Subfornical organ disconnection alters Fos expression in the lamina terminalis, supraoptic nucleus and area postrema after intragastric hypertonic NaCl.

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Running head: CVOs and Osmoreception.

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Abstract

The lamina terminalis was severed by a horizontal knife cut through the anterior commissure in order to determine the effect of a disconnection of the subfornical organ (SFO) on drinking and Fos-like immunoreactivity (Fos-ir) in the rat brain in response to an intragastric load of hypertonic saline (5 ml/kg of 1.5 M NaCl by gavage). After an initial load, knife-cut rats drank significantly less water than sham-cut rats, thus confirming a role for the SFO in osmotic drinking. After a second load at least a week later, the rats were not allowed to drink after the gavage and were perfused for analysis of Fos-ir at 90 min. Compared to sham-cut rats, the knife-cut rats displayed significantly elevated Fos-ir in the main body of the SFO, in the dorsal cap of the organum vasculosum laminae terminalis, and in the ventral median preoptic nucleus after the hypertonic load. The knife cut significantly decreased Fos-ir in the supraoptic nucleus. Fos-ir was expressed mainly in the mid-coronal and caudal parts of the area postrema of sham-cut rats, and this expression was greatly reduced in knife-cut rats. These findings strengthen the case for the presence of independently functioning osmoreceptors within the SFO and suggest that the structures of the lamina terminalis provide mutual inhibition during hypernatremia. They also demonstrate that the Fos-ir in the area postrema after intragastric osmotic loading is heavily dependent on the intact connectivity of the SFO.

Keywords: Organum vasculosum laminae terminalis, immediate early genes, osmoregulation, circumventricular organs
Osmoreceptive cells have been identified in a variety of neural structures, but early work in dogs and sheep demonstrated that the osmoreceptors that are most important for eliciting drinking and neurohypophyseal responses must reside outside the blood-brain barrier (17, 29). Since that time, much evidence has been presented to support the claim for osmoreceptor (or sodium-receptor) functions in the sensory circumventricular organs of the brain (1, 18), which lack a blood-brain barrier, and in the region of the gut or portal vein in the abdomen (2, 12, 21, 31).

Intragastric osmotic loads may stimulate drinking behavior or the secretion of vasopressin and oxytocin through either the gut or circumventricular routes. Intragastric fluid loads that increase osmolality in the portal circulation without elevating systemic osmolality can cause drinking and neuroendocrine secretions and can increase the expression of Fos in related areas of the hypothalamus and brain stem (2, 12, 31). These effects appear to be mediated by abdominal neural osmoreceptors or sodium receptors that signal the brain via the vagus or splanchnic nerves. Larger intragastric loads that are sufficient to increase plasma osmolality, and therefore to engage osmoreceptors in the circumventricular organs, also cause robust drinking responses and neurohypophyseal secretions. Responses to hypernatremia in general may be blunted by lesions of one or more of the sensory circumventricular organs (3, 9, 10, 15, 16, 20, 23, 25, 26, 28, 30). These organs include the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT) in the forebrain, and the area postrema (AP) in the hindbrain, all of which may contain Fos-like immunoreactivity (Fos-ir) after appropriately large doses of intragastric sodium (6, 7, 11, 19, 22, 23, 27).

Studies have revealed that osmotic stimulation must be great in order to induce Fos expression in the forebrain circumventricular organs (7, 27). Small osmotic loads that cause
significant amounts of Fos expression in the supraoptic (SON) or paraventricular (PVN) nuclei of the hypothalamus may induce little Fos-ir at all in the SFO or OVLT. This finding is curious if these two circumventricular organs indeed contain the principal osmoreceptors for generating drinking and neurohypophyseal activation. A possible contrary conclusion is that these circumventricular organs express Fos only as a consequence of synaptic drive from other more osmosensitive areas of the brain. Neither the lesion studies nor the Fos studies prove that these circumventricular organs contain important osmoreceptors that can function independently from other structures in the brain.

In a recent study, we disconnected the SFO from the rest of the brain with a wire knife cut at the level of the dorsal median preoptic nucleus (MnPO) or anterior commissure and examined the effects of the cut on Fos-ir in the hypothalamus following a mild osmotic load delivered into the stomach (26). Fos-ir was very sparse in the SFO and OVLT of both knife-cut and sham-cut rats. Nevertheless, the cut greatly blunted Fos-ir in the SON and PVN compared with the sham-cut rats. This demonstrated that an intact connectivity of the SFO was critical for a full expression of Fos-ir in the hypothalamus at this level of stimulation even though the circumventricular organs themselves were not showing strong Fos-ir. The results provided some evidence in favor of the location of principal osmoreceptors in the circumventricular organs, but they did not prove the case.

Our experiment (26) would have made a stronger case for independently functioning osmoreceptors in the SFO if we had been able to demonstrate that the osmotic stimulus caused an expression of Fos-ir in the SFO that was not eliminated by a disconnection of the SFO from the rest of the brain. Because the Fos-ir was absent in the SFO of intact rats, our previous study was not able to address that question. In the present study, we repeated that
experiment with a higher dose of intragastric hypertonic NaCl in an attempt to produce more Fos-ir in the circumventricular organs. If we found that a larger osmotic load caused Fos-ir in the SFO that was eliminated by a disconnection, it would question whether the SFO contained independently functioning osmoreceptors. If the Fos-ir continued to be expressed in SFO after the disconnection, the results would support the existence of independent osmoreceptors in that circumventricular organ.

**Materials and Methods**

**Animals**

Subjects were 21 male Long-Evans rats weighing 300-500 g at the beginning of the experiments. They were obtained from the vivarium at the University of Washington Department of Psychology and housed individually in hanging wire mesh cages with Harlan Teklad laboratory chow and tap water continuously available except during experiments. Room lights were on 12 h per day and temperature was held at 23 °C. Sample sizes after histology are given in the results. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Washington.

**Surgery**

Rats were anesthetized with Equithesin (0.35 mg/kg) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda, and a 2.5 mm hole was drilled immediately caudal to bregma. The sinus was retracted, and a knife cut of the rostroventral pole of the SFO was made using a Tungsten wire knife sheathed in the needle of a 1-µl syringe (26). The transection was aimed at the rostral wall of the third ventricle ventral to the SFO near the anterior commissure. The needle was lowered below the sinus at 1.6-1.8
mm caudal to bregma. The wire knife was then extended in an arc 2 mm rostrally at the midline and then rotated 90° to the left and to the right. Sham-cut surgery consisted of retracting the sinus and lowering the needle at 1.6-1.8 mm caudal to bregma just enough to penetrate the cerebral cortex. The hole in the skull was sealed with bone wax and the wound was closed with Vetbond. Betadine was applied topically to the wound and the rats recovered on a warm heating pad prior to being returned to their home cages upon waking. Rats were allowed to recover for at least 2 weeks before experimentation, at which time all body weights were greater than or equal to post-operative levels.

*Intragastric treatments*

All rats underwent at least 5 practice gavage treatments during the weeks preceding testing in order to adapt them to the gavage procedure and thus reduce stress response to the treatment. Each practice gavage consisted of an infusion of 2 ml of tap water through a gavage tube. As a result of the practice procedure, the rats were docile during testing.

*Drinking Test*

The drinking test was administered at least 1 week before the c-Fos assay experiment. On the day of the experiment, the rats were presented with burettes filled with tap water 30 min before testing began in order to acclimate the rats to the fresh water. Rats were then administered an intragastric load of 1.5 M NaCl at 0.5% BW and water intakes were recorded to the nearest 0.1 ml at 15, 30, 60, and 90 min. At the end of testing, food and water were returned to the rats.

*Histology and Fos-ir Analysis*

After the second load at least a week later, the rats were not allowed to drink after the gavage and were perfused for analysis of Fos-ir at 90 min. Fos-ir was demonstrated using the
avidin-biotin-peroxidase technique. Rats were deeply anesthetized with pentobarbital and perfused through the heart with phosphate buffered saline (PBS, 0.1 M pH 7.4) for exsanguination followed by 4% paraformaldehyde in PBS. Brains were removed and post-fixed in 4% paraformaldehyde overnight and then in a 25% sucrose and PBS solution for an additional 24 h for cryoprotection. The tissue was sectioned coronally on a freezing microtome at 50 µm. Sections were rinsed in PBS, soaked in a methanol-hydrogen peroxide solution for 20 min, soaked in 3% goat serum for 60 min, and incubated chilled with primary Fos antibody (rabbit polyclonal IgG, diluted 1:14,000, Santa Cruz Biotechnology), goat serum, and Triton X-100 for 48 h. Rinsed sections were then incubated in biotinylated goat antirabbit antibody for 1 h and processed using the Vectastain ABC kit (Vector laboratories) for 1 h. They were rinsed again, treated with hydrogen peroxide with diaminobenzadine as the chromogen for 2-3 min, mounted on subbed slides, dried, and coverslipped. Hindbrain sections were counterstained with neutral red before coverslipping. Both treatments were included in each batch.

Brains were also examined for placement of the knife-cut after the Fos-ir assay was complete. Knife-cuts were considered complete if the cut encompassed the tissue between the columns of the fornix, ventral to the SFO and near the anterior commissure. The cuts were large in order to increase the likelihood of completely damaging the connectivity of the SFO, and as a consequence there was often collateral damage observed in the septum and the ventral diagonal band. This should be considered when interpreting the results.

The nuclei of interest were the hypothalamic SON and PVN, SFO, MnPO, OVLT, AP, and the nucleus of the solitary tract (NTS). For SFO, OVLT, and MnPO all Fos grains were counted within the borders of the nucleus in all sections. The data were averaged separately
for sections from the rostral and caudal SFO, the dorsal cap and the main body of OVLT, and the ventral and dorsal parts of the MnPO. For PVN, the data were averaged from at least two bilateral sections containing the largest lateral magnocellular subnucleus at about -1.80 mm from Bregma (24). In order to standardize counting of three bilateral subregions of PVN, Fos-positive cells were counted within a circle 167 µm in diameter in the lateral magnocellular PVN and dorsal cap regions and within a square 200 µm on a side in the ventromedial parvocellular PVN (24). The SON was counted bilaterally and averaged for at least 3 sections. Three sections of the AP were selected representing the most rostral, medial, and caudal parts of the nucleus. The NTS was counted bilaterally in these same three sections. It is important to note that “rostral NTS” throughout this paper does not refer to the most rostral region of the NTS in the brain but rather to the level of the NTS in the same section as the most rostral AP. Thus, rostral AP and rostral NTS refer to the AP and NTS in the same section. All nuclei were photographed using a light microscope and digital camera, and were analyzed in a computer running Scion Image processing software. For SON, SFO, MnPO, OVLT, AP, and NTS, the area of the nucleus was measured by the imaging software after tracing its outline with a drawing tool. The areas were compared among the experimental groups to assure that they were not significantly different in size before comparing the counts. The density of Fos-ir was then calculated by dividing the total number of Fos-positive cells by the area. The densities are reported as cells per 10,000 square µm per section for the SFO, MnPO, or OVLT or per side for the bilateral structures.

**Statistical Analyses**

Non-parametric tests were used for a majority of the analyses in the present study. The non-parametric statistical tests used include Friedman, Wilcoxon, Mann-Whitney, and Kruskal-
Wallis. The decision to use primarily non-parametric tests was based on the discovery that, in many cases, the data were widely heterogeneous in their variances. To maintain consistency, we then chose to use non-parametric tests for all of the statistical analyses of that type. Pearson correlation coefficients were calculated between each of the different regions or subnuclei in which c-Fos expression was examined. The alpha level was at .05. All tests carried out were two-tailed, with the exception of those used to analyze the drinking data where previous research indicated the direction of difference to be expected and thus allowed for one-tailed testing (20, 25, 26).

Results

Based on histology, the final sample sizes were 10 sham-cut animals and 11 knife-cut animals. A photomicrograph of the typical knife cut is given in Figure 1. The cut always damaged the anterior commissure and sometimes included a piece of the dorsal MnPO. In every case, the cut isolated the SFO and some fragment of the dorsal part of the MnPO from the rest of the brain (13, 14). Damage was also observed in variable degrees in the septum and ventral diagonal band. In the drinking test, knife-cut animals drank significantly less than sham-cut animals during the time intervals of 0-15, 15-30, and 30-60 min (see Figure 2). The knife-cut animals increased intake gradually during the session, but the cumulative 90-min intake was still significantly less in knife-cut rats.

The results of the c-Fos assay can be seen in Figures 3 through 7. Fos-ir density was significantly enhanced throughout several regions of the lamina terminalis in knife-cut animals compared to sham-cut animals (Figure 3), and representative photomicrographs are shown in Figure 4. Specifically, Fos-ir density was greater in the dorsal cap of the OVLT ($p = .04$),
ventral MnPO ($p = .049$), and the main body of the SFO ($p = .006$) in knife-cut animals when compared to the Fos-ir density found in sham-cut animals.

Fos-ir density in AP was significantly diminished at the mid-coronal and caudal levels of AP ($p = .049$ and .024, respectively) in knife-cut animals compared to sham-cut animals (Figure 5). Representative photomicrographs of three coronal levels of the AP are shown in Figure 6. Further statistical analysis demonstrated that Fos-ir density was different at the various coronal levels of the AP in the sham-cut animals, Friedman Chi Square ($df = 2$) = 14.6, $p < .001$. Within-subject followup comparisons in the sham-cut group alone using the Wilcoxon Matched Pairs Signed Ranks Test ($n = 10$) demonstrated that the Fos-ir density was greater in the caudal sections than in the mid-coronal sections ($Z = 1.99$, $p = .047$), and greater in the mid-coronal sections than in the rostral sections ($Z = 2.60$, $p = .009$). No differences were significant in the knife-cut group.

Figure 7 shows the distribution of Fos-ir in a horizontal section of AP in a neurologically intact rat treated with intrastric hypertonic saline. Note the sparse Fos-ir in the rostral part near the fourth ventricle and the increasing density from the mid-coronal AP to the caudal AP. This animal was representative of a sham-vagotomized group from a previous study (27, see Discussion).

Finally, Fos-ir density was significantly reduced by the knife cut in the SON of the hypothalamus ($p = .011$) but not in any subregion of the PVN (Figure 8).

A Pearson correlation matrix was generated to test the degree of relationship between the Fos-ir density in the various brain areas that were measured. This was done on the assumption that areas that are “working together” or have a functionally dependent relationship should show similar levels of activity from rat to rat. Table 1 lists all of the
statistically significant correlations that were observed between pairs of structures in the sham-cut group followed by their respective correlations in the knife-cut group. Among the sham-cut rats, the highest significant correlations tended to be between subareas within a structure or between nearly adjacent structures. These included the different levels of AP either among themselves or paired with levels of the NTS; the OVLT or SFO paired with the MnPO; or the SON paired with the lateral magnocellular PVN. There were a few significant correlations between hindbrain regions of AP or NTS with the regions of the MnPO or PVN in the forebrain. Curiously, given the knife-cut findings presented above, all six of the possible correlations between pairs of the two regions of SFO with the three regions of AP were non-significant negative correlations ranging from -.17 to -.40. The correlations of the dorsal part of MnPO with the three levels of AP were also nonsignificant, ranging from -.09 to +.22.

Of the 19 significant correlations of Fos-ir density among pairs of areas in the sham-cut group, 12 were not significant in the knife-cut group. Many of these were directly attributable to the drastic restriction of the variability in Fos-ir in the mid-coronal and caudal AP after the knife cut. The correlations among the various areas of the SON and PVN were maintained after the knife cut, and some significant positive correlations persisted even between areas on either side of the knife cut, such as the OVLT and the dorsal part of the MnPO.

Table 2 lists all of the remaining significant correlations for Fos-ir between pairs of brain areas in the knife-cut group that were not significant in the sham-cut group. Positive associations were greatly strengthened by the knife cut between the hypothalamic areas and the two remaining connected circumventricular organs, the AP and OVLT. Also, correlations between several areas of the lamina terminalis on either side of the knife cut that were not significant in sham-cut rats became significant in the knife-cut rats (e.g., rostral SFO with either
part of the OVLT or with the ventromedial parvocellular PVN; main body of SFO, and ventral MnPO). The significance of these correlations probably resulted at least in part from the greater variability of Fos-ir in the lamina terminalis.

There were no significant negative correlations between any of the pairs of areas in either experimental group.

Discussion

The goal was to study osmotically-induced drinking behavior and Fos expression after a disconnection of the afferent and efferent fiber pathways of the SFO in rats (13, 14). The stimulus was a large load of NaCl solution delivered as a bolus by gavage. As expected from previous studies, the knife cut significantly reduced both the drinking response and the expression of Fos in the SON. The principal novel findings of the experiment are: (1) Fos-ir densities in the main body of SFO, the dorsal cap of OVLT, and the ventral MnPO were all significantly enhanced in response to the osmotic stimulus in knife-cut rats; (2) Fos expression was observed principally in the mid-coronal and caudal parts of the AP of sham-cut rats, and this expression was dramatically reduced in knife-cut rats; and (3) Significant positive correlations existed between various brain areas in response to the osmotic stimulus in neurologically intact rats, and the patterning of these relationships was altered by the knife cut. The findings provide further evidence for the hypothesis that principal osmoreceptors — i.e., those that are important for drinking and neurohypophyseal functions — may reside in the forebrain circumventricular organs such as the SFO. If osmotically responsive neurons in the SFO were heavily dependent on synaptic input from elsewhere in the brain, those cells should have exhibited a decrease in Fos expression after the SFO was disconnected. Instead, we
saw no change in Fos expression in the rostral SFO and a large increase in expression in the main body.

Drinking

Drinking and neurohypophyseal secretions after intragastric osmotic loads of this magnitude are probably caused by a summation of several afferent inputs. Interference with visceral afferents by a lesion of the abdominal vagus or splanchnic nerves blunts Fos expression and drinking after small loads that do not greatly affect systemic osmolality (2, 12) and also after larger loads that do (27). This argues for at least a facilitatory role of these gut afferents if not for outright gut osmo- or sodium-receptors.

Similarly, SFO lesions or disconnections consistently reduce water intake in rats after intragastric osmotic loads that raise plasma osmolality (25, 26). SFO lesions have never been tested with the smaller loads. We recently compared the effects of SFO lesions, OVLT lesions, or combined lesions of both on drinking responses after hypernatremia induced by SC hypertonic saline (4). SFO lesions reduced drinking early in the test, but the drinking of these rats rebounded later and cumulative intake was not significantly reduced at 90 min. Small OVLT lesions that did not destroy more than the bottom third of the ventral MnPO had no consistent effect on osmotic drinking in these rats (see also 5), and a combined lesion of SFO and OVLT was no more effective than a lesion of the SFO alone on drinking in response to hypernatremia. Dipsogenic osmoreceptors might not be ruled out in the OVLT, however, because many OVLT-lesioned rats had very high unstimulated daily water and saline intakes and this may have confounded the results of the experiment on hypernatremia (4). OVLT lesions alone have profound effects on osmotic drinking in dogs (30), so the results may be specific to the species. Even the AP may contribute to osmotic thirst, because rats with AP
lesions that voluntarily drank large volumes of strong hypertonic saline did not bother to correct their elevated plasma osmolality by drinking sufficient additional water (3).

Fos-ir

In our previous study (26) we examined the effects of a mild load of salt (0.6M NaCl at 0.5% of body weight) on Fos-ir in the brains of knife-cut and sham-cut rats. Although the knife cuts did affect the expression of Fos in the hypothalamic nuclei, very little if any Fos-ir was observed in the main body of SFO or in the OVLT. Thus, we can deduce that a knife cut alone is not responsible for increasing Fos-ir in the lamina terminalis as observed in the present study. We increased the dose of NaCl in the present study to 1.5M NaCl at 0.5% of body weight in an effort to generate more Fos-ir in the SFO and OVLT, and this effort was not entirely successful in the sham-cut rats (see Fig. 3). Fortunately, the increased Fos-ir in the circumventricular organs of knife-cut rats made that effort unnecessary. Because we did observe elevated Fos-ir in both forebrain CVOs after a load of 1M NaCl at 1% of body weight in sham vagotomized animals (27), the threshold for inducing Fos-ir in the circumventricular organs with this technique apparently falls between 0.75-1.00 mmol/100 g body weight.

As we noted in our previous paper (26), an absence of Fos-ir in the circumventricular organs does not mean that these structures are not responding to the stimulus. There are several possible, non-mutually exclusive scenarios that are consistent with the data, including: (1) Some osmoreceptive neurons may not express Fos-ir at all; (2) The level of stimulation is simply not robust enough to generate a noticeable amount of Fos-ir in the circumventricular organs, but the sum of all inputs from circumventricular organs, the gut, and intrinsic osmoreceptors in the SON and PVN is sufficient to generate much Fos-ir in those hypothalamic structures; or (3) The circumventricular organs provide tonic facilitating input to
the hypothalamus that sensitizes the intrinsic hypothalamic osmoreceptors. In the last case, the tonic nature of the input would not generate much Fos-ir in the circumventricular organs of sham-cut rats, but the loss of that input would make the hypothalamic osmoreceptors less sensitive to a given load so that they produce less Fos-ir there.

One interpretation of the robust elevation of Fos-ir in several areas of the lamina terminalis of knife-cut rats after an intragastric osmotic load is that an osmotic load generates considerable inhibitory activity within the lamina terminalis as well as excitatory activity in efferents leaving the lamina terminalis. The function of such inhibitory activity may be to moderate the activation in the lamina terminalis so that the physiological and behavioral responses are not out of proportion to the stimulus. This exaggerated activity in the OVLT and ventral MnPO in knife-cut rats may have compensated somewhat for the loss of efferents from the SFO in the production of Fos-ir in the PVN and SON and in the rebound of drinking at the end of the study.

Alternatively, the elevated Fos-ir in the lamina terminalis may result indirectly from some chronic change in the knife-cut animals as compared with sham cut animals. For example, knife cuts may reduce the excretory response to the sodium load and produce a larger rise in plasma osmolality that in turn produces a greater stimulation of the lamina terminalis. We previously found that sodium excretion and urinary sodium concentration were both decreased in SFO lesioned rats after stomach loads of hypertonic NaCl (25), so the same may occur with a knife cut.

The small reduction of Fos expression in the SON of knife-cut rats is consistent with our previous observation with a smaller salt load (26). However, that study also detected a significant decline of Fos expression in all regions of the PVN as well. The full expression of
Fos in the PVN in knife-cut rats of the present study may result from greater activation of the OVLT and MnPO as well as additive or synergistic contributions from the gut osmoreceptors and intrinsic osmoreceptors in the hypothalamus. As previously mentioned, we have observed that an abdominal vagotomy was able to reduce Fos expression in the hypothalamus after a smaller load but not after a larger load of hypertonic salt in the stomach (27). Larger loads may produce a “ceiling effect” of high Fos production that is able to be maintained even with fewer afferent inputs. An additional factor to consider is the timing of the perfusion for Fos-ir analysis at 90 min after the load. This probably revealed Fos expression more robustly from the earlier phase of absorption of the load, and the early activation of the SON is more likely to have resulted from SFO efferents than from direct stimulation of osmoreceptors in the SON itself. We have previously argued that a delayed activation of intrinsic osmoreceptors in SON and PVN may account for the delayed initiation of drinking in SFO lesioned rats after intragastric osmotic loads (4).

The activation of Fos expression mainly in the caudal, visceral half of the AP after an intragastric osmotic load is a novel observation as far as we are aware. It is consistent with the notion that this Fos activation in AP may rely on afferent input from gut osmoreceptors rather than intrinsic osmoreceptors in AP (11). This prompted us to reexamine slides of horizontal sections of hindbrains taken from rats with sham vagotomies in a previous study (27). In that intragastric salt loading study we analyzed Fos expression in the NTS in horizontal sections of the hindbrain, but we did not analyze the AP data. We now report that the Fos-ir in response to intragastric sodium loading was mainly limited to the caudal AP of those neurologically intact rats as well (see Figure 7).
The disconnection of the SFO in the forebrain greatly reduced the dense expression of Fos in the caudal AP. The meaning of this interesting result is impossible to say without further study, but the implications are several. It suggests that the interactions among the sensory circumventricular organs are powerful, even across the distance from the forebrain to the hindbrain. We know that at least some of the Fos expression in AP may arise from an activation of gut receptors rather than plasma osmolality (2, 23), so the present results suggest that the sensitivity of the AP to these visceral signals may be modulated by inputs from the SFO. This notion is reinforced by the observation of Hochstenbach and Ciriello (8) that a lesion of the SFO, or even of most of the lamina terminalis, did not alter Fos expression in AP after a large intravenous infusion of hypertonic saline, i.e., after a load that bypassed the stomach.

Correlations

Aside from the large group differences in Fos-ir already discussed, we also observed significant positive correlations among brain areas in the magnitude of Fos expression (Figures 1 and 2). There was a patterning to the responses in the sham-cut group, and this patterning was considerably altered in the knife-cut group. That is to say, the correlations were not a simple function of some animals having greater Fos expression in all target nuclei and others having less Fos expression the same areas. If that were the case, all nuclei should correlate positively with all others. Instead, we found high correlations among certain pairs of areas and essentially zero correlations among other pairs.

For example, in the sham-cut rats the Fos-ir in the SON and in the lateral magnocellular PVN were highly correlated. High correlations were also found among subregions of the lamina terminalis and among subregions of the AP and NTS. However, neither the lamina
terminalis nor the hindbrain correlated significantly with the SON and lateral PVN; nor did any subregion of the lamina terminalis correlate significantly with any measured region of the hindbrain. Thus, closely related areas tended to be stimulated similarly, but since these different clusters of activity were probably responding to different aspects of the overall stimulus — plasma osmolality, gastric distention, baroreceptor input, volume expansion — the clusters did not necessarily correlate well with each other. The mainly neurosecretory nuclei of the hypothalamus received and integrated the signals from a constellation of areas. Although the SON and PVN themselves interpreted these integrated signals quite similarly, the final output did not necessarily correlate well with any one of the individual inputs.

The patterning of these correlations was quite different in the knife-cut rats. Several correlations among areas that had been significant in the sham-cut group were virtually zero in the knife-cut group. This was particularly evident in the low correlation coefficients among the different levels of AP and NTS that resulted from the restricted range of Fos-ir densities in the caudal half of the AP in the knife-cut rats. Interestingly, as these correlations disappeared, other strong correlations emerged in the knife-cut group. For instance, the mid-coronal section of the AP correlated better with the hypothalamic nuclei after the cut than it did with other areas of the hindbrain. Also, the main body of the OVLT, which had very little Fos expression and very few positive correlations in the sham-cut group, developed significant correlations with several other forebrain structures in the knife-cut animals.

Among the more interesting correlations that occurred in the knife cut group were those of the SFO or the dorsal part of the MnPO with other areas of the brain even though these pairs of areas were completely disconnected (Tables 1 and 2, bold type). For example, different areas of SFO or the dorsal MnPO developed significant correlations with the body of
OVLT, the dorsal OVLT, or the ventromedial parvocellular PVN. We interpret this as evidence of independent but similar osmoreceptor functioning in the disconnected areas of SFO/dorsal MnPO and the OVLT/ventral MnPO.

Another interesting finding was the fact that the parts of SFO or the dorsal MnPO never correlated positively with any of the parts of AP in the sham-cut rats despite the fact that the Fos-ir in AP was demonstrated to be heavily dependent on its connectivity with the SFO or dorsal MnPO. This suggests that the activity in the SFO-MnPO nexus was not simply driving, in lock step, the activity in the AP. Perhaps the connections from SFO or dorsal MnPO acted to sensitize the caudal AP to other inputs, such as gut osmolality or volume expansion. In that case, the level of activity could be dependent on the connectivity with the forebrain without correlating highly with it.

It is likely that we observed a number of hypothesis-testing errors of both the I and II types after the analysis of a large number of correlations in two different groups with small sample sizes without a correction for the inflation of alpha, so we do not want to overstress the importance of any particular finding. What did impress us was the large number of significant correlations that emerged after the knife cut from essentially zero correlations in the sham-cut group, and also the number of correlations that existed between various nuclei that were completely separated by the knife cut. These findings suggest redundancy and a plasticity of function after the lesion.
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Figure Captions

Figure 1. Photomicrograph of a coronal section of a representative knife cut through the anterior commissure. Bar = 0.1 mm.

Figure 2. Drinking responses by knife-cut and sham-cut rats after an intragastric load of 0.5% body weight of 1.5 M NaCl at each of the time intervals measured (left panel) and cumulatively at 90 min (right panel). *P < .05, one-tailed.

Figure 3. Fos-ir density for the lamina terminalis of knife-cut and sham-cut rats. Units: Cells per 10,000 square µm. Abbreviations: OVLTb and OVLTd, body and dorsal cap of OVLT; MnPOv and MnPOd, ventral and dorsal MnPO; SFOr and SFOb, rostral and main body of SFO. *P < .05, two-tailed.

Figure 4. Representative photomicrographs of Fos-ir in the lamina terminalis of sham-cut (left) and knife-cut (right) rats in coronal sections. Top, OVLT; middle, rostral SFO; bottom, main body of SFO. Bar = 0.1 mm. Fos-ir was significantly increased in dorsal cap of the OVLT and the main body of the SFO in knife-cut rats.

Figure 5. Fos-ir density for different levels of AP and NTS in knife-cut and sham-cut rats. Units: Cells per 10,000 square µm. Abbreviations: r, rostral; m, mid-coronal; c, caudal level of AP. NTSr refers to the same section as the AP r, not to the most rostral level of the NTS. *P < .05, two-tailed.
Figure 6. Representative coronal photomicrographs of Fos-ir in the AP of sham-cut (left) and knife-cut (right) rats. Rostral (top), mid-coronal (middle), and caudal (bottom) sections are shown. Bar = 0.1 mm. Fos-ir was significantly decreased in the mid-coronal and caudal levels of AP in knife-cut rats.

Figure 7. Horizontal section of AP from previously unpublished work by Starbuck, Wilson, and Fitts following a large hypertonic dose of intragastric NaCl in a neurologically intact rat. The fourth ventricle is at the top. Note the regional distribution of Fos-ir in the central and caudal regions and the paucity of Fos-ir in the rostral part of AP. Bar = 0.1 mm.

Figure 8. Fos-ir density in the SON and PVN regions of the hypothalamus in sham-cut and knife-cut rats. Units: Cells per 10,000 square µm. Abbreviations: lm, lateral magnocellular; dc, dorsal cap; vp, ventromedial parvocellular. *$P < .05$, two-tailed. Fos-ir was significantly reduced in the SON of knife-cut rats compared with sham-cut rats.
Figure 1. Photomicrograph of a coronal section of a representative knife cut through the anterior commissure. AC, anterior commissure; 3v, third ventricle; OX, optic chiasm.
Figure 2. Drinking responses by knife-cut and sham-cut rats after an intragastric load of 0.5% body weight of 1.5 M NaCl at each of the time intervals measured (left panel) and cumulatively at 90 min (right panel). *$P < .05$, one-tailed.
Figure 3. Fos-ir density for the lamina terminalis of knife-cut and sham-cut rats. Units: Cells per 10,000 square µm. Abbreviations: bOVLT and dOVLT, body and dorsal cap of OVLT; vMnPO and dMnPO, ventral and dorsal MnPO; rSFO and bSFO, rostral and main body of SFO. *P < .05, two-tailed.
Figure 4. Representative photomicrographs of Fos-ir in the lamina terminalis of sham-cut (left) and knife-cut (right) rats in coronal sections. Top, OVLT; middle, rostral SFO; bottom, main body of SFO. Bar = 0.1 mm. Fos-ir was significantly increased in dorsal cap of the OVLT and the main body of the SFO in knife-cut rats.
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Table 1. List of all significant correlations (Pearson $r$) for For-ir between pairs of structures in the sham-cut group and the corresponding correlations in the knife-cut group.

<table>
<thead>
<tr>
<th>Nucleus 1</th>
<th>Nucleus 2</th>
<th>Sham (df = 8) $r$</th>
<th>$p$</th>
<th>Cut (df = 9) $r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APc</td>
<td>NTSc</td>
<td>.65</td>
<td>.04</td>
<td>-.01</td>
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<tr>
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<td>.03</td>
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<td>n.s.</td>
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<td>APc</td>
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<td>.04</td>
<td>.50</td>
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<tr>
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<td>.003</td>
<td>.34</td>
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</tr>
<tr>
<td>APr</td>
<td>APc</td>
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<td>.02</td>
<td>.31</td>
<td>n.s.</td>
</tr>
<tr>
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<td>NTSr</td>
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<td>.008</td>
<td>-.18</td>
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<td>MnPOd</td>
<td>MnPOv</td>
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<td>.01</td>
<td>.61</td>
<td>.05*</td>
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<td>MnPOv</td>
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<td>NTSm</td>
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<td>.05</td>
<td>.72</td>
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<td>OVLTb</td>
<td>MnPOd</td>
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<tr>
<td>OVLTd</td>
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<td>.01</td>
<td>.70</td>
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<td>PVNdc</td>
<td>PVNvp</td>
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<td>.62</td>
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<td>PVNvp</td>
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<td>PVNlm</td>
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<td>.004</td>
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</tbody>
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*Bold type* indicates significant correlations between nuclei on either side of the knife cut.

Abbreviations: rAP, mAP, cAP, rNTS, mNTS, cNTS: AP or NTS counted in sections at the level of the rostral, mid-coronal, or caudal AP; MnPOd, v: dorsal or ventral MnPO; PVNdc, lm,
VP: dorsal cap, lateral magnocellular, or ventromedial parvocellular PVN; SFOb, r: main body or rostral stalk of SFO; OVLTb, d: main body or dorsal cap of OVLT.
Table 2. List of significant correlations for Fos-ir between pairs of structures in the knife-cut group that were not significant in the sham-cut group.

<table>
<thead>
<tr>
<th>Nucleus 1</th>
<th>Nucleus 2</th>
<th>Sham (df = 8)</th>
<th>p</th>
<th>Cut (df = 9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>APm</td>
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<td>OVL Tb</td>
<td>PVNlm</td>
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<td>.05</td>
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<tr>
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<td>.68</td>
<td>.03</td>
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<td>n.s.</td>
<td>.73</td>
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<td>n.s.</td>
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<td>n.s.</td>
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<td>PVN vp</td>
<td>.62</td>
<td>n.s.</td>
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