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ABSTRACT

In the clinical setting, acute injuries in hypothalamic mediobasal regions with polydipsic and polyuric symptoms have been observed in patients with cerebral salt wasting (CSW). CSW is also characterized by hypovolemia and hyponatremia due to an early increase in natriuretic peptide activity. Salt and additional amounts of fluid are the main treatment for this disorder. In parallel, lesions to these brain regions, which include the median eminence (ME), produce a well-documented neurological model of polydipsia and polyuria in rats, which is preceded by an early sodium excretion of unknown cause. Hence, the main objective of this study was to prolong the hydromineral imbalances observed in ME-lesioned rats by systemic administration of the natriuretic peptide oxytocin (OT) and examine their intake of water and hypertonic NaCl intakes **in the absence and presence of food**. Electrolytic lesion of the ME produced an increase in water intake, urinary volume, and sodium excretion of food-deprived rats and a decrease in their urinary osmolality **and estimated plasma sodium concentration.** OT administration at 8 h post-surgery reduced water intake, urine output, and plasma sodium concentration and increased urine osmolality and urine sodium excretion between 8 and 24 h post-lesion**.** From 24 to 30 h, more water and hypertonic NaCl was consumed by OT than by physiological saline (PS) treated-ME lesioned animals**.** Food availability from 30 to 48 h reduced the intake of hypertonic saline solution by ME/OT animals, which increased their water and food intake during this period. **OT administration therefore appears to enhance the natriuretic effect of ME lesion, producing hydroelectrolytic changes that reduce the water intake of food-**

deprived animals. Conversely, the presence of hypertonic NaCl increases the fluid intake of these animals, possibly due to the plasma sodium depletion and hypovolemic states previously generated. Finally, the subsequent increase in food intake by ME/OT animals reduces their need for hypertonic NaCl but not water, possibly in response to osmotic thirst. These results are discussed in relation to a possible transient activation of the ME with the consequent secretion of natriuretic peptides stored in terminal swellings, which would be augmented by OT administration. Electrolytic lesion of the ME may therefore represent a useful neurobiological model of CSW.

Keywords: Hydromineral regulation, oxytocin, sodium appetite, water intake, median eminence, natriuresis, **cerebral salt wasting, plasma sodium depletion**.

INTRODUCTION

Polydipsic/polyuric symptoms are observed in patients **with acute brain injury** involving disruption of hypothalamic mediobasal renal pathways. [1-3] **In some cases**, **t**hese patients are diagnosed with cerebral salt wasting (CSW) syndrome, characterized by hyponatremia and hypovolemia due to early natriuresis/diuresis secondary to increased natriuretic peptide activity. [4-6] **In these conditions, early sodium therapy should be initiated with additional amounts of fluid to prevent further complications. [1, 6, 7] Other patients with similar lesions can develop central diabetes insipidus (CDI). In this neurobiological syndrome, the polydipsic disorder is accompanied by the uncontrolled excretion of dilute urine and hypernatremia due to a reduction in the antidiuretic hormone (ADH) or vasopressin. [8, 9] ADH is** synthesized in the perikarya of magnocellular neurons of hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. [10, 11] **Axonal swellings containing ADH have been identified in the median eminence (ME) and neurohypophysis, permitting access of this neurohormone to the bloodstream and kidney. [11, 12] Acquired** CDI **can be experimentally induced in animals by electrolytic lesions of** different hypothalamic basal structures, including the ME. [13-16] **Parallel to observations in humans, a** marked polydipsic and diuretic response is observed in the animals after mediobasal ME lesions [13-15, 17] **that affect the posterior- hypothalamus-pituitary axis and interrupt ADH secretion. [1, 18, 19] Interestingly, this** neurogenic hypothalamic polydipsia is frequently preceded by sodium excretion during the first few hours post-surgery [15], **but** the precise mechanisms underlying the high rate of early sodium excretion in ME-lesioned animals remain unknown. Research results to date have not ruled out transient activation of the affected neural tissue by the **electric current [20-22]**, which might generate the increased natriuretic response **[15] observed in CSW syndrome. [4-6] Besides producing ADH secretion, it is possible that transient ME activation can also stimulate the secretion of OT, a neuropeptide found in the ME together with ADH. [23] OT participates in sodium excretion either alone [17, 24-26], in synergy with ADH [27, 28], and/or indirectly through the cardiac secretion of atrial natriuretic peptide (ANP). [29-31] It has also been related to CDI symptoms induced by electrolytic lesion of the ME. [32]**

Given the above data, the augmented renal sodium and water losses induced by OT administration could be expected to **prolong** the hydroelectrolytic abnormalities **(volemic deficit and possibly hyponatremia)** in these animals during the first few hours post-surgery. If this is confirmed, it can be hypothesized that ME-lesioned animals receiving **OT** may

increase both their sodium appetite and water intake in order to restore the volume and composition of extracellular body fluid.

MATERIALS AND METHODS

Animals

Male Wistar rats (300-370 g) from a breeding colony of the University of Granada were randomly assigned to one of four experimental groups (n = 22). Two groups, **ME1 and ME2**, received anodic lesions in the ME coordinates, while two non-lesioned control groups, **SHAM1 and SHAM2,** underwent the same surgery but received no electrolytic lesion. Animals were housed in individual cages with unlimited access to food and water (Union Alimentaria Sanders, Madrid, Spain) unless otherwise indicated. The room was maintained at 21-23 ºC on a 12/12 h light-dark cycle. Utilization and handling of the animals followed guidelines for research with animals established by the European Union (2010/63/EU) and Spanish Law (53/2013) and were approved by the Ethics Committee for Animal Experimentation of the University of Granada (10-301). All efforts were made to minimize animal suffering and reduce the number of animals used.

Surgical procedure

ME lesions were made under sodium thiopental anesthesia (Lab. Abbot, Madrid, Spain) induced by intraperitoneal (i.p) injection (50 mg/kg). After placing the animals in a stereotaxic device (Stoelting Co. 51.600, IL, USA), a DCML-5 lesion-maker (Grass Instruments, Quincy, MA, USA) was used to administer bilateral anodic current (1.5 mA) application for 15 s, [13-15, 17, 33, 34] using a 00 stainless steel electrode insulated except at its tip. Stereotaxic coordinates, obtained from the atlas of Paxinos and Watson, [35] were 6.44 mm anterior to interaural line, 0.4 mm lateral to midline, and 0.2 mm dorsal to interaural line. Non-lesioned control groups underwent the same surgery as the lesioned rats except that the vertical coordinate was 0.5 mm above the lesion coordinates and no current was applied. All animals received an intramuscular (i.m.) injection of 0.1 mL penicillin at 250,000 IU/mL (Penivel retard, Lab. Level, S. A., Barcelona, Spain) as a prophylactic measure against infection.

Experimental procedure (Figure 1)

All animals had a 10-day adaptation period before the surgery with *ad libitum* access to tap water and food (Sandermus dry chow, Sanders, S. A. Madrid, Spain). Water and food intake and body weight during the 4 days before surgery were recorded daily at 09:00 h (baseline).

After surgery, animals were housed in metabolic cages (3701MO-000, Tecniplast, Milan, Italy) with *ad libitum* access to water but complete food deprivation for 24 h. Rats were randomly assigned to one of four experimental groups: ME-lesioned (**ME1, n = 6 and ME2, n = 6**) and SHAM-lesioned (**SHAM1, n = 5 and SHAM2, n = 5**) groups. **At 8 h post-surgery, the ME1 and SHAM1 groups** were administered with an i.p. injection of 1.0 mL of OT (22 mg/mL, Laboratories Iven, Madrid) [ME/OT and **SHAM/OT]**, whereas the **ME2 and SHAM2** groups received an i.p. injection of 1.0 mL physiological saline (PS) [ME/PS and **SHAM/PS]** (Apiroserum, Lab. Ybis. Madrid) following the same procedure.

Data were collected on water intake, urinary volume excretion, urinary osmolality, and urinary sodium excretion of all animals at 8 h (0-8 h post-surgery) and 24 h post-surgery (data for the 16-h period between 8 and 24 h post-surgery). Selection of these time points was based on the time course of behavioral effects observed in previous studies. [13-15, 33] Urinary osmolality (mOsm/kg) was measured with an osmometer (Osmostat OM-6020, Kyoto, Japan) using the freezing-point method. Urinary sodium **concentration was measured with an** automatic analyzer (Beckman Instruments, Synchron CX3 Delta, California, USA), and **urine sodium excretion** was calculated by multiplying the urine sodium **concentration** by the urine volume.

On day 2, animals were returned to their habitual methacrylate cages where they had access to a hypertonic saline solution (1.5 % NaCl) and water, recording the volumes consumed at 30 h and 48 h post-surgery. **Animals remained under** food deprivation from 24 h to 30 h post-surgery. Food was then available *ad libitum* in their cages, recording the food intake of all animals during the 30-48 h period post-surgery.

Statistical analysis

Data were expressed as means ± SEM and analyzed using Statistica software for Windows (6.0; StatSoft, Inc., OK). Non-parametric tests were applied because of the non-normal distribution of the data and the large differences in variances between groups. [36] Body weight, water, NaCl and food intake, urinary volume, water "balance" (water intake minus urine volume), urinary sodium excretion, urinary osmolality, **and estimated plasma sodium concentration** at each study time point were analyzed across all groups using the Kruskal-Wallis one-way ANOVA test. The Mann-Whitney *U* test was used for pairwise comparisons between groups. The level of significance was set at $p<0.05$ for all tests. **Plasma sodium concentration was estimated using the formula of Stricker [37]: 1000 × [(0.150) (0.69) (BW) + sodium "balance"] / [(0.69) (BW) + water "balance"], where BW is the body weight (g)**

of the animal at the time of testing, and the sodium "balance" (mEq) is computed as the difference between sodium intake and sodium loss in urine.

Histology

After completion of the experiment, all animals were overdosed with sodium pentothal (80 mg/kg) and transcardially perfused. The brain was removed from the skull and placed in 4% formaldehyde for at least 24 h and was then frozen and sliced into 40 nm sections (Leitz 1320, Wetlar, Germany). Slides were stained with cresyl violet, examined under light microscope (stereoscopic microscope UMZ-4F, Olympus, Tokyo, Japan), and microphotographed (Olympus Optical, mod PM-G, Tokyo, Japan) to determine the localiztion of lesions.

RESULTS

Histological analysis

The authors estimated the extent of damage in each animal by reconstructing the location and extent of the lesion on plates of the rat brain atlas.[35] Figure 2 depicts the maximum and minimum degree of damage produced by the ME lesions. Lesions in the two ME-lesioned groups were characterized by their caudal extension throughout the base of the brain, with damage to midline and basal hypothalamic structures (arcuate nucleus, dorsomedial hypothalamus and ventromedial hypothalamus) in some cases. No damage to supraoptic or paraventricular nuclei was observed. The ME was completely lesioned from its mid-position to its most posterior region, caudal to the site of stalk separation. The behavior of these animals did not differ according to the extent of ME damage; therefore, no animal was excluded from the study.

Analysis of baseline intake behavior

No significant differences were found among any of the study groups in baseline body weight or water or food intake (day -1: body weight $H_{3,22} = 0.73$, $p < 0.985$; water intake, $H_{3,22} = 1.29$, $p < 0.7309$; food intake, $H_{3,22} = 4.8147$, $p < 0.1859$).

*Effects of ME lesion on water and **sodium metabolism** at 8 h post-surgery (before OT or PS injections).*

The Kruskal-Wallis ANOVA test showed significant differences in water intake [($H_{3,22} = 15.88$, $p < 0.0012$), urinary volume ($H_{3,22} = 16.01$, $p < 0.0011$), water "balance" ($H_{3,22} = 12.05$, $p < 0.0072$), osmolality ($H_{3,22} = 16.34$, $p < 0.0010$), sodium excretion ($H_{3,22} = 15.71$, $p < 0.0013$], **and estimated plasma sodium concentration ($H_{3,22} = 11.06$, $p < 0.0114$).**

Pairwise comparisons revealed a **greater** water intake (Fig. **3**A, all, $p < 0.01$) and urine volume (Fig. **3**B, all, $p < 0.01$) for ME-lesioned *versus* **SHAM** groups between 0 and 8 h post-surgery. The water balance during this period was more negative in ME-lesioned *versus* **SHAM groups** (Table 1, $p < 0.05$), and urinary osmolality was **lesser** in ME-lesioned *versus* **SHAM** groups (Fig. **3**C, all, $p < 0.01$). During the same period (0–8 h post-surgery) urinary sodium excretion was **greater** in ME-lesioned *versus* **SHAM** groups (Fig. **3**D, all, $p < 0.01$), whereas **estimated plasma sodium was lesser in ME-lesioned *versus* SHAM groups (Table 1, all, $p < 0.01$). No significant differences were observed between ME1- and ME2-lesioned groups during this period.**

*Effects of OT administration on water **and sodium** metabolism in ME-lesioned and SHAM animals at 24 h post-surgery (from 8 h to 24 h).*

The groups significantly differed in water intake ($H_{3, 22} = 18.53$, $p < 0.0003$), urinary volume ($H_{3, 22} = 19.74$, $p < 0.0002$), water "balance" ($H_{3, 22} = 10.93$, $p < 0.0121$), osmolality ($H_{3, 22} = 17.47$, $p < 0.0006$), sodium excretion ($H_{3, 22} = 19.71$, $p < 0.0002$), and **estimated plasma sodium concentration ($H_{3,22} = 16.51$, $p < 0.009$).**

The water intake remained **greater** in ME/PS animals than in **SHAM**/PS ($p < 0.01$) or ME/OT ($p < 0.01$) animals during this period (8-24 h post-surgery, Fig. **4**A). Therefore, OT administration reduced water intake in the lesioned group, which showed no significant differences with the **SHAM** group at 24 h after surgery. Water intake was significantly **greater** in the **SHAM**/OT *versus* **SHAM**/PS groups ($p < 0.01$).

Between 8 and 24 h post-surgery, a greater diuretic response was observed in ME-lesioned *versus* **SHAM** groups (Fig. **4**B, all, $p < 0.01$); however, the polyuric response was **reduced** in ME/OT animals in comparison with those receiving PS ($p < 0.01$). This was not observed in non-lesioned groups, with a **greater** urine volume being observed in **SHAM**/OT *versus* **SHAM**/PS groups ($p < 0.01$). The water balance was more negative in OT-administered *versus* PS-administered groups (Table 1, all $p < 0.05$).

Post-hoc analysis showed that urinary osmolality was **lesser** in lesioned *versus* SHAM groups (Fig. **4**C, all, $p < 0.01$) and in ME/PS *versus* ME/OT animals ($p < 0.01$), whereas it was greater in **SHAM**/OT *versus* **SHAM**/PS groups ($p < 0.05$).

The natriuretic response was **greater** in ME/OT animals than in **SHAM/OT** or ME/PS animals during this period (Fig. **4**D, all, $p < 0.01$), while urinary sodium excretion was significantly **lesser** in ME/PS *versus* **SHAM**/PS animals ($p < 0.01$). Pairwise comparisons revealed a greater natriuretic response in the **SHAM**/OT group than in the **SHAM**/PS group ($p< 0.01$) **and a less estimated plasma sodium concentration in the ME/OT group than in the SHAM/OT or ME/PS groups (Table 1, all $p < 0.01$). With respect to the SHAM animals, the estimated plasma sodium concentration was reduced in those receiving OT (SHAM/OT) than in those receiving PS (SHAM/PS) (Table 1, $p < 0.01$).**

Water and NaCl (1.5 %) intake of food-deprived ME-lesioned and SHAM animals at 30 h post- surgery (from 24 to 30 h).

Significant differences were found among groups in water intake [$H_{3,22} = 19.72$, $p < 0.0002$] and NaCl intake [$H_{3,22} = 19.78$, $p < 0.0002$]. As shown in Fig. **5**A, pairwise comparisons revealed a **greater** water intake by lesioned groups than by their respective **SHAM** groups (all, $p < 0.01$). *Post-hoc* analysis showed that water intake during this period (24-30 h post-surgery) was **greater** in groups receiving OT than in those receiving PS (all, $p < 0.01$).

With regard to the appetite for NaCl (Fig. **5**A), the intake of hypertonic saline solution was **increased** in lesioned groups *versus* their respective **SHAM** groups (all, $p < 0.01$) and in OT-administered *versus* PS-administered groups during the 6-h period between 24 and 30 h post-surgery (all, $p < 0.01$).

Water, NaCl (1.5 %), and food intake of ME-lesioned animals at 48 h post-surgery (from 30 to 48h)

Intake behavior significantly differed among the groups (water intake [$H_{3,22} = 18.63$, $p < 0.0003$], NaCl intake [$H_{3,22} = 15.27$, $p < 0.0016$], food intake [$H_{3,22} = 17.02$, $p < 0.0007$]. *Post-hoc* analysis revealed a **greater** water intake in ME-lesioned groups than in their respective **SHAM** groups (Fig. **5**B, all, $p < 0.01$) and in ME/OT *versus* ME/PS animals ($p < 0.01$). No difference was observed between **SHAM** groups during the 30-48 h period post-surgery. NaCl consumption (1.5 %) during this period was **greater** in the ME/OT group than in the **SHAM/OT** group (Fig. **5**B, $p < 0.01$) but not in comparison to the ME/PS group. No significant difference was observed between ME/PS and **SHAM**/PS groups or between the two **SHAM** groups.

Pairwise comparisons showed that food intake during the 30-48 h period post-surgery was **greater** by ME-lesioned *versus* **SHAM** animals (Table 1, all, $p < 0.01$) and **greater** by ME/OT *versus* ME/PS animals ($p < 0.01$). No significant difference was observed between **SHAM** groups.

DISCUSSION

Lesions in the ME of rats produce a well-documented neurological model of polydipsia and polyuria. ME lesions may interrupt some of the brain systems (e. g. posterior hypothalamus-hypophyseal axis) involved in hydromineral regulation, causing major derangements in the neuroendocrine control and regulation of water and sodium metabolism. [13-15, 17, 18, 32, 33, 38, 39] Besides this alteration in fluid retention mechanisms, the present study verifies previously published findings of excess renal sodium excretion by ME-lesioned animals during the first few hours post-surgery. [15] The precise mechanisms underlying this early natriuretic response have not yet been elucidated. It is therefore possible that the **electric current** used in the present study may also produce a transient activation of the affected neural tissue [20-22] **and a resulting secretion of natriuretic agents during the first few hours post-surgery. In fact, terminals of the affected axons, which end in the ME and neurohypophysis, are known to contain not only ADH but also natriuretic agents such as ANP and OT.** [23, 40] **Moreover, ADH and OT have been proposed to have synergic natriuretic effects, with their combined administration exerting an effect of greater intensity and longer duration than the sum of the effects of each neurohormone.** [27, 28]

Recent clinical data indicate that patients with the CSW syndrome, which is produced by acute brain injury (e.g., to mediobasal hypothalamic regions), **present with polydipsic and polyuric** symptoms. [41, 42] **These symptoms have been related to the excretion of large** amounts of sodium during the perioperative period, attributable to the increased activation of natriuretic peptides [2, 6, 7] (e. g. ANP or OT). In some cases of CSW, the presence **of ADH** has **also** been observed in the patients. [1, 3] **These data may suggest a parallelism between ME-lesioned animals and CSW patients. In the present study, OT was administered to ME-lesioned animals in order to examine its possible involvement in sodium excretion.**

The findings obtained confirm the natriuretic capacity of OT [17, 25, 31, 43] with ME-lesioned animals showing greater sodium excretion (vs. SHAM/OT or ME/PS) and urinary osmolality (vs. ME/PS group) at 24 h post-surgery (8-24 h period).

However, the greater natriuretic response of the ME-lesioned group receiving OT was accompanied, as previously observed [17, 44], by a marked reduction in their urine excretion and water intake, which was not observed in the lesioned group receiving saline or in the sham group receiving oxytocin. This antidiuretic effect, which has been related to the action of OT on vasopressin receptor 2, [17, 29, 45-47] would in part compensate the hypovolemic state of these animals during the 0-8 h period**;** however, it would not resolve **the hyponatremia (decreased plasma sodium, quantitatively estimated in the present study; see also [17])** induced by the ME lesion and aggravated by OT administration. This would explain the reduced water intake of the lesioned ME/OT group during the 8-24h period post-surgery**, given that the problem could be aggravated by the intake of water, the only alternative under these conditions of hyponatremia and food deprivation (48).**

All of these hydroelectrolytic abnormalities generate regulatory responses in food-deprived ME-lesioned animals to restore the volume and composition of extracellular body fluid, of which sodium is one of the main constituents. [49] Thus, the **early losses of body sodium and water in ME/PS animals would explain their greater intake of hypertonic saline solution from 24 to 30 h post-surgery in comparison to the SHAM/PS group.**
We **especially** highlight the elevated consumption of hypertonic sodium (1.5 % NaCl) from 24 h to 30 h post-surgery by ME/OT animals in comparison to SHAM/OT and ME/PS animals, which may possibly be **induced by more marked sodium losses and reductions in plasma sodium (from 8 to 24 h).**
It is likely that these behavioral responses, which may **constitute** optimal strategies to correct the volemic and electrolytic deficits observed, [44, 50, 51] **may be mediated by activation of the renin-angiotensin-aldosterone system. Indeed, the sodium depletion and hypovolemia induced by the natriuretic effects of ME lesion and OT administration and the non-availability of food are potent stimuli for activation of the renin-angiotensin-aldosterone system, [1, 51, 52] see also CSW patients, [53] stimulating sodium appetite and water intake. [54, 55]**

The greater consumption of hypertonic saline solution **as well as water by ME/OT animals helps to increase their low plasma sodium concentrations and restore extracellular volume more effectively. [56]**

The present experiment confirms previous reports on the high food consumption of ME-lesioned animals between 30-48 h post-surgery. [13-15, 17, 32, 33] This may result from the impact of the lesion on mediobasal-periventricular hypothalamic centers that critically participate in

food intake, [57, 58] including the ME. Leptin receptors are abundant in these centers and may serve as a pathway for leptin to enter the hypothalamus to exert its anorectic effect. [59, 60]

The **greater** consumption of food/sodium alongside the intake of hypertonic saline solution by ME/OT animals during the nighttime period of maximum activity (30-48 h post-surgery) and the consequent increase in osmolality/plasma sodium concentration may be responsible for the magnitude of their polydipsic response. These results support the close relationship observed by various authors between the sodium intake and polydipsic response of animals with mediobasal hypothalamic lesions. [15, 32, 61]

It is possible that electrolyte concentration imbalances in the organism can be better resolved in the present situation by the availability of food (containing 0.25 % Na), saline solution (1.5 % NaCl, aversive taste), and water.

These results are consistent with the most common approach to the restoration of volume and sodium levels in patients with CSW, who are treated with hypertonic saline solution, water, and salt tablets to minimize the pathological natriuresis and diuresis observed in this disorder. [49, 62]

Evidence has been published on the importance of the brain OT system in the regulation of sodium appetite. Thus, the capacity of OT to inhibit salt intake has been demonstrated in studies of its central administration and of its brain secretion by the parvocellular component of the PAV [52, 63-66] as well as by genetic manipulation experiments (OT gene-disruption model, see Amico et al., [67-69] However, the present findings appear to be more compatible with previous observations of sodium appetite stimulation in animals with electrolytic lesion in the posterior hypothalamus (e.g. medial tuberomammillary nuclei (E3), see [70] or mediobasal hypothalamus, including the ME (present data), as well as in intact animals. [71] The behavioral effects observed in these studies is likely related to the renal effects of OT (natriuresis) rather than to its central action. [24, 25] It should be borne in mind that OT has a short half-life in plasma when administered peripherally [10] and it is very difficult for it to reach the brain. [72]

In conclusion, electrolytic lesion of the ME interrupts both ADH and OT secretions [73, 74] and causes polydipsia, preceded by polyuria, [13, 17, 33, 34] and it is a widely used animal model for the study of neurogenic diabetes insipidus. According to the present results, the electric current may also induce natriuretic activity in these animals, possibly due to

transient activation of the affected tissue, and may therefore be useful for the study of CSW.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Figure Legends

Figure 1. Time-line of the experimental procedure followed in experiment. During the four baseline days (each small vertical bar represents one day), the animals were kept in their individual cages with food and water available. On day 0, the corresponding ME lesion or SHAM lesion was induced. At 8 h post-surgery groups were administered with oxytocin (OT) or physiological saline (PS). During the first 24 h post-surgery, animals remained in metabolic cages with ad libitum access to water but complete food deprivation. On day 2 post-surgery, animals were returned to their habitual cages with water and hypertonic saline solution (1.5% NaCl). Animals remained under food deprivation from 24 h to 30 h post-surgery. Food was available ad libitum in their cages, during the 30-48 h period post-surgery.

Figure 2: Sequential series of schematic drawings of the smallest (gray areas) and largest (hatched areas) ME lesions in a representative ME-lesioned rat (A-D). Plates correspond to coronal sections approximately -2.30 (A), -2.56 (B), -2.80 (C) and -3.14 (D) caudal to bregma according to the atlas of Paxinos and Watson (1986). (Arc) arcuate hypothalamic nucleus; (DA) dorsal hypothalamic area; (DMD) dorsomedial hypothalamic nucleus, dorsal part; (ME) median eminence; (VMH) ventromedial hypothalamic nucleus. The arrowhead indicates the lesion.

Figure 3: Water intake (A), urinary volume (B), osmolality (C), and sodium excretion (D) in rats with median eminence lesions (ME) or sham lesions (SHAM) at 8 h after surgery (before administration of Oxytocin (OT) to ME1 and SHAM1 groups and Physiological saline (PS) administration to ME2 and SHAM2 groups). Data are shown as means +SEM. When the SEM is very small, it is not visible on the graph. * Significantly different from SHAM group, $P < 0.05$ (Mann-Whitney *U* test).

Figure 4: Water intake (A), urinary volume (B), osmolality (C), and sodium excretion (D) in rats with median eminence lesions (ME) or sham lesions (SHAM) from 8 to 24 h post-surgery. At 8 h post-surgery, rats were treated with Oxytocin (OT) or Physiological saline (PS). Data are shown as means +SEM. * Significantly different from SHAM group, $P < 0.05$ (Mann-Whitney *U* test). # Significantly different from ME/PS group, $P < 0.05$ (Mann-Whitney U test). ■ Significantly different from SHAM/PS group, $P < 0.05$ (Mann-Whitney U test).

Figure 5: Amounts of water and NaCl (1.5%) (mL) ingested by oxytocin (OT) or physiological saline (PS)-treated median eminence (ME) and sham-lesioned rats from 24 to 30 h (food-deprived) (A) and from 30 to 48 h (food available) (B). Data are shown as means + SEM. When the SEM is very small, it is not visible on the graph. *Significantly different from SHAM group, $P < 0.05$ (Mann-Whitney *U* test). # Significantly different from ME/PS group, $P < 0.05$ (Mann-Whitney *U*

test). ■ Significantly different from SHAM/PS group, P < 0.05 (Mann-Whitney U test).

Table Legends

Table 1
Food intake, water "balance" and estimated plasma sodium at baseline and at different time points during the first 48h after surgery.

Values are means ± SEM in rats with median eminence lesions (ME1 and ME2) and sham rats (SHAM1 and SHAM2) treated with oxytocin (ME/OT, n = 6; SHAM/OT, n = 5) or physiological saline (ME/PS, n = 6; SHAM/PS, n = 5).
a Significantly different from SHAM group, P < 0.05 (Mann-Whitney *U* test).
b Significantly different from ME/PS group, P < 0.05 (Mann-Whitney *U* test).
c Significantly different from SHAM/PS group, P < 0.05 (Mann-Whitney *U* test).

		Baseline (day -1)	8h post-surgery		24h post-surgery		48h post-surgery
	n	Food intake (g)	Water "balance" (ml)	Estimated plasma sodium (mequiv./l)	Water "balance" (ml)	Estimated plasma sodium (mequiv./l)	Food intake (g)
ME1	6	28.00±0.67	-11.50±1.20 a	137.9±1.8 a	-2.50±3.78 b	134.9±2.2 a, b	43.83±1.64 a, b
SHAM1	5	27.60±0.83	-4.20±0.87	142.6±1.3	4.60±1.08 c	139.2±1.4 c	25.80±1.66
ME2	6	29.80±0.55	-11.30±1.10 a	138.6±1.8 a	16.70±3.88	141.8±2.1	34.80±1.42 a
SHAM2	5	29.40±1.25	-6.20±1.07	143.8±2	10.80±1.82	142.7±1.4	25.20±1.56

		Baseline (day -1)	8h post-surgery		24h post-surgery		48h post-surgery
	n	Food intake (g)	Water "balance" (ml)	Estimated plasma sodium (mequiv./l)	Water "balance" (ml)	Estimated plasma sodium (mequiv./l)	Food intake (g)
ME1	6	28.00±0.67	-11.50±1.20 a	137.9±1.8 a	-2.50±3.78 b	134.9±2.2 a, b	43.83±1.64 a, b
SHAM1	5	27.60±0.83	-4.20±0.87	142.6±1.3	4.60±1.08 c	139.2±1.4 c	25.80±1.66
ME2	6	29.80±0.55	-11.30±1.10 a	138.6±1.8 a	16.70±3.88	141.8±2.1	34.80±1.42 a
SHAM2	5	29.40±1.25	-6.20±1.07	143.8±2	10.80±1.82	142.7±1.4	25.20±1.56

Table Legends

Table 1

Food intake, water "balance" and estimated plasma sodium at baseline and at different time points during the first 48h after surgery.

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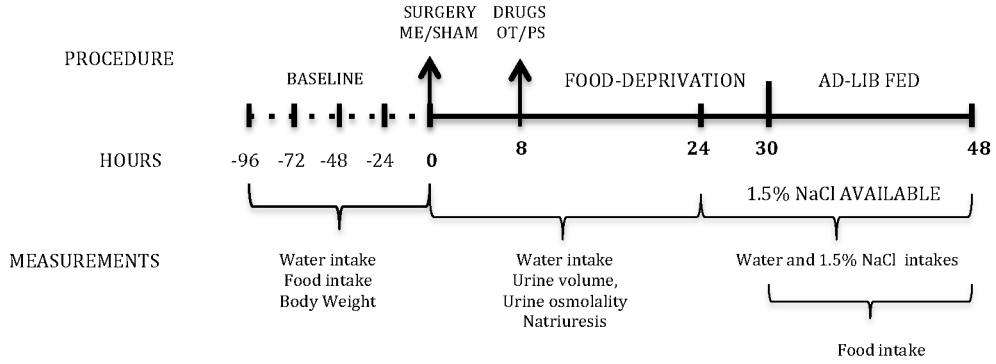
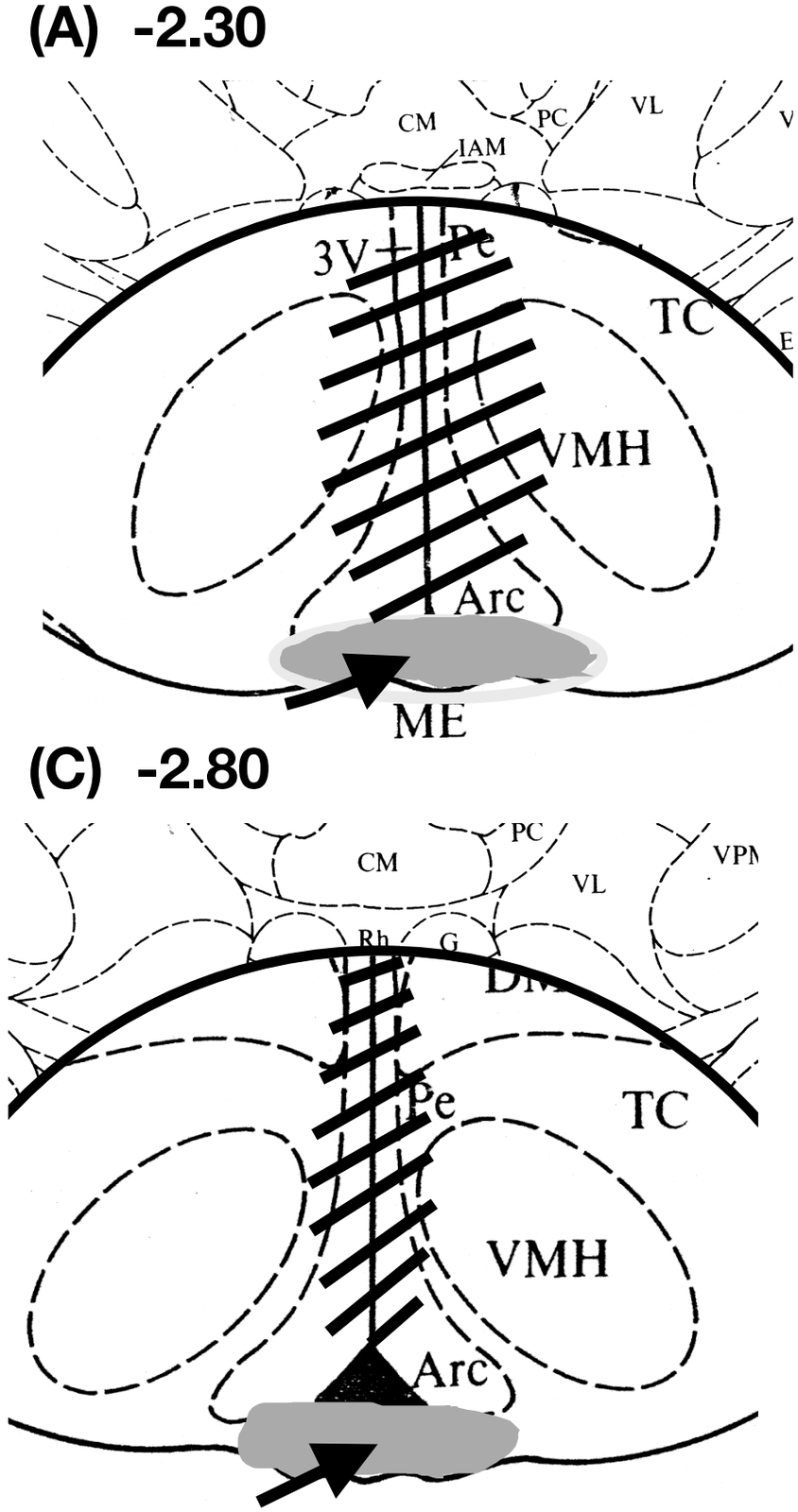
a Significantly different from SHAM group, $P < 0.05$ (Mann-Whitney *U* test).

b Significantly different from ME/PS group, $P < 0.05$ (Mann-Whitney *U* test).

c Significantly different from SHAM/PS group, $P < 0.05$ (Mann-Whitney *U* test).


SURGERY ME/SHAM
DRUGS OT/PS
PROCEDURE
BASELINE
FOOD-DEPRIVATION
AD-LIB FED
HOURS
-96 -72 -48 -24 0 8 24 30 48
1.5% NaCl AVAILABLE
MEASUREMENTS
Water intake
Food intake
Body Weight
Water intake
Urine volume,
Urine osmolality
Natriuresis
Water and 1.5% NaCl intakes
Food intake


(A) -2.30
CM
PC
VL
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Arc
ME
(C) -2.80
CM
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Rh
G
DM
Pe
TC
VMH
Arc
(B) -2.56
CM
PC
VL
CM
DA
3V
DMD
f
Pe
TC
VMH
Arc
(D) -3.14
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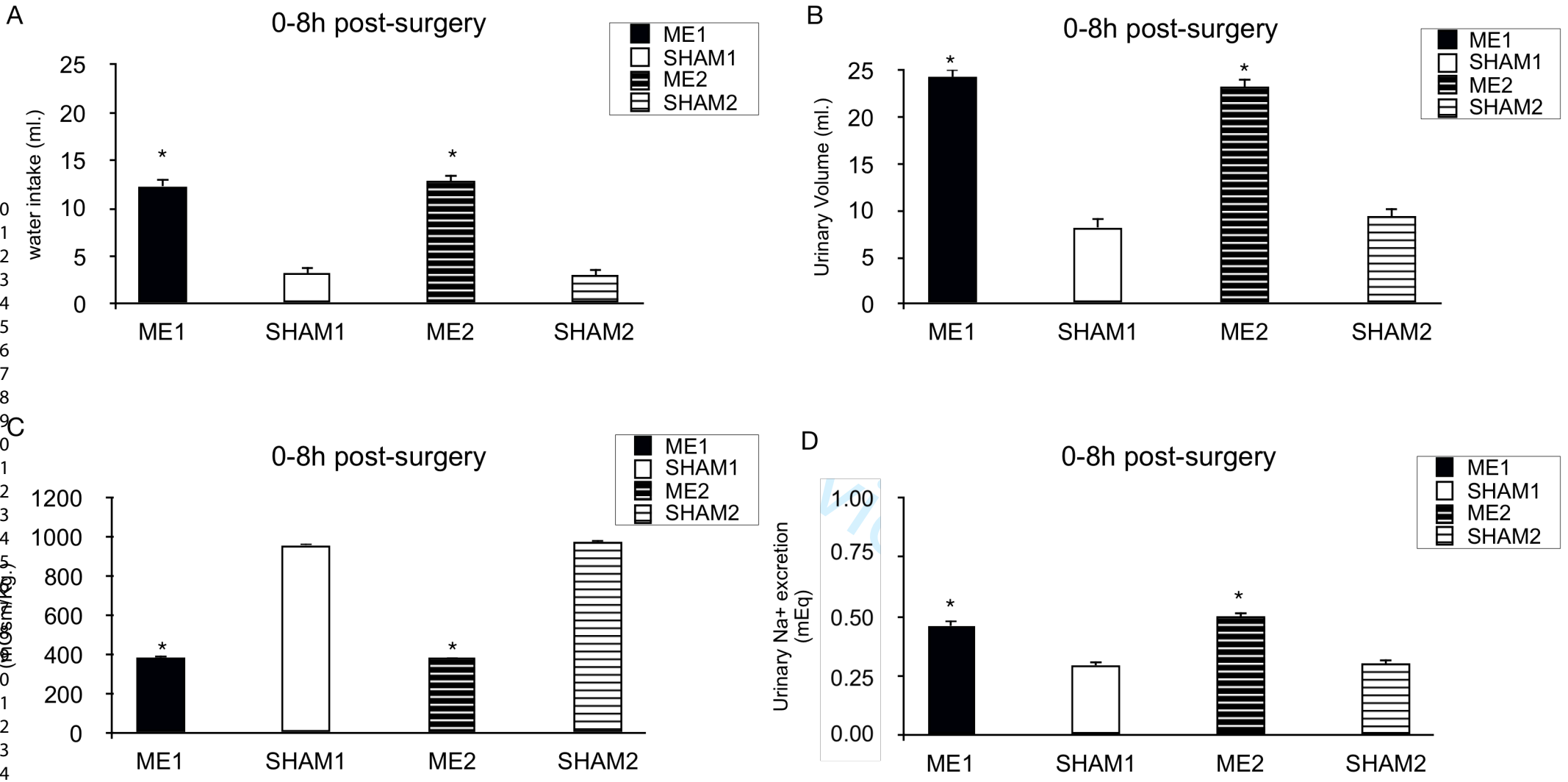
A
0-8h post-surgery
water intake (ml.)
25
20
15
10
5
0
ME1
SHAM1
ME2
SHAM2
*
*
B
0-8h post-surgery
Urinary Volume (ml.)
25
20
15
10
5
0
ME1
SHAM1
ME2
SHAM2
*
*
C
0-8h post-surgery
Urinary Osmolality (mOsm/Kg.)
1200
1000
800
600
400
200
0
ME1
SHAM1
ME2
SHAM2
*
*
D
0-8h post-surgery
Urinary Na+ excretion (mEq)
1.00
0.75
0.50
0.25
0.00
ME1
SHAM1
ME2
SHAM2
*
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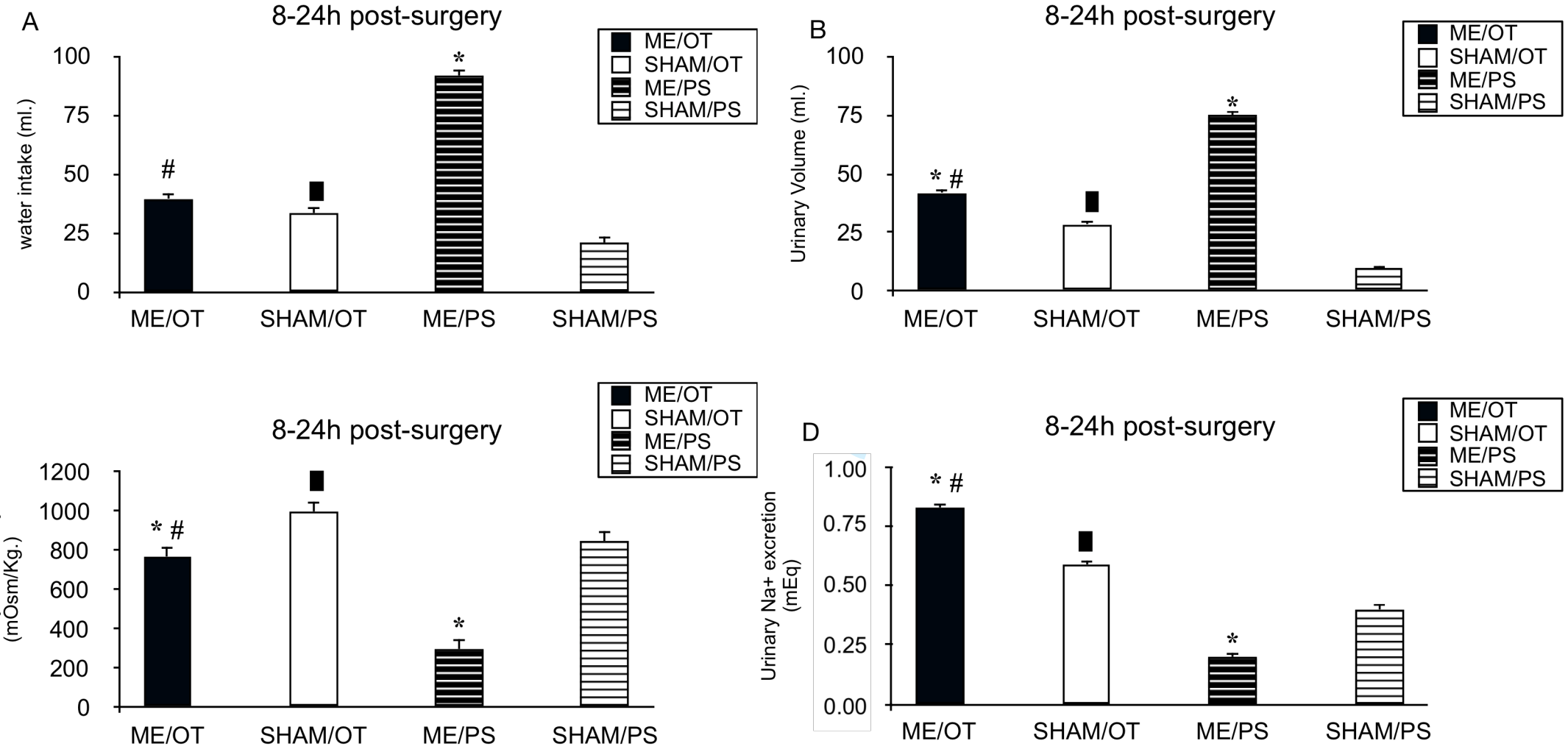
A
8-24h post-surgery
water intake (ml.)
100
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ME/OT
SHAM/OT
ME/PS
SHAM/PS
B
8-24h post-surgery
Urinary Volume (ml.)
100
75
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0
* #
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ME/OT
SHAM/OT
ME/PS
SHAM/PS
C
8-24h post-surgery
Urinary Osmolality (mOsm/Kg.)
1200
1000
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ME/OT
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8-24h post-surgery
Urinary Na+ excretion (mEq)
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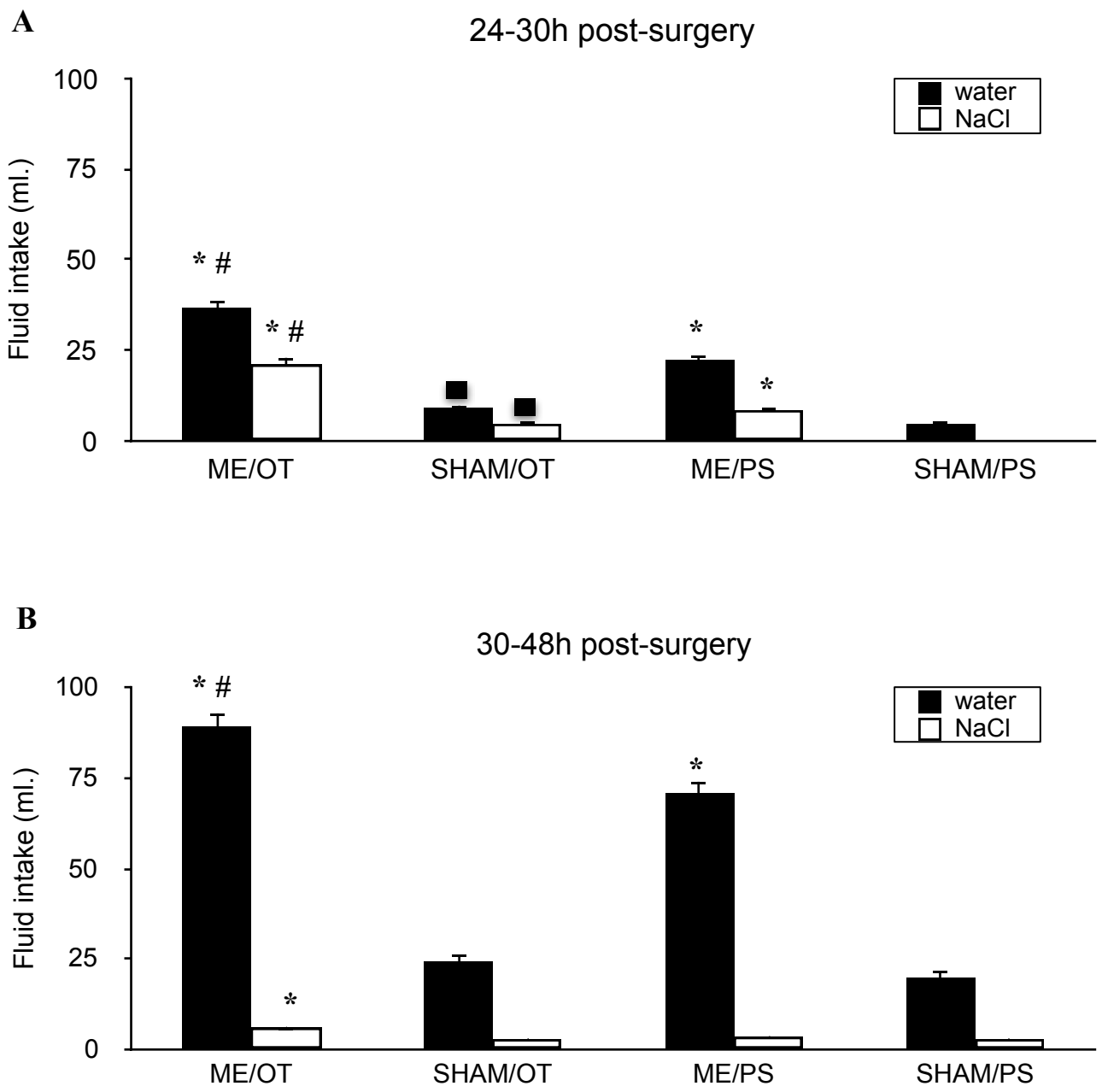
SHAM/PS


A
24-30h post-surgery
Fluid intake (ml.)
100
75
50
25
0
water
NaCl
* #
* #
*
*
ME/OT
SHAM/OT
ME/PS
SHAM/PS
B
30-48h post-surgery
Fluid intake (ml.)
100
75
50
25
0
water
NaCl
* #
*
*
ME/OT
SHAM/OT
ME/PS
SHAM/PS