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Mapping brain Fos immunoreactivity in response to water deprivation and partial rehydration: Influence of sodium intake

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HIGHLIGHTS

- Water intake after water deprivation increased Fos in numerous rat brain areas.
- Isotonic NaCl intake immediately after such water intake altered Fos.
- Sodium intake decreased Fos in brain areas known to facilitate sodium appetite.
- It also decreased Fos in most, but not all, inhibitory areas.
- Sodium intake decreased Fos in a brain reward area (accumbens).

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ABSTRACT

Water deprivation (WD) followed by water intake to satiety, produces satiation of thirst and partial rehydration (PR). Thus, WD–PR is a natural method to differentiate thirst from sodium appetite. WD–PR also produces Fos immunoreactivity (Fos-ir) in interconnected areas of a brain circuit postulated to subserve sodium appetite. In the present work, we evaluated the effect of sodium intake on Fos-ir produced by WD–PR in brain areas operationally defined according to the literature as either facilitatory or inhibitory to sodium intake. Isotonic NaCl was available for ingestion in a sodium appetite test performed immediately after a single episode of WD–PR. Sodium intake decreased Fos-ir in facilitatory areas such as the lamina terminalis (particularly subfornical organ and median preoptic nucleus), central amygdala and hypothalamic parvocellular paraventricular nucleus in the forebrain. Sodium intake also decreased Fos-ir in inhibitory areas such as the area postrema, lateral parabrachial nucleus and nucleus of the solitary tract in the hindbrain. In contrast, sodium intake further increased Fos-ir that was activated by water deprivation in the dorsal raphe nucleus, another inhibitory area localized in the hindbrain. WD–PR increased Fos-ir in the core and shell of the nucleus accumbens. Sodium intake reduced Fos-ir in both parts of the accumbens. In summary, sodium intake following WD–PR reduced Fos-ir in most facilitatory and inhibitory areas, but increased Fos-ir in another inhibitory area. It also reduced Fos-ir in a reward area (accumbens). The results suggest a functional link between sodium intake and the activity of the hindbrain–forebrain circuitry subserving reward and sodium appetite in response to water deprivation.

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1. Introduction

Humans and laboratory rats usually have sufficient access to sodium, but both may go into a transient negative sodium balance because of the obligatory sodium loss in urine during dehydration [1]. Accordingly, restricted access to water produces thirst and water intake that often precede an increase in sodium consumption in humans [2]. Similar to humans, rats living under laboratory conditions respond with sodium appetite to a sequence of water deprivation (WD) followed by water intake to satiety (i.e., partial rehydration or PR). Water intake corrects extracellular osmolality and rehydrates the intracellular compartment, but this rehydration is partial because sodium intake is also necessary for complete restoration of extracellular fluid (ECF) [3,4].

The WD–PR model provides convenient temporal resolution for interventions aiming to understand neuroendocrine and neural mechanisms subserving thirst and sodium appetite [3,5–7]. For example, antagonism of the renin–angiotensin system (RAS) after the rat has satiated its thirst during PR shows that subsequent sodium intake in the sodium appetite test depends on activation of brain angiotensin II

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AT1 receptors [5]. WD increases plasma renin activity (PRA), which remains elevated at the end of PR [3]. WD–PR also changes neuronal activity as measured by Fos immunoreactivity (Fos-ir) in key brain areas that control body-fluid balance, thirst and sodium appetite [4,8–10].

Water deprivation activates the lamina terminalis, a structure that forms part of the anterior wall of the third cerebral ventricle [3,4,9]. The lamina terminalis has two sensory circumventricular organs, the organum vasculosum of the lamina terminalis (OVLT) and subfornical organ (SFO), which monitor humoral (e.g. angiotensin II and osmolality) factors in blood [8,10,11]. Through the median preoptic nucleus (MnPO), those organs signal second order neurons projecting to the hypothalamic paraventricular nucleus (PVN) to elicit compensatory behavioral and physiological responses to dehydration [8,10,11]. Such responses originating in the lamina terminalis involve water and sodium intake, sympathetic activation, vasopressin secretion and renal water reabsorption. However, in spite of the complete correction of blood osmolality and partial ECF rehydration at the end of WD–PR, the lamina terminalis continues to display marked increases in Fos-ir [3,4,9].

WD–PR, but not WD alone, activates Fos-ir in the central nucleus of the amygdala (CeA) and hindbrain areas such as the nucleus of the tractus solitarius (NTS), lateral parabrachial nucleus (LPBN), dorsal raphe nucleus (DRN), and area postrema (AP) [4,9]. The AP also monitors blood osmolality, thus contributing to vasopressin secretion [10]. Signals from AP, as well as second order neurons in the NTS that sense blood volume through baroreceptors, send projections that reinforce sympathetic activation of the cardiovascular system, and ascend to control thirst as well as vasopressin and oxytocin secretion [8,10]. AP, NTS, DRN, LPBN and CeA, in addition to the lamina terminalis and PVN, belong to overlapping circuits postulated to control neurohypophysial hormone secretion, thirst and sodium appetite [4,10–14].

Thus, at the end of WD–PR, we find increased Fos-ir in the lamina terminalis, CeA, NTS, LPBN, DRN and AP. In contrast, water intake during the thirst test nearly abolishes Fos-ir in magnocellular PVN and hypothalamic supraoptic nuclei, which directly control vasopressin and oxytocin secretion [3,4,9]. This finding correlates with correction of blood osmolality and reduced vasopressin secretion [3,4]. On the other hand, as mentioned above, there is a persistent increase in PRA after WD–PR [3]. Such increase is likely important in maintaining peripherally mediated effects on arterial pressure because intracranial injections of angiotensin II antagonists fail to produce hypotension in ECF-dehydrated rats [15]. The increase in PRA is also associated with the production of sodium appetite in the WD–PR model [5]. Thus, rats subjected to water deprivation end a thirst test with elevated PRA and mild hypovolemia, along with activation in the lamina terminalis, parvocellular PVN, CeA and hindbrain areas. In spite of the link between thirst and sodium appetite and motivation [12,16–19], it is not known what effects WD–PR have on central areas involved with reward such as the nucleus accumbens (Acb). It is also not known how subsequent sodium intake affects neuronal activity in the abovementioned areas.

Although these areas play a broader role in body fluid homeostasis, for the purposes of the present study, we have operationally defined them in the context of the control of sodium appetite as either facilitatory or inhibitory, based on previous studies that employed lesions or neurochemical manipulations [8,14]. We consider lamina terminalis and CeA facilitatory to sodium intake, and NTS, LPBN, DRN and AP inhibitory to sodium intake. The PVN, particularly its parvocellular division, has been included in a hypovolemic thirst circuit connected to lamina terminalis and descending autonomic circuits [8,12]. Moreover, water intake may alter Fos-ir in this brain area in some rat strains [9]. Therefore, at least for the purposes of the present work, we consider parvocellular PVN as facilitatory to sodium intake. In addition, we here performed more detailed analysis of parvocellular PVN subdivisions (parvocellular medial, PaMP; ventral, PaV; posterior, PaPo) based on a previous work [20]. Given the role of these areas in the control of sodium appetite, we hypothesize that sodium intake reduces Fos-ir in facilitatory areas and increases Fos-ir in inhibitory areas. For-ir expression in a reward area such as Acb, should also change in response to changes in behavior (water or sodium intake) [16,18,19].

Evaluation of the effect of sodium intake on brain Fos-ir should benefit from the recent demonstration that isotonic NaCl is preferred in the sodium appetite test performed immediately after WD–PR [6]. Unlike hypertonic NaCl intake [4,11,21–23], isotonic NaCl intake should not induce thirst, vasopressin and oxytocin secretion, or hypertonicity-associated Fos-ir in key brain areas (e.g. lamina terminalis, SON, PVN, LPBN, NTS and AP). Moreover, NaCl intake after WD–PR increases as the concentration of the ingested sodium solution ingested decreases, as has been observed in other protocols of stimulated salt intake [6]. Therefore, the total amount of ingested sodium is similar, independent of its concentration, thereby guaranteeing appropriate replacement of the sodium lost during water deprivation [3]. Thus, isotonic NaCl intake in the sodium appetite test is an expression of sodium appetite with the advantage of avoiding the confounding effects of hypertonic NaCl intake.

Brain activation in the WD–PR protocol has not been examined when rats are permitted to drink isotonic NaCl. Thus, the objective of the present work was to further evaluate the changes in brain Fos-ir associated with WD–PR. We specifically investigated if isotonic NaCl intake in the sodium appetite test alters the pattern of Fos-ir expression associated with one episode of WD–PR.

2. Material and methods

2.1. Animals

Wistar male rats (240–350 g) were used. All animals were individually housed in stainless steel cages in a temperature-controlled environment, with a 12:12 h light/dark cycle. Standard chow diet (Cargill Inc. Argentina, containing approx. 0.18% NaCl), tap water and 0.15 M NaCl solution were available ad libitum during a 5-day adaptation period. All experimental protocols were approved by the Ferreyra Institute animal care and use committee, which follows the guidelines of the Public Health Service Guide for the Care and Use of Laboratory Animals (NIH, USA), and both the discomfort and number of animals used were minimized.

2.2. Water deprivation–partial rehydration (WD–PR), and thirst and sodium appetite tests

Animals were deprived of all fluids (WD), but had free access to standard diet for 36 h. Then, food was removed and the cages were rinsed with water. A glass burette with 0.1 ml divisions fitted with a stainless steel spout and containing filtered water was used as a drinking bottle. The bottle was offered immediately at the end of WD and water intake recorded after 90 min (thirst test). The sodium appetite test (WD–PRS) began immediately after completion of the thirst test. An identical drinking bottle containing 0.15 M NaCl was offered for the duration of the 120-min test. Immunohistochemical procedures to evaluate Fos-ir in selected brain areas were performed at the end of WD, WD–PR, and sodium appetite test (WD–PRS), as detailed below.

2.3. Effect of ingesting sodium on brain Fos-ir

After the adaptation period, the animals were separated into four groups: hydrated, i.e., non-fluid deprived with 36 h of free access to water and NaCl 0.15 M (H, $n = 5$); 36 h-fluid deprived (WD, $n = 4$); 36 h-fluid deprived followed by a 90-min thirst test (WD–PR, $n = 4$); 36 h-fluid deprived followed by a 90-min thirst test and 120-min sodium appetite test after the thirst test (WD–PRS, $n = 5$). One animal from each group was tested on a given day, and on these days, animals were perfused at the same time. Perfusion for immunohistochemical procedures started 210 min after termination of the thirst test or 90 min

after termination of the sodium appetite test. Fig. 1 describes the time interval for each group.

2.4. Fos immunohistochemistry (Fos-ir)

Animals in the WD, WD–PR, WD–PRS, or H (hydrated) condition (please see timing details in Section 2.3) were anesthetized with chloral hydrate (0.6 ml/100 g b.w.) and perfused transcardially with normal saline followed by 4% paraformaldehyde. Phosphate buffer with pH adjusted to 7.2 (PB), at the concentration of 0.1 M, was used to dissolve the paraformaldehyde and other reagents as well. The brains were removed, fixed in the perfusion solution overnight and stored at 4 °C in PB containing 30% sucrose. Coronal sections (40 μm) were cut with a freezing microtome. Immediately after, sections were placed in a solution of 10% H_2O_2 and 10% methanol in distilled water until bubbles ceased appearing. They were then incubated for 1 h in 10% normal horse serum (NHS; Gibco, Life Technologies) in PB and agitated to block sites of nonspecific binding. Fos-ir was detected using a standard avidin–biotin peroxidase protocol. Free-floating sections were incubated overnight at room temperature in an antibody raised in rabbits against a synthetic 14-amino acid sequence corresponding to residues 4–17 of human Fos (Oncogene Science, Manhasset, NY) diluted 1:10,000 in a solution of PB containing 2% NHS and 0.3% Triton X-100. The next day, after being washed in PB (0.01 M), the sections were incubated in biotin-labeled anti-rabbit immunoglobulin (1:200; Jackson Immunoresearch Laboratories) for 1 h at room temperature. Then, after washing again in PB, they were incubated with the avidin–biotin peroxidase complex (Vector, 1:200 dilution in PB-1% NHS) for 1 h at room temperature. The peroxidase label was detected using diaminobenzidine hydrochloride (DAB; Sigma) intensified with 1% cobalt chloride and 1% nickel ammonium sulfate to produce a blue–black nuclear reaction product. Finally, the free-floating sections were mounted on gelatinized slides, air-dried overnight, dehydrated, cleared in xylene, and placed under a coverslip with DePeX. Controls for Fos-ir were conducted by placing sections in a primary Fos antibody that had been preadsorbed with an excess of the Fos peptide or by processing sections without the primary antiserum. No Fos immunoreactive neurons were observed after either of these control procedures.

Fos immunoreactive nuclei were counted using a computerized system that includes a Zeiss microscope equipped with a DC 200 Leica digital camera attached to a contrast enhancement device. Images were digitalized and analyzed using Scion Image PC, based on the NIH 1997 version. Fos immunoreactive cells in each section were counted by setting a size range for cellular nuclei (in pixels) and a threshold level for staining intensity. The representative section image for each brain area studied was obtained with Adobe Photoshop Image Analysis Program, version 5.5, based on stereotaxic coordinates [24]. Fos immunoreactive nuclei were counted at only one level (one representative section for each nucleus, because of the anatomical and functional segregation found within the antero-posterior axis) in 4–5 animals from each condition. The counting procedure was repeated at least twice on each section to ensure that the numbers of profiles obtained were similar. The investigator who conducted the counting of Fos immunoreactive cells was blind to the experimental group. The brain nuclei expressing Fos-ir were identified and delimited according to a rat brain atlas [24].

2.5. Statistical analysis

Data are expressed as mean ± SE. Data were analyzed by one-way ANOVA. Post-hoc comparisons were made using the Least Significant Difference (LSD) test. Significance levels were set at $p < 0.05$ for all tests.

3. Results

3.1. Water and sodium intake during the thirst and sodium appetite tests

There was a significant effect of treatment [$F_{(2,10)} = 77.9$; $p < 0.05$] on water intake in the 90-min thirst test. Water intake increased in both WD–PR (6.6 ± 0.2 ml/100 g b.w.) and WD–PRS (6.7 ± 0.7 ml/100 g b.w.) groups compared with H (0.2 ± 0.1 ml/100 g b.w.) in the 90-min thirst test ($p < 0.05$). There was no difference between WD–PR and WD–PRS groups.

In addition, there was a significant effect of treatment [$F_{(1,7)} = 27.2$; $p < 0.05$] on sodium intake during the intake test. Isotonic NaCl intake increased in the WD–PRS group (15.4 ± 1.4 ml/100 g b.w.) compared with H (1.0 ± 0.5 ml/100 g b.w.) in the 120-min sodium appetite test ($p < 0.05$).

3.2. Fos immunoreactivity in the forebrain

Our results showed a significant effect of treatment on Fos-ir in each component of the lamina terminalis [OVLT: $F_{(3,12)} = 18.6$; MnPO: $F_{(3,15)} = 26.1$; SFO: $F_{(3,14)} = 60.7$; $p < 0.05$]. In this sense, Fos-ir increased Fos-ir in the OVLT, MnPO and SFO of the WD compared to H group ($p < 0.05$, Fig. 2, left). Water intake by the WD–PR group did not alter such Fos-ir, which remained elevated compared to H ($p < 0.05$). Isotonic NaCl intake by the WD–PRS group reduced Fos-ir in the SFO and MnPO by about 70% ($p < 0.05$), with no effect in the OVLT ($p > 0.05$).

There was also a significant effect of treatment on Fos-ir in each magnocellular hypothalamic nucleus [SON: $F_{(3,16)} = 555.7$; PaLM: $F_{(3,15)} = 780.4$; $p < 0.05$]. Fos-ir increased in the SON and PaLM of the

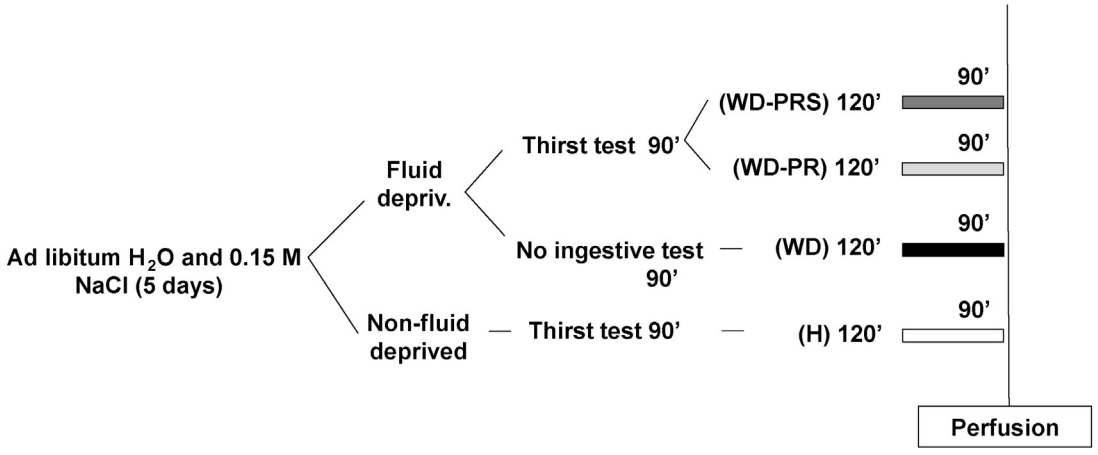

Fig. 1. Timeline representing the protocol employed to collect brains for Fos-ir. The protocol included: hydration (H), water deprivation (WD), water deprivation partial repletion with thirst test (WD–PR), and water deprivation partial repletion with thirst test followed by sodium appetite test (WD–PRS) conditions.

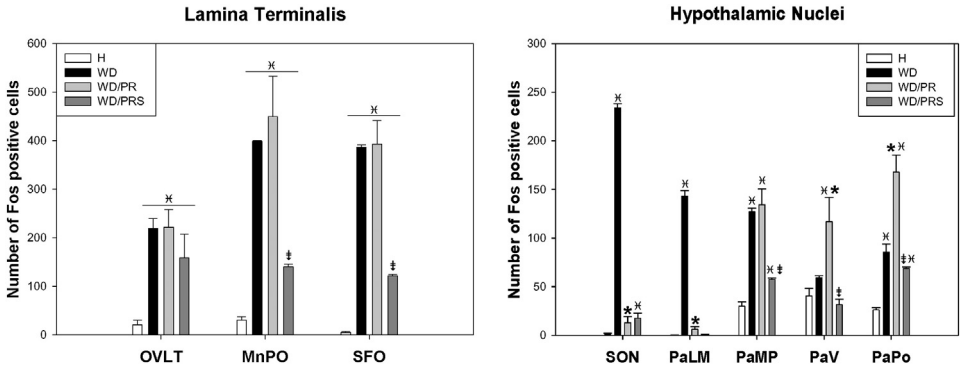

Fig. 2. Number of Fos-ir positive cells in organum vasculosum of the lamina terminalis (OVLT), median preoptic nucleus (MnPO) and subfornical organ (SFO) (left), and supraoptic (SON) and paraventricular hypothalamic nuclei (right) of hydrated control (H), water deprived (WD), water deprived-partially rehydrated (WD/PR) and water deprived-partially rehydrated animals following the sodium appetite test (WD/PRS). Paraventricular nucleus subdivisions: magnocellular (PaLM), and parvocellular (medial, PaMP; ventral, PaV; posterior, PaPo). ⚹ vs. H; ‡ vs. WD and WD/PR [except in the SON which differs only from WD]; and ✱ vs. WD; $p < 0.05$. n = 4–5.

WD compared to H group ($p < 0.05$, Fig. 2, right), and water intake by the WD–PR group reduced Fos-ir in both nuclei near to that observed in the H group ($p < 0.05$). Isotonic NaCl intake did not alter Fos-ir ($p > 0.05$).

There was an effect of treatment on Fos-ir in each subdivision of parvocellular PVN [PaMP: $F_{(3,15)} = 63.7$; PaPo: $F_{(3,15)} = 65.0$; PaV: $F_{(3,15)} = 13.2$; $p < 0.05$]. Fos-ir increased only in two subdivisions of parvocellular PVN, PaMP and PaPo, of the WD compared to H group ($p < 0.05$, Fig. 2, right). Water intake by the WD–PR group had no effect on such increase in both PaMP and PaPo ($p > 0.05$), but it induced Fos-ir in PaV ($p < 0.05$). Isotonic NaCl intake by the WD–PRS group reduced Fos-ir by 50% in PaMP and PaPo, and by 100% in PaV, compared with the WD–PR group ($p < 0.05$).

The results also showed a significant effect of treatment on Fos-ir in AcbC [$F_{(3,14)} = 129.5$; $p < 0.05$]. Fos-ir increased in AcbC of the WD compared to H group ($p < 0.05$, Fig. 3, left), and water intake did not alter this increase ($p > 0.05$). Isotonic NaCl intake reduced Fos-ir in the AcbC by about 50% ($p < 0.05$).

There was an effect of treatment on Fos-ir in AcbSh [$F_{(3,14)} = 54.1$; $p < 0.05$]. Fos-ir increased in AcbSh of the WD compared to H group ($p < 0.05$, Fig. 3, left). Water intake by the WD–PR group slightly enhanced such increase in Fos-ir ($p < 0.05$). Isotonic NaCl intake reduced Fos-ir in the AcbSh by about 40% ($p < 0.05$).

Finally, there was a significant effect of treatment on Fos-ir in CeA [$F_{(3,14)} = 100.0$; $p < 0.05$]. Fos-ir increased in CeA of the WD compared to H group ($p < 0.05$, Fig. 3, right). Water intake by the WD–PR group enhanced such increase in Fos-ir by 3.5-fold ($p < 0.05$). Isotonic NaCl intake by the WD–PRS group reduced by 40% the Fos-ir in CeA ($p < 0.05$).

3.3. Fos immunoreactivity in the hindbrain

Our results showed a significant effect of treatment on Fos-ir in DRN [$F_{(3,14)} = 255,7$; $p < 0.05$]. In this sense, Fos-ir increased in DRN of the WD compared to H group ($p < 0.05$, Fig. 4, left). Water intake by the WD–PR group slightly decreased such Fos-ir ($p < 0.05$). Isotonic NaCl intake by the WD–PRS group produced a 3.8-fold increase in Fos-ir in DRN ($p < 0.05$).

In addition, there was a significant effect of treatment on Fos-ir in each of the other three hindbrain areas [LPBN: $F_{(3,16)} = 62.4$; NTS: $F_{(3,12)} = 41.3$; AP: $F_{(3,12)} = 94.0$; $p < 0.05$]. Fos-ir slightly increased in AP ($p < 0.05$), but did not alter in LPBN and NTS, of WD compared with H (Fig. 4, right). Water intake by the WD–PR group strongly increased Fos-ir in LPBN, NTS and AP ($p < 0.05$). Isotonic NaCl intake by the WD–PRS group decreased Fos-ir in LPBN (74%), NTS (63%) and AP (63%) ($p < 0.05$).

3.4. Photomicrographs

Photomicrographs of sections illustrating Fos-ir in SFO, SON, PVN and LPBN and DRN are depicted in Figs. 5 and 6 respectively.

4. Discussion

Isotonic NaCl intake during the sodium appetite test had a pronounced effect on the mapping of brain Fos-ir associated with WD–PR. It reduced the activity in most facilitatory and inhibitory areas, but increased the activity in one inhibitory area, and reduced the activity

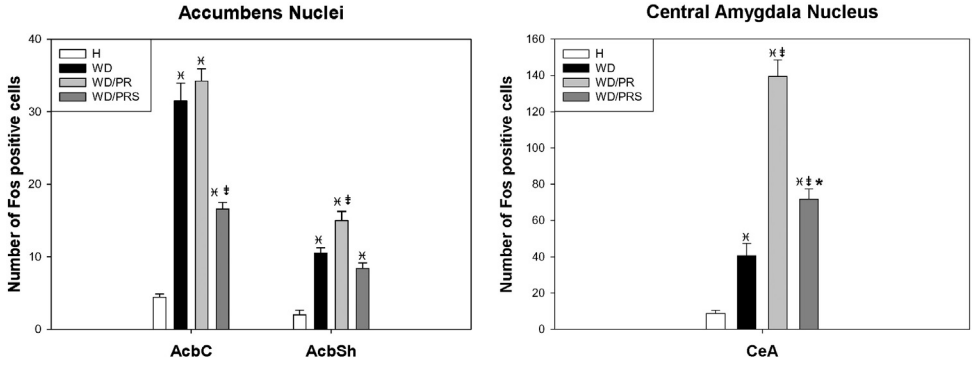

Fig. 3. Number of Fos-ir positive cells in nucleus accumbens, core (AcbC) and shell (AcbSh) (left) and central amygdala (CeA) (right) of hydrated control (H), water deprived (WD), water deprived-partially rehydrated (WD/PR) and water deprived-partially rehydrated animals following the sodium appetite test (WD/PRS). ⚹ vs. H; ‡ vs. WD–PR; and ✱ vs. WD; $p < 0.05$. n = 4–5.

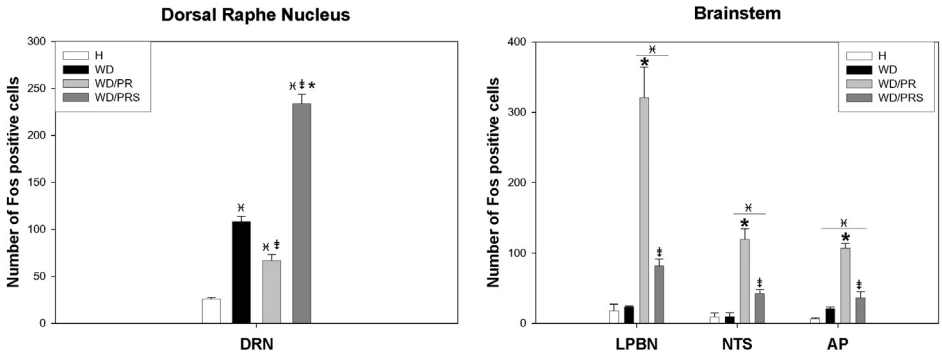

Fig. 4. Number of Fos-ir positive cells in dorsal raphe nucleus (DRN) (left) and other brainstem nuclei: lateral parabrachial nucleus (LPBN) nucleus of the solitary tract (NTS) and area postrema (AP) (right) of hydrated control (H), water deprived (WD), water deprived-partially rehydrated (WD/PR) and water deprived-partially rehydrated animals following the sodium appetite test (WD/PRS). ⚹ vs. H; ‡ vs. WD and WD/PR; ★ vs. WD; $p < 0.05$. $n = 4–5$.

in a reward area. Specifically, isotonic NaCl intake decreased Fos-ir in areas known to be activated at the end of WD–PR [3,4,9]: the lamina terminalis (particularly SFO and MnPO), parvocellular PVN, and CeA in the forebrain and LPBN, NTS and AP in the hindbrain. On the contrary, sodium intake further increased Fos-ir that was activated by water deprivation in the hindbrain inhibitory area, DRN. Water deprivation increased Fos-ir in both the core and shell of the Acb, and water intake slightly altered this effect. Interestingly, subsequent sodium intake decreased Fos-ir in both parts of the Acb. Thus, the present results show that sodium intake alters Fos-ir expressed in forebrain and hindbrain areas after satiation of thirst, and the ingestion of an isotonic NaCl solution is fundamental to support this conclusion.

We next discuss how Fos-ir observed in the present work relates to physiological responses to dehydration. Then, we detail and interpret the results of Fos-ir for each group of brain areas in the context of thirst and sodium appetite. Finally, we summarize the conclusion and overall significance of the present findings.

As delineated in the Introduction, dehydration not only produces behaviors, such as water and sodium intake, but also influences physiological responses (e.g. neuroendocrine secretion and autonomic output). Thus, it is not a simple task to establish a strict correspondence between neural activity and behavior, or to separate it from the control of physiological responses. Thus, a discussion about the potential relation between the observed brain Fos-ir and physiological responses associated with WD–PR should help the interpretation of the results in the context of thirst and sodium appetite.

Consistent with findings from the literature, water intake practically eliminated Fos-ir in SON and magnocellular PVN (PaLM) ([3,4,9], present results), two areas which constitute the source of vasopressin and oxytocin released from the neurohypophysis [4]. Several areas

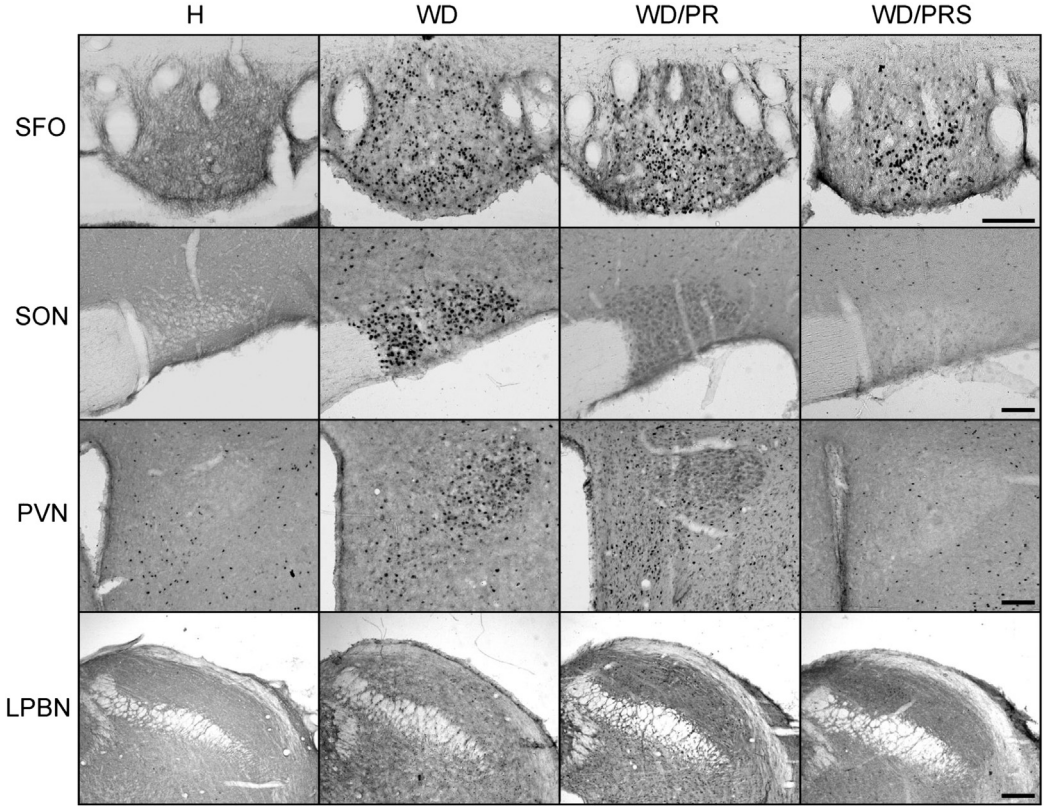

Fig. 5. Photomicrographs of selected coronal sections of the subfornical organ (SFO, top), supraoptic and paraventricular nucleus of the hypothalamus (SON and PVN, middle) and lateral parabrachial nucleus (LPBN, bottom) showing Fos-ir in: hydrated (H), water deprived (WD), water deprived-partially rehydrated (WD–PR) and water deprived-partially rehydrated animals following the sodium appetite test (WD–PRS). Scale bar: 50 μm.

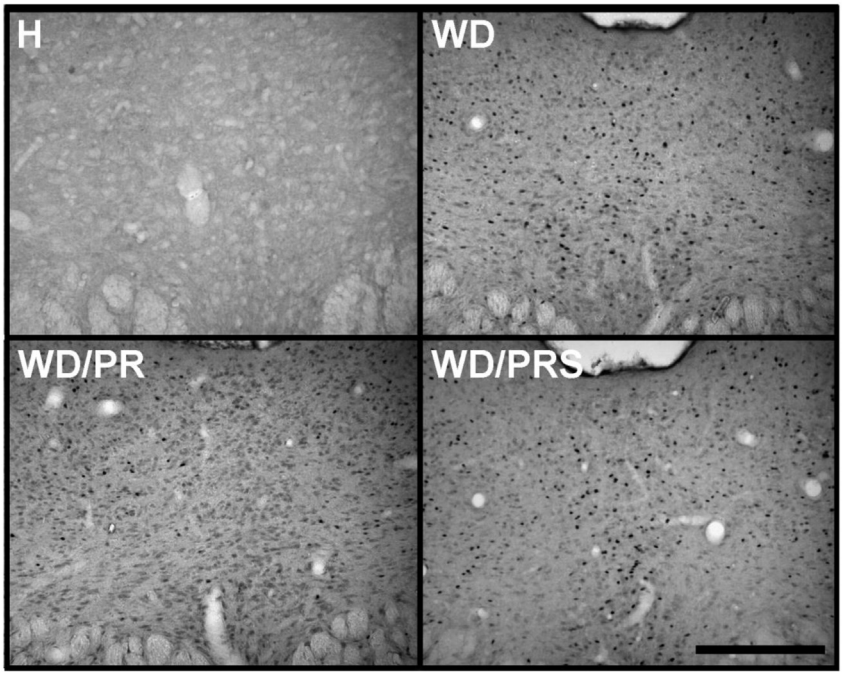

Fig. 6. Photomicrographs of selected coronal sections of the dorsal raphe nucleus (DRN) showing Fos-ir in: hydrated (H), water deprived (WD), water deprived-partially rehydrated (WD–PR) and water deprived-partially rehydrated animals following the sodium appetite test (WD–PRS). Scale bar: 50 μm.

under study in the present work, from the hindbrain (DRN, LPBN, NTS, AP) to forebrain (OVLT, MnPO, SFO), also participate in the control of vasopressin and oxytocin secretion, arterial blood pressure and gastrointestinal function [8,10,11,25,26]. Yet, the pattern of Fos-ir was not necessarily the same for all areas, whether compared among themselves or to SON and magnocellular PVN. It is well known that, in addition to altering Fos-ir in the brain, water intake after dehydration partially replenishes the ECF and, at the same time, corrects blood osmolality [3,4]. Sodium intake produces the final repletion of ECF [3,4]. However, ECF repletion was likely not a predominant effect on Fos-ir because of the opposite patterns of Fos-ir produced by water and sodium intake in some areas. Satiety of thirst, or oral and gastrointestinal stimulation produced by water intake may have also induced Fos-ir at the end of WD–PR [25–27], but again the effect on brain Fos-ir was different from that produced by sodium intake, suggesting that gastrointestinal fill was not the source of the alteration in Fos-ir in all areas.

Another physiological response common to dehydration is renin secretion. PRA remains elevated at the end of WD–PR and is a causal factor in both thirst and sodium appetite [3,5]. Moreover, angiotensin II induces a pattern of Fos-ir in both the forebrain and hindbrain similar to that found at the end of WD–PR [28,29]. Isotonic sodium intake drastically reduces PRA in water-deprived rats [4], and our results show that it consistently reduced Fos-ir in most brain areas, except in the DRN.

We may conclude that, in spite of sharing similar somatic motor responses and underlying physiological stimuli (e.g. blood refilling, gastrointestinal distention, and angiotensin II), water and sodium intake produced remarkably different patterns in brain Fos-ir.

Water deprivation, or water deprivation followed by water intake, activated brain areas known to facilitate sodium appetite (SFO, MnPO, CeA) [8,12,13], and subsequent sodium intake reduced Fos-ir in these areas. As expected from previous studies, water intake — which is accompanied by a complete reversal of blood hyperosmolality [3,4,9], practically eliminated Fos-ir in SON and PaLM. Water intake had no effect on Fos-ir in the lamina terminalis or PaMP of water deprived animals, but further increased Fos-ir in CeA, PaV and PaPo. Importantly, the present work showed that isotonic NaCl intake had no effect on Fos-ir in SON and PaLM, but reduced Fos-ir in the lamina terminalis, CeA and parvocellular PVN of WD–PR animals.

The effect of sodium intake on activity in parvocellular PVN (PaMP, PaV and PaPo) suggests a role for this part of PVN in the behavioral control of hypovolemia. The parvocellular PVN forms a nodal connection between the lamina terminalis and limbic areas that control thirst induced by hypovolemia and angiotensin II [8,12]. The present results show that WD activated Fos-ir only in part of parvocellular PVN (PaMP, PaPo). Water intake did not affect Fos-ir in PaMP, but induced Fos-ir in PaV and further increased it in PaPo. Subsequent sodium intake reduced Fos-ir in all three areas, suggesting that parvocellular PVN is also sensitive to sodium intake and thus, somehow linked to sodium appetite.

Increased Fos-ir in one inhibitory area, the DRN, accompanied the decreased Fos-ir found in facilitatory areas. Neurons in DRN are sensitive to body sodium status and inhibit sodium appetite [14,22,23]. Similar to patterns of activity in facilitatory areas in the forebrain, WD induced Fos-ir in the DRN. Water intake in the subsequent PR produced a slight decrease in Fos-ir in the DRN; however, sodium intake produced a further increase in Fos-ir that easily surpassed the one produced in response to WD. The decreased activity in facilitatory areas and increased activity in the DRN, an inhibitory area, marked the reduction in sodium appetite, as one would predict from the functions of those areas.

In contrast to Fos-ir in the DRN, water intake and sodium intake respectively increased and decreased Fos-ir in other brain areas considered to be inhibitory to sodium appetite, the LPBN, NTS, and AP [4,8,9]. The effect of sodium intake on Fos-ir in LPBN, NTS, and AP was not predicted, given that all those areas inhibit sodium appetite [8]. Rather, we would have expected Fos-ir in these areas to exhibit the same pattern as in DRN (increased by sodium intake). However, we found that sodium intake decreased Fos-ir in LPBN, NTS and AP.

The decreased Fos-ir in LPBN, NTS, and AP after sodium intake suggests that these areas have both facilitatory and inhibitory influences on sodium intake. Evidence from the literature points to the same direction. For example, sodium-sensitive neurons are present in the NTS and AP [13,30], and methysergide injection into the LPBN increases Fos-ir in the NTS and AP, in addition to increasing sodium intake [31,32]. In

addition, damage to the CeA reduces the increased sodium intake produced by deactivation of the LPBN inhibitory mechanism [33]. The present results, together with data from the literature, suggest that LPBN, NTS and AP belong to circuits that both facilitate and inhibit sodium appetite, but such hypothesis is still open for further investigation.

We also found that changes in Fos-ir in the Acb were similar to those in facilitatory areas, particularly in the SFO and MnPO. Water deprivation increased Fos-ir in the core (AcbC) and shell (AcbSh) of the Acb. Water intake produced a slight further increase in Fos-ir only in the AcbSh. Thus, Fos-ir in the Acb remained at its peak after the dehydrated animal ingested water. Isotonic NaCl intake then reduced Fos-ir, both in the AcbC and AcbSh. Brain circuits that control the body-fluid balance make anatomical connections with areas that control reward, such as the Acb [12,13]. The Acb also receives projections from putative sodium sensitive cells in the AP, responds with increased Fos-ir to repeated episodes of sodium depletion, and has neuronal excitability modulated by sodium taste or body sodium balance [17–19,30]. Moreover, sodium intake in response to single sodium depletion produces Fos-ir in both AcbC and AcbSh of non-satiated rats with an open gastric fistula [16]. According to the authors of that study, this production of Fos-ir in the Acb represents unsatisfied motivation to replace the lost sodium or failure to obtain the expected reward, in this case sodium. In the same study [16], the authors suggest that the Fos-ir that they found in the Acb resembles that produced in the same area in response to the administration of drugs of abuse – drugs which hardly produce satiety – or dopaminergic neurons firing in response to "violation" of reward [34,35]. It is possible that the same type of failure to obtain the expected reward happened when the water-deprived rat ingested only water in the present study. Thus, the persistent increase in Fos-ir in the Acb after water intake would represent the expectation of the sodium reward. Another possibility, not mutually exclusive, is that Fos-ir in the Acb, similar to Fos-ir in the SFO and MnPO, was the response to a persistent physiological signal such as angiotensin II. The peptide has a dipsogenic effect dependent on dopamine receptors in the Acb and its concentration in the blood decreases only in response to sodium intake [3,4,36].

In contrast to the other two components of the lamina terminalis (SFO, MnPO), Fos-ir in the OVLT remained elevated after the thirst and sodium appetite tests. It is not clear why OVLT responded differently from SFO since both organs presumably sense the same humoral factors [8,11]. The persistent Fos-ir in OVLT after sodium intake also coincided with a residual Fos-ir found in other brain areas. Moreover, water deprivation activates most cells that project from OVLT to SON [37]. Thus, the drastic and persistent reduction in Fos-ir in SON after water intake, and the reduction in Fos-ir in SFO after sodium intake, suggests that cells in OVLT are, at least in part, working independently from these two areas. However, we still do not know what this means. It could relate to satiation of sodium appetite or reflect differences in Fos-ir decay or a more complex functioning of the organ (e.g. temporal dynamics of group cell activity) not easily detectable using Fos-ir.

The present work demonstrates distinct patterns of brain Fos-ir that change according to changes in both internal deficits and motivational status of the rat. As pointed out before, WD–PR reveals two distinct drives produced in response to water deprivation, thirst and sodium appetite [6]. Thus, our results have important implications for the concept of goal directed behavior embedded in theories of motivation [38].

Several lines of evidence converge to suggest a circuit formed by LPBN, NTS and AP coupled to Acb, CeA, parvocellular PVN and lamina terminalis [8,12–14,22,23,28–32,39,40]. The present work mapped brain Fos-ir associated with that circuit and WD–PR. In particular, it showed how sodium intake influences Fos-ir observed after water-deprived rats satiated their thirst. Although it is still early to determine whether a causal relationship exists, the findings suggest neurological correlates of thirst and sodium appetite associated with that circuit during WD–PR. They also provide a framework for testing further hypotheses about how several brain areas operate and produce reward to control sodium appetite.

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