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The effects of lesions of the bed nucleus of the stria terminalis on sodium appetite

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Short communication

Abstract. Lesions of the bed nucleus of the stria terminalis result in decrements in sodium ingestion. Specifically, sodium ingestion that is aroused by mineralocorticoids, in addition to sodium depletion, was reduced in rats with large lesions of the bed nucleus of the stria terminalis. Need-free salt ingestion was also reduced. These results suggest that the bed nucleus of the stria terminalis may be involved in the regulation of sodium appetite.

Key words: sodium appetite, mineralocorticoids, bed nucleus of the stria terminalis

The levels of both adrenal steroid hormones (aldosterone and corticosterone) in addition to angiotensin are elevated during body fluid loss (e.g. Denton, 1982). These hormones physiologically conserve sodium, in addition to activating the behavioural state of sodium ingestion in repairing and maintaining body fluid balance (e.g. Rice and Richter 1943, Wolf and Handel 1966, Fluharty and Epstein 1983, Fregly and Rowland 1985).

The central and medial amygdala contain both adrenal steroid hormones, in addition to containing angiotensin receptors and cell bodies (Lind et al. 1985). Lesions of the amygdala are known to reduce sodium ingestion (e.g. Chiaraviglio 1971, Zolovick et al. 1977, Cox et al. 1978). Evidence suggests that increased sodium ingestion following mineralocorticoid treatment, or central infusions of renin-angiotensin, is abolished following central nucleus lesions (Galaverna et al. 1992). Other evidence has demonstrated that lesions of the medial region of the amygdala abolished mineralocorticoid-induced, but not angiotensin elicited sodium ingestion (Zhang et al. 1993). Interestingly, implants of aldosterone into the medial amygdala elicit the appetite for sodium (Reilly et al. 1993).

Knife-cut studies have revealed that while the stria terminalis pathway is not essential in response to these natriorexigenic signals (Black et al. 1992), the amygdalafugal pathway is (Chiariviglio 1971, Alheid et al. 1992). Specifically, knife-cuts of the amygdalafugal pathways decreased both mineralocorticoid and angiotensin in addition to sodium depletion-induced sodium appetite (Alheid et al. 1992).

In further elucidating the neural substrates of sodium appetite, we inquired into whether the bed nucleus of the stria terminalis was an important part of the anatomical circuit underlying this behaviour. We did so because (1) the bed nucleus is continuous with the amygdala and is therefore called "extended amygdala" (e.g. Alheid and Heimer 1988), (2) the bed nucleus contains corticosteroid receptors (Coirini et al. 1988) and has angiotensin receptors and cells (Lind et al. 1985) and (3) there is evidence that bed nucleus is part of a neural circuit that includes the medial and central amygdala that underlies other hormonally-induced behaviours (e.g. male copulatory behaviours to testosterone in hamsters, see Lehman and Winnans 1980, Lehman et al. 1983). Therefore, in the following study we lesioned the bed nucleus and determined its effects on mineralocorticoid-induced and sodium depletion-induced sodium appetite.

Male Sprague-Dawley rats weighing between 300-400 g were used. They were individually housed in wire mesh cages in a temperature controlled room, maintained under a 12:12 light: dark cycle. Except when indicated, rats were maintained on Purina Chow, water and 3% NaCl *ad lib*. The water and salt were given in drinking tubes attached to the front of the cage.

Surgery was performed under ketamine hydrochloride (70 mg/kg) and acepromazine maleate (1.0 mg/kg) which were administered IM. Anodal lesions were induced with insulated stainless steel electrodes which were bared at the tip (0.5 mm). Two lesions were made on each side. The current we delivered was 1.0 mA for 15 s. The coordinates were the following: 1.2 mm lateral to the midline, 6.5 mm below dura and 0.04 and 0.06 mm posterior to bregma. Several (four) of the animals expressed a mild adipsia and aphagia for several days following surgery. During this time they were maintained on a wet mash diet until they resumed normal water and food intake. At the time of the first test (3 weeks post surgery) all animals were drinking the same amount of water and their body weights were comparable.

Rats were injected subcutaneously with either 2.5 or 5.0 mg of deoxycorticosterone weekly. Each rat received three injections one daily on successive days with either dose. A one week rest period was imposed between tests, and rats were treated in a counterbalanced design. Ingestion of salt was recorded for three days before treatment, and then during the treatment. Ingestion was averaged over this time period.

Two weeks following the above treatment, all rats were treated with the diuretic and natriuretic agent furosemide. Briefly, each rat received two

subcutaneous injections of 5 mg of furosemide in an isotonic vehicle, separated by 2 h. The Purina Chow was removed, the rats were placed on a sodium deficient diet, and the salt was removed from the cage. The following day the rats were one again given access to the salt; they were prodded with the salt to alert them to its presence. Their intakes of salt and water were then recorded at 15, 30, 60 and 120 min. Their normal food was returned to them and 24 h later their intake of salt water were recorded.

At the termination of the experiment, rats were sacrificed. They were anaesthetized with 0.05 ml of Socumb/6 GR and perfused through the heart with isotonic saline followed by 10% formalin. Celloidine was used to fix the brains for slicing. Brains were cut on a cryostat at 32 μm sections. The sections were stained with cresyl violet.

We used standard two-way analysis of variance and *t* test in the analysis of the data.

Following surgery, lesioned rats typically reduced their ingestion of water and food for several days, but within 5 days the rats were eating and drinking normally, and their body weights were comparable to control rats.

Of the rats lesioned in the study, sixteen had lesions that damaged the bed nucleus. Eight rats had large lesions and eight had small lesions. The small lesions were mainly confined to the medial region. The large lesions generally destroyed portions of both the medial and lateral division of the bed nucleus (Fig. 1). In both the small and the large lesion groups there was damage to the anterior commissure. Two rats had damage outside the bed nucleus and four rats were sham operated controls. These later two groups were not distinguishable from operated controls (eight), so they were collapsed together.

Figure 2 shows that need-free salt intake (baseline intake) was reduced in the large bed nucleus lesioned group when compared to controls ($P < 0.05$). Figure 2 also shows that the salt intake following the mineralocorticoid treatment of the large lesion group was reduced when compared to controls ($P<0.01$). The ingestion of salt was significantly different in the large lesion group from the other two groups at both doses ($P<0.01$). Both controls and the small lesion group increased their salt ingestion in a dose dependent manner. The large lesion group only marginally increased their salt intake at the high dose, but it was not significant.

Figure 3 shows that the sodium intake following sodium depletion was different in the large lesion group from the other two groups ($P<0.01$). While all groups increased their salt and water intake over

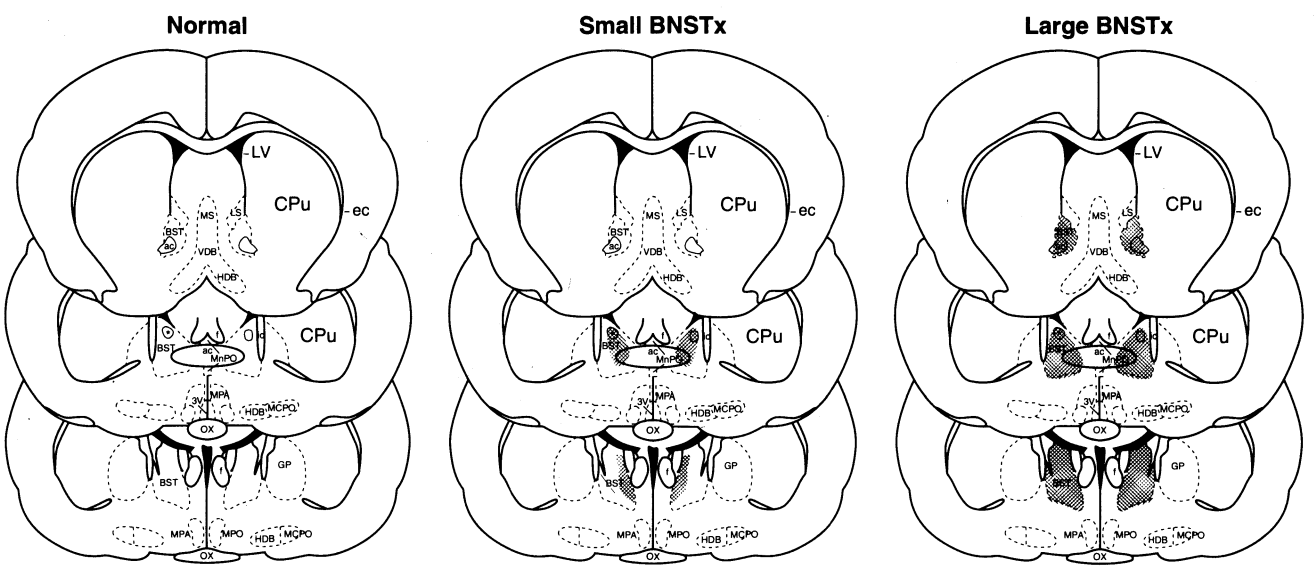

Fig. 1. The range of the small and large lesions of the bed nucleus (BNSTx). Shaded area shows the lesioned region in the small and large BNST lesion.

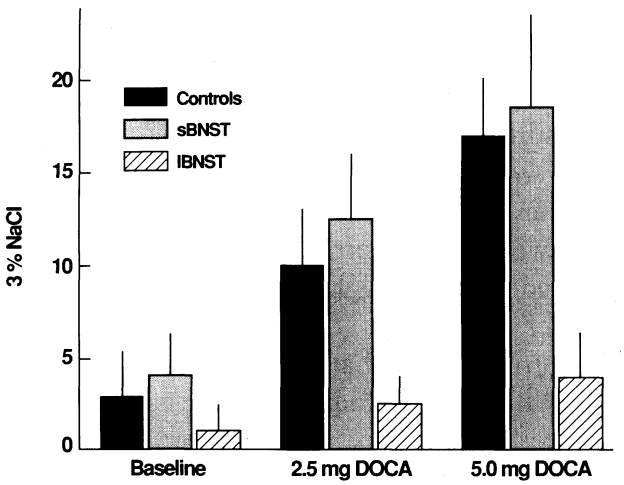

Fig. 2. 3% NaCl solution intake (ml) in the three groups in response to the deoxycorticosterone (DOCA) injection. Small lesion: sBNST; large lesion: IBNST.

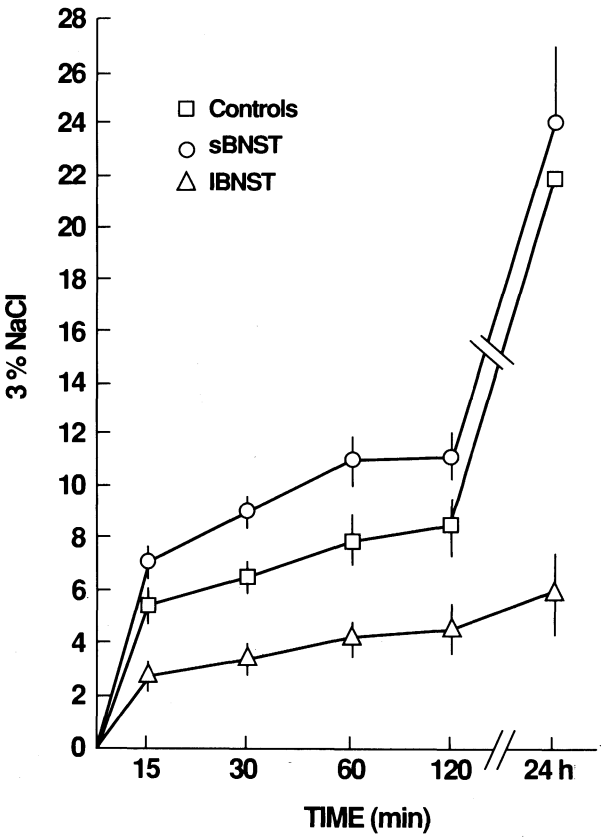

Fig. 3. 3% NaCl solution intake (ml) of the three groups in response to sodium depletion. Small lesion: sBNST; large lesion: IBNST.

baseline ($P<0.01$), the salt intake was dramatically reduced only in the large lesion group. Water ingestion was comparable in all groups (means: 65±8.5 large BNST, 58±6.2 small BNST and 62±4.5 for the controls).

Our results show that extensive damage to the bed nucleus of the stria terminalis interfered with the expression of mineralocorticoid-induced sodium appetite and sodium depletion-induced sodium appetite. These results extend and corroborate the findings of Zaardetto-Smith et al. (1991) who found decreased sodium appetite following sodium depletion in rats with bed nucleus lesions. We now add that the natriorexegenic effects of one of the primary hormones (mineralocorticoids) are compromised with large bed nucleus lesions.

Our lesion destroyed large portions of both medial and lateral divisions of the bed nucleus. In many cases the anterior commissure was damaged in addition to portions of the internal capsule. Moreover, it was only when there was extensive damage to the bed nucleus that sodium ingestion was altered. The anatomical specificity of our findings is unclear.

These results are comparable though not identical to what we found in our earlier studies with medial and central amygdala damage. We found that mineralocorticoid-induced sodium appetite was either abolished or reduced with medial or central amygdala damage (Schulkin et al. 1989, Black et al. 1992, Galaverna et al. 1992). Sodium depletion-induced sodium appetite was expressed in both the medial and central amygdala lesioned rats, but reduced when compared with controls (Galaverna et al. 1992). Taken together they suggest that while sites in either the central or medial amygdala are critical for the full expression of adrenal steroid-induced sodium appetite the bed nucleus is not, though it appears to play a role. Interestingly, the tachykinins when injected into either the medial bed nucleus or medial amygdala reduced the salt appetite aroused by sodium depletion (Mass et al. 1990, Pompei et al. 1992).

Because of its anatomical connectivity to amygdala, hypothalamus and lower brainstem sites such as the solitary nucleus and parabrachial nucleus, the bed nucleus is strategically tied and perhaps functionally part of the autonomic nervous system. Regions of the bed nucleus not only concentrate the adrenal steroid hormones, in addition to angiotensin but also receive gustatory input from these same brainstem sites (Norgren 1976). They also contain oxytocin cells and receptors, which have been functionally tied to other behavioural systems (Insel 1990). Perhaps the loss of oxytocin in the bed nucleus due to the lesion counteracted the inhibition that results from oxytocin on sodium appetite in rats (Stricker and Verbalis 1987) and thereby prevented further decrements in sodium ingestion.

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