngII on the
18 SFO. For the OVLT, SFO, NTS and area postrema, the number of neurons expressing
19 Fos in response to AngII was similar for each corresponding brain region across the four
20 groups of rats that had access to water to drink during experiments. This result indicates
21 that the hormonal and neural signals from the periphery that reach the CNS were similar
22 across groups, and not affected by LPBN lesions. However, there was significant
23 attenuation of neuronal activity in the MnPO, PVN, SON, BST and CeAm in response
24 to SC AngII in rats with ablation of the LPBN compared with controls. Additionally,
25 less Fos was observed within all regions of the PVN with very few neurons activated in
26 both the dorsal parvocellular and posterior magnocellular and limited activation in the
27 medial parvocellular subnuclei (Fig. 3C, D). This decrease in neuronal activation was
28 unexpected in LPBN-lesioned rats because neurons located in the MnPO, PVN, SON,
29 BST and CeAm have been associated with conditions where intake of water increases,
30 and removal of an inhibitory pathway could be predicted to increase neuronal
31 activation. To this end, we have previously shown that blocking serotonergic
32 mechanisms in the LPBN combined with SC isoproterenol causes a robust salt appetite
33 and potentiates Fos expression in these brain regions (Davern and McKinley, 2010).

1 It is possible that neurons in these brain regions may normally be selectively
2 influenced by an excitatory ascending LPBN mechanism controlling thirst, and that
3 ablation of the LPBN removes such excitation. This notion is supported by the known
4 neural connections of the LPBN with neurons located in the OVLT, MnPO, SFO, BST,
5 PVN, SON, CeAm and NTS (Fulwiler and Saper, 1984, Herbert et al., 1990, Moga et
6 al., 1990, Gu and Ju, 1995). However, in the light of our results in LPBN-lesioned rats
7 that were prevented from drinking, a more likely explanation of the decreased levels of
8 neuronal activation in these brain regions is that inhibitory influences arising secondary
9 to water intake override excitatory drive in response to increased plasma levels of
10 AngII. It is known that withholding water for drinking purposes and water deprivation
11 followed by rehydration with water in rats causes differences in Fos expression in a
12 number of brain regions. Dehydration for approximately 48 hours increases Fos labelled
13 neurons in the MnPO, PVN, SON, OVLT, NTS and area postrema and following 2
14 hours of rehydration decreases Fos in the PVN and SON but further increases levels in
15 the NTS and area postrema (Ji et al., 2005, Gottlieb et al., 2006, Ji et al., 2007).
16 Therefore, it was necessary to further investigate whether the results observed in our
17 study were an effect of LPBN ablation or the result of secondary effects induced by
18 water ingestion.

19 To further elucidate the role of the LPBN in the neuronal activation in response
20 to AngII, LPBN-lesioned rats were injected with AngII but denied access to water. In
21 contrast to LPBN-lesioned rats allowed water to drink in response to AngII, LPBN-
22 lesioned rats administered SC AngII, but denied water to drink, showed selective
23 increases in the number of neurons expressing Fos in three forebrain regions compared
24 with controls. Notably, greater neuronal excitation was identified in the MnPO, PVN
25 and SON suggesting that normally, neurons located within these brain regions are
26 possible targets for inhibitory inputs arising from the LPBN in conditions of increased
27 arterial pressure. These forebrain regions are well recognised for their roles in
28 cardiovascular regulation and fluid balance (Buggy and Fisher, 1976, Swanson and
29 Sawchenko, 1983, Bellin et al., 1987). One likely possibility is that an inhibitory
30 pathway via the LPBN, maybe GABAergic, influences these forebrain regions in
31 response to arterial baroreceptor stimulation induced by increased BP resulting from
32 increased levels of peripheral AngII. Direct projections arise from the LPBN and
33 innervate neurons located in the MnPO and PVN (Fulwiler and Saper, 1984). However,
34 there is little verification of direct LPBN inputs to the SON; therefore, an inhibitory

LPBN mechanism may influence neurons located in the SON possibly via the MnPO (Oldfield et al., 1991a, Oldfield et al., 1991b).

If it is assumed that the reduced activity of neurons in the MnPO, PVN and SON in LPBN-lesioned rats that drank, was a consequence of the greater drinking response to AngII, it follows that these effects were not mediated by inhibitory neural signals from the LPBN. The SFO and the MnPO are recognised for their roles in regulating thirst in response to increased levels of circulating AngII (Mangiapane et al., 1983, Cunningham et al., 1992, Fitzsimons, 1998, McKinley et al., 2001) and it is well known that these brain regions influence magnocellular regions in the PVN and SON to mediate vasopressin release (Mangiapane et al., 1983, Cunningham et al., 1992). When dehydrated animals drink water, there is a rapid decrease in vasopressin secretion and this effect is mediated by an oropharyngeal reflex that is probably independent of changes in arterial pressure (Blair-West et al., 1985, Thrasher et al., 1987). LPBN-lesioned rats drank significantly more water by 15 min after SC AngII, than did non-lesioned rats. Therefore, the marked decrease in Fos expression detected in LPBN-lesioned rats given access to water compared with all other AngII treated rats that drank water was likely to reflect a rapid decrease in activity and vasopressin secretion of posterior magnocellular neurons located in the PVN and SON and signalled via reduced activity from neural inputs coming from the MnPO (Fig. 3).

There is considerable evidence to support the proposal that increased arterial pressure, following systemic administration of AngII, has an inhibitory influence (mediated by arterial baroreceptors) on the dipsogenic action of this peptide (Robinson and Evered, 1987). Lesions of the LPBN or the area postrema have been shown to increase drinking in response to increased levels of systemic AngII and it has been shown that these lesions do not reduce the pressor effect that arises with administering AngII (Ohman and Johnson, 1986) (Edwards et al. 1982). In the present study, increased Fos expression in both the NTS and area postrema of LPBN-lesioned rats administered AngII occurred only when animals did not drink. It is not clear why a lack of drinking influences the Fos expression in these hindbrain regions.

The most important finding from this study was the identification of an inhibitory mechanism, likely related to arterial baroreceptor stimulation, being relayed by neurons located in the LPBN to influence neural activity in the MnPO, PVN and SON responding to increased levels of circulating AngII. One possibility is that an ascending inhibitory pathway via the LPBN, maybe GABAergic, influences these

forebrain regions as a result of arterial baroreceptor stimulation caused by the pressor response to AngII. It is likely that this mechanism involves direct projections from the LPBN that innervate neurons located in the MnPO and the PVN. However, neurons located in the SON are probably indirectly influenced by the LPBN via projections from the MnPO. Our data confirm an inhibitory influence for the LPBN on AngII dependent water intake, they also show that decreased activity of neurons occurs in the OVLT, SFO, BST and CeAm in response to water ingestion. This decreased neural activity is not due to altered neural signals relayed through the LPBN.

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FIGURE LEGENDS

Fig. 1 – Schematics indicating the extent of LPBN lesions in the experimental group of rats as depicted by dashed lines. LPBN-lesioned rats were designated as those rats with electrolytic ablation of brain tissue that included the central, dorsal and external LPB subnuclei at a level mid (Bregma -9.3mm) to mid-caudal (Bregma -9.8mm) through the LPBN. Abbreviations: MPBN, medial parabrachial nucleus; SCP, superior cerebellar peduncle.

Fig. 2 – Effect of bilateral LPBN lesions on cumulative water intake induced by subcutaneous injection of Angiotensin II. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ v control.

Fig. 3 - Photomicrographs of coronal sections through the median preoptic nucleus (MnPO) (A, B), paraventricular nucleus of the hypothalamus (PVN) (C, D) and supraoptic nucleus (SON) (E, F) of rats that were all administered subcutaneous Angiotensin II (0.5mg/kg), following no surgery (A, C, E) or recovery following electrolytic bilateral lesion of the LPBN (B, D, F). Markedly less neurons, identified by Fos-immunoreactivity (black dots), appear activated in rats pretreated with bilateral LPBN lesions (B, D, F) compared with more activated cells in control rats (A, C, E). Abbreviations: dp, dorsal parvocellular PVN; mp, medial parvocellular PVN; pm, posterior magnocellular PVN; pv, periventricular PVN; 3V, third ventricle. Magnification bar=100 μ m.

Fig. 4 – Mean number of nuclei detected as Fos-positive following an injection of SC AngII in rats allowed access to drinking water (n=4 per group). Control groups include: No Lesion, indicating rats administered SC AngII only; Sham, indicating rats where an

1 electrode was lowered to the lesion coordinates but no current was passed; Miss, rats
2 with lesions made outside the LPBN. The experimental group underwent bilateral
3 LPBN lesions. Abbreviations: SC AngII, subcutaneous injection of angiotensin II;
4 MnPO, median preoptic nucleus; PVN, paraventricular nucleus of the hypothalamus;
5 SON, supraoptic nucleus; BST, bed nucleus of the stria terminalis; Ce, central nucleus
6 of the amygdala. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with all controls.

7 Fig. 5 - Photomicrographs of coronal sections through the median preoptic nucleus
8 (MnPO) (A, B), paraventricular nucleus of the hypothalamus (PVN) (C, D) and
9 supraoptic nucleus (SON) (E, F) of rats that were all administered Angiotensin II (0.5
10 mg/kg) and were not permitted to drink, following no surgery (A, C, E) or recovery
11 following electrolytic bilateral lesion of the LPBN (B, D, F). A markedly greater
12 number of neurons, identified by Fos-immunoreactivity (black dots), appear activated
13 in rats pretreated with bilateral LPBN lesions (B, D, F) compared with less activated
14 cells in control rats (A, C, E). Abbreviations: dp, dorsal parvocellular PVN; mp, medial
15 parvocellular PVN; pm, posterior magnocellular PVN; pv, periventricular PVN.
16 Magnification bar=100 μ m.

17 Fig. 6 – Mean number of nuclei detected as Fos-positive following an injection of SC
18 AngII in rats denied access to drinking water. The control group, No Lesion, was
19 administered AngII only (n=5) and the experimental group underwent bilateral LPBN
20 lesions (n=3). Abbreviations: SC AngII, subcutaneous injection of angiotensin II;
21 MnPO, median preoptic nucleus; PVN, paraventricular nucleus of the hypothalamus;
22 SON, supraoptic nucleus; NTS, nucleus of the solitary tract; AP, area postrema.
23 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with no lesion controls.

Figure 1

Figure 1. (A) Photomicrographs of coronal sections illustrating a typical bilateral electrolytic lesion in the LPBN that includes ablation of the central, dorsal and external PBN. Arrows illustrate the lesion site. a and b represent the left and right side of the brain and at higher power in c and d. Magnification bar=100 μ m. (B) Schematics indicating the extent of LPBN bilateral lesions in the experimental group of rats (n=4) as depicted by dashed lines from mid (Bregma -9.3mm) to mid-caudal (Bregma -9.8mm) LPBN sections. Abbreviations: LC, locus coeruleus; MPBN, medial parabrachial nucleus; SCP, superior cerebellar peduncle.

Figure 2

Figure 3

Fig. 4 – Mean number of nuclei detected as Fos-positive following an injection of SC Ang II in rats allowed access to drinking water (n=4 per group; 4 sections per region per brain). Control groups include: No Lesion, indicating rats administered SC Ang II only; Sham, indicating rats where an electrode was lowered to the lesion coordinates but no current was passed; Miss, rats with lesions made outside the LPBN. The experimental group underwent bilateral LPBN lesions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with all controls (top panel) and no differences between groups (lower panel). Abbreviations: SC Ang II, subcutaneous injection of angiotensin II; MnPO, median preoptic nucleus; PVN, paraventricular nucleus of the hypothalamus; SON, supraoptic nucleus; BST, bed nucleus of the stria terminalis; Ce, central nucleus of the amygdala; OVLT, vascular organ of the lamina terminalis; SFO, subfornical organ; NTS, nucleus of the solitary tract; AP, area postrema.

Figure 5

Fig. 6 – Mean number of nuclei detected as Fos-positive following an injection of SC Ang II in rats denied access to drinking water. The control group, No Lesion, was administered Ang II only (n=5) and the experimental group underwent bilateral LPBN lesions (n=3; 4 sections per region per brain). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with no lesion controls (top panel) and no differences between groups (lower panel). Abbreviations: SC Ang II, subcutaneous injection of angiotensin II; MnPO, median preoptic nucleus; PVN, paraventricular nucleus of the hypothalamus; SON, supraoptic nucleus; NTS, nucleus of the solitary tract; AP, area postrema; OVLT, vascular organ of the lamina terminalis; SFO, subfornical organ; Ce, central nucleus of the amygdala; BST, bed nucleus of the stria terminalis.

- LPBN lesions increase AngII-induced water intake and reduce forebrain Fos
- Reduction in Fos is secondary to lesions possibly due to excessive water ingestion
- LPBN lesions directly increase AngII-induced Fos in rats without water
- LPBN influences the MnPO, PVN and SON to increases in peripheral AngII
- LPBN inhibitory mechanism is likely related to arterial baroreceptor stimulation

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