Dehydration followed by sham rehydration contributes to reduced neuronal activation in vasopressinergic supraoptic neurons after water deprivation.

**Abbreviated title:** Sham rehydration and the SON

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Abstract

This experiment tested the role of oropharyngeal and gastric afferents on hypothalamic activation in dehydrated rats instrumented with gastric fistulae and allowed to drink water or isotonic saline compared with euhydrated controls (CON). Rats were water deprived for 48h (48 WD) or 46h WD with 2h rehydration with water (46+W) or isotonic saline (46+S). 46+W and 46+S rats were given water with fistulae open (46+WO/46+SO, sham) or closed (46+WC/46+SC). Compared to CON, water deprivation increased and water rehydration decreased plasma osmolality while sham rehydration had no effect. Water deprivation increased Fos staining in the lamina terminalis. However, none of the sham or rehydration treatments normalized Fos staining in the lamina terminalis. Analysis of AVP and Fos positive neurons in the SON revealed reduced co-localization in 46+WO and 46+SC rats compared to 48 WD and 46+SO rats. However, 46+WO and 46+SC rats had higher Fos staining in the SON than 46+WC or CON rats. Examination of Fos in the perinuclear zone (PNZ) revealed that sham and rehydrated rats had increased Fos staining to CON while 48 WD and 46+SO rats had little or no Fos staining in this region. Thus, pre-absorptive reflexes contribute to the regulation of AVP neurons in a manner independent of Fos expression in the lamina terminalis. Further, this reflex pathway may include inhibitory interneurons in the PNZ region surrounding the SON.
Introduction

In response to dehydration, plasma osmolality and hematocrit rise as fluid balance shifts towards the negative. These hemodynamic changes occur in spite of compensation at the level of the kidney due to the hormone arginine vasopressin (AVP). AVP is an anti-diuretic hormone of hypothalamic origin produced by the paraventricular (PVN) and supraoptic (SON) nuclei (3, 29, 34). Understanding the neurohumoral regulation of AVP output from these neurons is critical to determining CNS control of fluid and electrolyte homeostasis. Inappropriate AVP release contributes to the pathophysiology of several chronic disease states including congestive heart failure and hepatic cirrhosis (50, 56, 57, 69). Several physiological stimuli participate in the physiological regulation of AVP neurons, but the relative contributions of pre- and post-absorptive reflexes associated with fluid intake have not yet been determined.

The lamina terminalis is a key CNS region involved in the regulation of osmolality by way of modulating AVP release from the hypothalamus (9, 49). In particular, the dorsal cap of the organum vasculosum of the lamina terminalis (OVLT) and the ventral median preoptic nucleus (MnPO) contain osmo-sensitive neurons which project to the SON and PVN (42-44). The OVLT is a circumventricular organ which can respond directly to changes in plasma osmolality by way of its extensive vascular bed. On the other hand, the MnPO is bordered by an intact blood-brain barrier and receives neural input from the OVLT, among other afferent sources (35, 40). In addition to control of body fluid homeostasis, chronic activation of these areas has been associated with cardiovascular disease states such as hypertension (10, 11).
Water rehydration, particularly following bouts of dehydration, significantly reduces circulating AVP levels across several species (2, 5-7, 21, 31, 34, 53, 59, 63, 65). In addition to osmoregulatory control from the lamina terminalis, oropharyngeal afferent pathways are activated during the cephalic phase of rehydration and appear to play an important, pre-absorptive role in the regulation of AVP in many of these species (2, 7, 21, 53, 63, 65). Later, the more potent, post-absorptive inhibition of AVP release after water intake is mediated by the gastrointestinal system in the rat (59, 60), although studies using anesthetized preparations indicate that oropharyngeal afferents may contribute to the regulation of magnocellular neurosecretory cells in the rat (1, 55).

In the present study, we tested the hypothesis that sham rehydration following dehydration would contribute to the inhibition of SON magnocellular neurons. In rodents implanted with gastric fistulae, we removed water access for 46 hours. Then, we presented these rodents with either water or physiological saline for a 2 hour rehydration period while shunting ingested water through the gastric fistulae away from the intestinal tract.
Methods

All experiments were conducted on adult male Sprague-Dawley rats (200-300 g bw, Charles River). Prior to surgery, rats were individually housed in a temperature-controlled room on a 14:10 light:dark cycle with light onset at 700 h. Food and water were available *ad libitum* except during the dehydration experiments; food was not re-supplied during rehydration periods. Experiments involving rehydration were conducted and terminated within the early portion of the light phase. Water and saline were provided at room temperature. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio according to NIH guidelines.

**Gastrointestinal fistulation**

Each rat was anesthetized with isofluorane (drop jar) and maintained with 2% isofluorane delivered by an atomizer with O₂. An abdominal midline incision was made and rats were implanted with a 14mm gastric fistulae (UTHSCSA Instrumentation Services) through the non-glandular gastric mucosa as previously described (19). The 6mm opening in each fistula was kept sealed by a 10mm screw until opened for the experimental protocol. Animals were allowed to recover from the surgery until food and water intake returned to pre-surgical levels (2 week minimum).

**Protocol**

Fistulated rats were randomly divided into five treatment groups: Control (CON), 48 hour water deprived (48 WD), 46 hour deprived followed by 2 hours rehydration with fistulae closed (46+WC), 46 hour deprived followed by 2 hours sham rehydration with fistula open (46+WO), 46 hour deprived followed by 2 hours rehydration with isotonic
(0.9%; w:v) saline with fistula closed (46+SC), and 46 hour deprived followed by 2 hours sham rehydration with isotonic saline with fistula open (46+SO). All rats were pre-acclimated and housed in individual metabolic cages that allowed for the collection of recovered fluids and measurement of fluid intake. To ensure the patency of open fistulae, animals were periodically monitored during rehydration to allow for adequate shunting of ingested fluid. During the 2 hour rehydration period, recovered fluid and fluid intake were also measured and compared to determine efficacy of the sham rehydration; sham-rehydrated (46+WO, 46+SO) rats that ingested 3ml greater than the volume of the recovered fluid were eliminated. In order to control for unrecovered fluid, a separate group of unoperated rats were dehydrated for 46 hours and given 2 hours of access to 3ml of water (46+3ml). Due to the preceding 46 hour deprivation period, the presence of urine in the recovered fluid was negligible.

Immediately following the experiment, rats were anesthetized with thiobutabarbital (Inactin from Sigma, 100mg/kg ip) and a 1-2 ml sample of whole blood was collected via cardiac puncture for measuring plasma osmolality and hematocrit as previously described (22). Plasma osmolality was measured using a vapor pressure osmometer (Wescor, Logan, UT). Rats were perfused transcardially with PBS followed by 4% paraformaldehyde. Brains were removed and placed in PBS with 30% sucrose for 2-3 days until sunk. Brains were then sectioned in a cryostat. Three sets of coronal 40 μm sections were preserved in cryoprotectant and stored at -20°C until they were processed for immunohistochemistry.
Immunohistochemistry

Separate sets of serial forebrain sections were stained for c-Fos (Rabbit anti-c-Fos Ab5, Calbiochem, San Diego, CA) as previously described (29, 34). Sections were incubated with the primary antibody (1:30,000) for 72 h at 4°C. After rinsing, the sections were processed with a biotinylated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS for 2 h at room temperature. Sections were reacted with an avidin-peroxidase conjugate (Vectastain ABC Kit; Vector Laboratories) and PBS containing 0.04% 3,3’–diaminobenzidine hydrochloride (DAB) and 0.04% nickel ammonium sulfate for 10 to 11 minutes. Sections were processed for vasopressin immunofluorescence using a Guinea pig anti-(Arg8) Vasopressin primary antibody (T-5048; Peninsula Laboratories, San Carlos, CA, 1:10,000) and a Cy3 conjugated anti-Guinea pig secondary antibody (1:250, Jackson ImmunoResearch, West Grove, PA). Sections were mounted on gelatin coated slides, air dried for 1-2 days and the slides were cover slipped with Permount.

Image processing and Co-localization

Tissue sections containing regions of interest were inspected using an Olympus microscope (BX41; Olympus Imaging, Center Valley, PA) equipped for epifluorescence. Digital images were acquired using an attached camera (Olympus DP70) connected to a Dell GX260 running Digital Photo Plus imaging software (v.2.2.1.227). Images were adjusted for uniform brightness and contrast. Regions of interest were identified using the rat brain stereotaxic atlas of Paxinos and Watson (51). One to three sections of the OVLT, two to four sections of the ventral MnPO, and four to six sections of the SON and
PNZ were analyzed from each rat and the number of Fos+ cells was recorded for each section and averaged.

Sections containing the supraoptic nucleus were analyzed for Fos and AVP double immunostaining. High magnification images of multiple labeling were captured with a Qimaging camera attached to an IX50 Olympus converted to a DSU confocal microscope with an attached mercury lamp for fluorescence. Because Fos was analyzed on a bright field, the image was inverted and adjusted to remove background artifacts. This inverted image was pseudo-colored green and merged with the AVP image on a dark field to observe co-localization. Because Fos (green) is a nuclear stain and AVP (red) is a cytoplasmic stain co-localization does not often merge to yellow. Therefore, cells with a green nucleus and red cytoplasm are considered “co-localized”. Percent co-localization in AVP neurons is expressed as: \#Fos^+AVP^+ cells / \# total AVP^+ cells * 100. Percent colocalization in Fos^+ neurons is expressed as: \#Fos^+AVP^+ cells/ \#total Fos^+ cells * 100.

Statistics

Data were analyzed by one-way analysis of variance with student Newman-Keuls (SNK) test for posthoc analysis of significant main effects (SigmaStat, v. 2.03, Systat Software Inc., Point Richmond, CA). Significance was set at p < 0.05. All values are presented as means ± SEM.
Results

Hemodynamic and Ingestive responses

As expected, water deprivation (48 WD) increased plasma osmolality and hematocrit. Each of the rats provided with 3 ml of water (46 +3 ml) drank the entire volume of fluid and their plasma osmolality and hematocrit was not different from 48 WD (Table 1). Plasma osmolality and hematocrit in rats rehydrated with physiological saline (46 + SC) were not different from control (Table 1). Further, water rehydration (46+WC) significantly reduced osmolality below euhydrated CON rats (Table 1). On the other hand, sham rehydration with either water (46+W0) or saline (46+SO) did not significantly reduce osmolality or hematocrit compared to 48 WD rats. Following deprivation, 46+SC drank slightly more fluid than 46+WC rats but the difference was not significant. On the other hand, 46+W0 and 46+SO rats ingested significantly more fluid than the corresponding groups with closed fistulas (Table 1).

Lamina Terminalis

Controls showed very little Fos immunoreactivity in either the MnPO or OVLT. As expected, 48 hour water deprivation increased Fos staining in both of these regions. Further, 2 hour rehydration and sham rehydration treatments did not affect Fos staining in the MnPO or OVLT (Fig. 1). There were no detectable differences in Fos staining in the dorsal cap and lateral margin sub-regions of the OVLT associated with any of the treatments (data not shown). Quantification of stained images for each treatment is displayed in Figure 2.

Supraoptic Nucleus
Again, there was negligible Fos immunoreactivity in the SON of control, euhydrated rats (Figure 3). Water deprivation increased Fos staining in the SON and both water rehydration and sham water rehydration significantly reduced Fos staining as compared to water deprivation (Table 2). In water rehydrated rats (46+WC), Fos staining in the SON was significantly decreased to the level of controls. However, Fos immunoreactivity in the SON of sham water rehydrated rats (46+WO) was significantly decreased when compared to water deprivation but still significantly higher than control (Table 2). On the other hand, providing rats with 3 ml of water following WD (46 + 3 ml) was associated with Fos staining significantly higher than control and not different from 48 WD (Table 2). Similarly, neither saline rehydration (46+SC) nor sham saline rehydration (46+SO) significantly reduced Fos staining in the SON as compared to water deprivation.

Water deprivation and all rehydration treatments had no significant effect on AVP staining (Table 2). Two hour water rehydration (46+WC) reduced %AVP colocalization to control levels while sham rehydration attenuated %AVP colocalization compared to water deprived levels but slightly elevated above control and 46+WC levels (Fig. 3). Saline rehydration (46+SC) also attenuated %AVP colocalization compared to water deprived rats but saline sham rehydration (46+SO) was not significantly different from 48 WD rats. Quantification of stained images for each treatment is noted for Fos and AVP immunoreactivity as well as % of Fos+ nuclei observed in AVP neurons and % of AVP neurons observed in total number of Fos+ neurons (Table 2). Representative images of co-localization are displayed in Figure 4.
Perinuclear Zone

The perinuclear zone (PNZ), defining the tissue region of the lateral hypothalamus surrounding the SON, was somewhat active at control levels (Fig. 5). Water deprivation reduced Fos staining in the (PNZ) to trace immunoreactivity while both water rehydration and sham water rehydration were increased compared to control. Saline rehydration significantly increased Fos staining in the PNZ to control levels while Fos staining following sham saline rehydration failed to reach control levels. Quantification of stained images of each treatment is displayed in Figure 6.
Discussion

There have been many studies concerning the role of the lamina terminalis and neurosecretory hypothalamic nuclei in the regulation of hydromineral balance \((18, 22, 29, 34, 37, 41, 42, 46, 52, 60, 62, 70)\). The presented evidence, however, furthers this body of work by using a unique, conscious animal approach to sham rehydration involving gastric fistulae that divert ingested fluid away prior to intestinal absorption. Further, the effects of isotonic sham rehydration are utilized to expand upon similar fistulation studies performed previously \((45, 54, 59)\) to determine the osmotic contribution of the pre and post-absorptive, rehydration stimuli on distinct forebrain pathways that regulate circulating levels of vasopressin.

According to the osmolality data, gastric fistulation successfully prevented any significant rehydration in sham-rehydrated animals whether they ingested water or saline. Further, the ingested volume for sham-rehydrated rats was increased compared to rehydrated rats with closed fistulae. These data suggest persistent thirst during sham rehydration with either water or saline as others have reported \((36, 61)\), which is consistent with hyperosmolality and elevated hematocrit. Dehydration reportedly increases sodium appetite \((17, 18)\) and stimulates natriuresis \((30)\); however we were unable to uncover a significant increase in saline intake following dehydration and osmolality in saline rehydrated rats returned to normal. Further, sham saline rehydrated rats drank less fluid than sham water rehydrated rats. This suggests that the effects of thirst or the effects of hedonic and appetitive behaviors on saline rehydration \((47)\) are minimal at this low saline concentration.
Two hours of rehydration following two days of deprivation were unable to reverse the increased Fos immunoreactivity in regions of the lamina terminalis that control AVP release and fluid intake. This lack of change includes sub-regions of the OVLT such as the robustly-osmosensitive dorsal cap (44, 49). This suggests that neither pre-absorptive nor hemodynamic stimuli are sufficient to inhibit activity in these regions within the time frame of this study. These observations are important because we have previously observed gradual stimulation of the OVLT and MnPO dependent upon the duration of dehydration (34). However, Fos staining has several limitations (16) and there is evidence that the OVLT contains both excitatory and inhibitory efferents (48), the balance of which may be shifted in the transition from dehydration to rehydration. The difference in Fos expression patterns between groups, as evidenced in Fig. 1, suggests that more thorough study of these responses are warranted.

In contrast to the lamina terminalis, Fos staining in the SON was significantly affected by rehydration and sham rehydration with water. As previously reported (22), water rehydration reduced Fos staining in the SON to control levels. Sham water rehydration also significantly reduced SON Fos immunoreactivity but to a lesser degree. The effects of sham water rehydration are not likely due to absorption of unrecovered water or changes in plasma volume or osmolality. This is based on the observation that rats ingesting 3 ml of water (the cut off used for inclusion in sham rehydration group) had significantly higher Fos staining in the SON compared to both water rehydration groups. Further, the average hematocrit and osmolality of the sham water rehydrated group was not different from values obtained after 48 WD. This suggests that both pre- and post- absorption signals associated with water intake can influence SON neurons.
Published research suggests that osmosensitive oropharyngeal reflexes participate in rapid inhibition of AVP neurons in the rat. Shingai et al (58) demonstrated that infusions of water into the oral cavity of anesthetized rats produces an increase in urine flow. Further, oral hypertonic or hypotonic fluid application increases or decreases the activity of magnocellular neurosecretory cells (1, 55). The osmotic contribution to these reflexes was further explored in this study. We demonstrated that supraoptic neurons are differentially regulated by water and saline rehydration. While SON Fos staining was not decreased by saline rehydration, there was an ~50% reduction in Fos$^+$ AVP$^+$ neurons (see Table 2), suggesting that saline rehydration increased Fos in oxytocin neurons of the SON. Although dehydration induces Fos in both oxytocin and vasopressin neurons, there seems to be a specific effect of saline rehydration on oxytocin cells in the SON as supported by our previous report (22). The current study suggests, however, that this effect is likely post-absorptive as sham saline rehydration had no effect on co-localization compared to dehydrated animals. Several studies support the notion that oxytocin plays a post-prandial, compensatory role on salt balance. For example, oxytocin stimulates natriuresis (30), including natriuresis as a result of volume expansion (26). Further, the post-prandial neuropeptide secretin increases oxytocin neuronal activity (68) and may be involved in co-release of AVP and oxytocin during dehydration (12).

The observations from the lamina terminalis coupled with the effects of sham rehydration suggest a purely systemic “osmo-sensing” hypothesis is too limited to explain regulation of magnocellular secretory neurons. In fact, these data suggest an alternative regulatory mechanism that decreases Fos expression in SON AVP neurons
following both sham and true rehydration, at least within a few hours. One possibility is
the presence of intrinsic osmo-sensation from the SON and PVN neurons themselves
(8, 9, 38). However, lesions of the OVLT and MnPO only partially attenuate AVP
release due to osmotic stimulation (39, 64) suggesting an independent pathway that
may not be regulated by changes in systemic osmolality.

While intrinsic osmosensitivity may explain changes in vasopressin that occur
independently of the lamina terminalis, it does not explain changes in this tone during
sham rehydration. In this situation, there is evidence to support an afferent reflex arc
from the nucleus tractus solitarius (NTS) tracing back to sensory afferents on the
superior laryngeal branch of nerve X, the pharyngeal branch of nerve IX, and the chorda
tympani of nerve VII (20, 27, 66). Oral application of water, a hypotonic stimulus, has
been demonstrated as a negative regulator of vasopressin release regardless of
whether or not this bolus is absorbed (1). Further, sham water deglutition stimulates
diuresis in anesthetized rats and this effect is blocked by bilateral sectioning of the
superior laryngeal nerve (58). Lastly, the NTS communicates extensively with a
network of nuclei participating in cardiovascular regulation of vasopressin release that
includes the Diagonal Band of Broca (DBB) (14, 23), locus ceruleus (LC) (24), and
parabrachial nucleus (PBN) (22). Therefore, these regions could participate in an
alternative pathway involved in pre-absorptive regulation of AVP and will be examined
in our future endeavors.

The previously-described network including the NTS modulates vasopressin in
response to changes in blood pressure (15, 23, 24) through the perinuclear zone, a sub-
region of the lateral hypothalamic area dorsomedial to the SON (13, 32, 33). The PNZ
contains inhibitory interneurons (28, 33, 67) that reduce activity of phasic AVP neurons in the SON (4, 14, 25). We have previously demonstrated that Fos staining is increased in the PNZ following water but not saline rehydration (22). Our current data is consistent with these observations and further suggests that pre-absorptive mechanisms contribute to rehydration-induced increases in PNZ Fos. Further, our data demonstrate that saline rehydration or sham saline failed to increase Fos in the PNZ suggesting that pre-absorptive increases in PNZ Fos are dependent on the osmolality of the ingested fluid.

Our data suggest several key findings: 1) the MnPO and OVLT may not be involved in osmosensitive, pre-absorptive reflexes or acute post-absorptive reflexes during rehydration, 2) SON AVP neurons are regulated by pre-absorptive and post-absorptive stimuli during rehydration, and 3) the PNZ may be involved in pre-absorptive regulation of vasopressin in an osmo-dependent manner. These results, taken with published data, suggest that capable, physiological regulation of vasopressin can occur in the absence of systemic, osmotic stimuli. However, additional studies looking directly at AVP release will be required to further test this hypothesis. In addition, this pre-absorptive regulation appears to be sensitive to the osmolality of the fluid exposed to the oropharyngeal reflex pathways, as evidenced by isotonic saline rehydration and sham saline rehydration. Therefore, future studies will be aimed at understanding the specific afferent and CNS components of this pre-absorptive reflex arc that contribute to reduced vasopressin signaling regardless of systemic osmolality.
Acknowledgments

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Figure Captions:

Figure 1: Representative photomicrographs depicting Fos staining in the MnPO (left column) and OVLT (right column) for each of the treatments labeled in the left-hand column. Treatment groups are Control (CON); 48h water deprived (48 WD); 46h water deprived + 2h H2O access with fistulae closed (46+WC); 46h water deprived + 2h H2O access with fistulae open (46+WO, sham); 46h water deprived + 2h 0.9% SAL access with fistulae closed (46+SC); 46h water deprived + 2h 0.9% SAL access with fistulae open (46+SO, sham). Scale bar = 100µm

Figure 2: Effects of dehydration and subsequent sham or true rehydration on Fos staining in the MnPO (A) and OVLT (B). Dividing line separates experimental controls from rehydrated groups. Data are expressed as means ± sem. *p<0.05 vs. all.

Figure 3: Representative photomicrographs depicting Fos staining, AVP staining, and composite images of Fos+AVP (merge; Fos is pseudo-colored green) in the supraoptic nucleus (SON) for the treatments labeled on the left hand side. Treatment groups are Control (CON); 48h water deprived (48 WD); 46h water deprived + 2h H2O access with fistulae closed (46+WC); 46h water deprived + 2h H2O access with fistulae open (46+WO, sham); 46h water deprived + 2h 0.9% SAL access with fistulae closed (46+SC); 46h water deprived + 2h 0.9% SAL access with fistulae open (46+SO, sham). Scale bar = 100µm

Figure 4: Representative photomicrographs depicting composite confocal images of Fos+AVP staining (Fos is pseudo-colored green) in the SON for the treatments labeled on the left hand side. Treatment groups are 48h water deprived (48 WD); 46h water deprived + 2h H2O access with fistulae closed (46+WC); 46h water deprived + 2h 0.9% SAL access with fistulae closed (46+SC); 46h water deprived + 2h 0.9% SAL access with fistulae open (46+SO). Not depicted: CON and 46+WC groups, which showed only trace co-localization. Scale bar = 25µm

Figure 5: Representative photomicrographs depicting Fos staining in the perinuclear zone (PNZ). Treatment groups are Control (CON); 48h water deprived (48 WD); 46h water deprived + 2h H2O access with fistulae closed (46+WC); 46h water deprived + 2h H2O access with fistulae closed (46+WO, sham); 46h water deprived + 2h 0.9% SAL access with fistulae closed (46+SC); 46h water deprived + 2h 0.9% SAL access with fistulae open (46+SO, sham). (Reference points key: SON=supraoptic nucleus; ot=optic tract). Scale bar = 100µm

Figure 6: Effects of dehydration and subsequent sham or true rehydration on Fos staining in the PNZ surrounding the SON. Dividing line separates experimental controls from rehydrated groups. Data are expressed as means ± sem. Treatments with unique subscripts are significantly different (p<0.05).
References


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<table>
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<td>45.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>296 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>48 WD</td>
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<td>307 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>46+3ml</td>
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<td>308 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>46+WC</td>
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<td>278 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>56 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
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Data are expressed as means ± sem. Values with unique superscript letters are significantly different (p<0.05). CON=Control; 48 WD = 48h water deprived; 46+3ml = 46h water deprived + 2h H2O access to 3ml of H2O; 46+WC = 46h water deprived + 2h H2O access with fistulæ closed; 46+WO = 46h water deprived + 2h H2O access with fistulæ open; 46+SC = 46h water deprived + 2h 0.9% SAL access with fistulæ closed; 46+SO = 46h water deprived + 2h 0.9% SAL access with fistulæ open. n=6-7.
Knight, et al. Figure 1

MNPO | OVLT

CON | 
48HWD | 
48+WC | 
48+WO | 
48+SC | 
48+SO |
Knight, et al. Figure 2

A. MNPO

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<tr>
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<tr>
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B. OVLT

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<td>46+SO</td>
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Table 2: Effect of Rehydration or Sham Rehydration on Fos and AVP co-localization in the SON

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<th>AVP+ cells</th>
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<td>CON</td>
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<td>49.7 ± 4.7</td>
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<td>56.4 ± 9.7a</td>
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<td>48 WD</td>
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</tr>
<tr>
<td>46+3ml</td>
<td>40.3 ± 5.3b</td>
<td>53.1 ± 3.5</td>
<td>43.9 ± 1.8b</td>
<td>58.9 ± 3.9a</td>
</tr>
<tr>
<td>46+WC</td>
<td>2.7 ± 0.5a</td>
<td>58.8 ± 3.6</td>
<td>2.3 ± 0.5a</td>
<td>54.1 ± 9.5a</td>
</tr>
<tr>
<td>46+WO</td>
<td>21.6 ± 7.1c</td>
<td>59.8 ± 4.0</td>
<td>20.6 ± 6.1c</td>
<td>59.4 ± 5.1a</td>
</tr>
<tr>
<td>46+SC</td>
<td>50.8 ± 4.8b</td>
<td>61.8 ± 1.5</td>
<td>28.0 ± 2.8c</td>
<td>27.9 ± 2.8b</td>
</tr>
<tr>
<td>46+SO</td>
<td>32.1 ± 8.7b</td>
<td>50.1 ± 3.9</td>
<td>41.2 ± 2.0b</td>
<td>48.7 ± 13.6a</td>
</tr>
</tbody>
</table>

Data are expressed as means ± sem. Values with unique superscript letters are significantly different (p<0.05). CON=Control; 48 WD = 48h water deprived; 46+3ml = 46h water deprived + 2h H2O access to 3ml of H2O; 46+WC = 46h water deprived + 2h H2O access with fistulae closed; 46+WO = 46h water deprived + 2h H2O access with fistulae open; 46+SC = 46h water deprived + 2h 0.9% SAL access with fistulae closed; 46+SO = 46h water deprived + 2h 0.9% SAL access with fistulae open.
Knight, et al. Figure 6

Avg # of PNZ c-Fos+ cells

46 WD + 2h RH:
- 46+WC
- 46+WO
- 46+SC
- 46+SO

CON
48 WD
48 WD + 2h RH: