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Evidence for Hippocampal Regulation of Neuroendocrine Neurons of the Hypothalamo–Pituitary–Adrenocortical Axis

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Expression of mRNAs coding for the ACTH secretagogues corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) was examined in the hypothalamic paraventricular nucleus (PVN) of rats bearing hippocampal lesions. Either total hippocampectomy (HPX) or extirpation of the dorsal hippocampus (DHPX) precipitated a 4-fold increase in CRF mRNA expression relative to sham-operated controls (SHAM), as determined by semiquantitative *in situ* hybridization histochemistry. AVP mRNA was localized to individual parvocellular neurons of the medial parvocellular division of the PVN in only the HPX and DHPX groups, consistent with enhanced production of AVP message in this neuronal population subsequent to hippocampal damage. HPX did not affect AVP mRNA content in magnocellular divisions of PVN. Plasma β-endorphin levels were significantly elevated in the HPX and DHPX groups relative to SHAM animals, indicating a chronic increase in release of proopiomelanocortin peptides from the anterior pituitary gland in response to hippocampal lesion. Circulating corticosterone levels were elevated in HPX rats as well. To control for effects of lesion size and location, additional animals received large ablations of cerebral cortex or cerebellum. In neither case was CRF or AVP mRNA significantly altered in the PVN. The results suggest that the hippocampus exercises a tonic inhibitory role on ACTH secretagogue production in neuroendocrine neurons promoting ACTH release.

Stimulation of glucocorticoid secretion by the hypothalamo–pituitary–adrenocortical (HPA) axis is instrumental in mediating adaptive physiological responses of vertebrate organisms to physiological and psychological stressors. Due perhaps to the catabolic nature of these steroid hormones, an elaborate system of hormonal negative-feedback regulation has evolved to limit the magnitude and duration of glucocorticoid stress responses. It is generally agreed that glucocorticoids act both at the pituitary and via the brain to inhibit ACTH release (Keller-Wood and Dallman, 1984; Dallman et al., 1985; Plotsky et al., 1987). At the level of the pituitary gland, glucocorticoids directly inhibit release of ACTH and production of the ACTH precursor proopiomelanocortin (POMC) (Dallman et al., 1985). While it is clear that glucocorticoids also act in the brain, the precise brain locus (or loci) subserving neuronally mediated feedback inhibition remain(s) to be definitively described.

ACTH release is believed to be primarily the province of corticotropin-releasing factor (CRF)-synthesizing neurosecretory neurons localized in the medial parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN) and projecting to the external layer of the median eminence (Makara et al., 1981; Antoni et al., 1983; Bruhn et al., 1984). In addition to producing CRF, this neuronal population has been demonstrated to contain numerous other neuropeptides, including arginine vasopressin (AVP) (Kiss et al., 1984a; Sawchenko et al., 1984), cholecystokinin (Kiss et al., 1984b), neurotensin (Jennes et al., 1982), and enkephalins (Khachaturian et al., 1983) by immunohistochemistry. The extent of colocalization of CRF with other neuropeptides and the intensity of staining of colocalized species vary with the physiological status of the animal (see Swanson et al., 1988). Importantly, AVP mRNA and peptide expression undergo a pronounced induction in CRF neurons of the medial parvocellular PVN under conditions of adrenal insufficiency (Kiss et al., 1984a; Sawchenko et al., 1984; Wolfson et al., 1985; Plotsky and Sawchenko, 1987; Sawchenko, 1987a). Adrenalectomy-induced up-regulation of CRF and AVP is blocked by treatment with large doses of exogenous glucocorticoids, either systemically or directly into the region of the PVN (Kovacs et al., 1986; Sawchenko, 1987b), suggesting an interaction between circulating glucocorticoid levels and synthesis/secretion of these neuropeptides. CRF and AVP are colocalized in the same neurosecretory granules in terminal fields of PVN neurons located in the external lamina of the median eminence, suggesting co-release of CRF and AVP into the portal circulation (Whitnall et al., 1987). The importance of colocalization and putative co-release of AVP and CRF in medial parvocellular PVN neurons toward regulation of the HPA axis is further highlighted by physiological studies indicating synergistic effects of CRF and AVP on ACTH release at the level of the adenohypophysial corticotroph (Gillies et al., 1982). It is therefore logical to assert that neuronal inhibitory influences on ACTH release must interact in some meaningful way with this population of neurosecretory neurons.

Numerous reports indicate that the hippocampal formation plays an important role in neuronal regulation of the HPA axis.

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Since the early 1960s it has been known that hippocampal removal (or section of the fornix) precipitates adrenal hypersecretion of glucocorticoids (Fendler et al., 1961; Knigge, 1961; Moberg et al., 1971; Fischette et al., 1980; Wilson et al., 1980; Feldman and Confronti, 1980; Sapolsky et al., 1984a; Magariños et al., 1987). This hypersecretion is mediated by increased release of ACTH by adenohypophysial corticotrophs (Wilson et al., 1980). Conversely, hippocampal stimulation has been shown to effectively diminish circulating corticosterone levels in anesthetized rats (Dunn and Orr, 1984). Putative hippocampal influences on the HPA axis are further supported by data documenting large populations of type 1 and type 2 glucocorticoid receptors in the hippocampus (McEwen et al., 1968; Sapolsky et al., 1983; Reul and deKloet, 1985), which regulate in response to stress or glucocorticoid removal (Olpe and McEwen, 1976; Tornello et al., 1982; Sapolsky et al., 1984b). Taken together, this body of data strongly suggests a role for the hippocampus in inhibition of the HPA axis, possibly mediated by glucocorticoid negative feedback.

The aim of this study was to examine the effect of hippocampal removal on expression of CRF and AVP mRNA in medial parvocellular PVN neurons, thereby testing whether the hippocampus tonically suppresses the synthetic activity of neurons issuing the primary ACTH secretagogues. POMC-derived peptide content of pituitary and plasma samples and plasma corticosterone levels were measured to assess the effects of hippocampectomy (HPX) on long-term regulation of the HPA axis.

Materials and Methods

Subjects. Subjects were male Sprague-Dawley rats, weighing between 250–275 gm at the time of surgery. All rats were housed in hanging wire cages (6 per cage) on a 12 hr light:dark cycle. Animals were allowed free access to food and water at all times.

Hippocampectomy. Animals were left undisturbed for 1 week prior to surgical procedures. On the day of surgery animals were anesthetized with a mixture of chloral hydrate (60 mg/kg) and pentobarbitol (25 mg/kg) and mounted in a Kopf stereotaxic apparatus with the incisor bar raised to the highest allowable position. The scalp was incised, 2 bone "windows," roughly 2 × 3 mm, were removed from the skull (anterior extent: 1 mm posterior to bregma; medial extent: 0.5 mm lateral to midline), and the underlying dura was incised. At this point, animals composing the sham-operated group (SHAM) had the "window" packed with Gelfoam and the skin sutured with wound clips. In the total HPX and dorsal hippocampectomy (DHPX) groups, the cortex overlying the hippocampus was aspirated by a suction device (an angled Pasteur pipette attached to a vacuum pump) to allow visualization of the alveus overlying the dorsal hippocampus. The Pasteur pipette was then placed directly on the hippocampus (angled posteroventrally) and used to complete the aspiration procedure. The hippocampectomy method reliably removed the majority of the dorsal hippocampus, but in 50% of the cases spared the ventral-most aspect of this structure; thus, the DHPX and HPX groups were defined post hoc based on histological specimens. Cortical control animals (CTX) received ablation of cortical tissue overlying the hippocampus, corresponding to the cortex removed in the process of HPX. Following ablation procedures, wounds were packed with sterile Gelfoam and skin incisions closed with wound clips.

Mortality rate following HPX was approximately 33%; the majority of morbid animals died within 1–4 d following surgery and generally exhibited extensive necrosis in cortical, thalamic, and midbrain regions upon post-mortem examination. All animals included in the data analysis had appeared overtly healthy at the time of death and had gained weight over the period between surgery and sacrifice. No animals exhibited seizure activity during the postoperative survival period.

Surgical controls. To obviate the possibility that changes observed following HPX may be the result of the surgical stress, 2 additional surgical groups were examined. One such group received large lesions of the cerebellum, the other large lesions of temporal and occipital cortices, both utilizing the same surgical protocal as used for HPX. In both groups, the amount of tissue damaged was similar to that caused by hippocampal ablation. Operated controls were anesthesized, a bone window overlying the cerebellum or lateral cortex was removed, the "window" packed with Gelfoam, and the wound closed with wound clips.

Death. Animals were killed 10 d after surgery by decapitation between 9:00 and 10:30 a.m. The brains were rapidly removed and frozen in isopentane cooled to −50°C on dry ice. Pituitaries were removed, dissected into anterior and neurointermediate lobes, and frozen on dry ice. Trunk blood was collected in evacuated tubes containing EDTA and spun for 5 min at 1500 × g', the plasma was acidifed in 0.1 N HCl and frozen on dry ice. Samples were stored at −80°C until processed.

To establish plasma steroid levels in the respective groups, additional rats were killed as above. Plasma was processed as above and corticosterone assayed by a competitive protein binding assay. Adrenal and thymus glands were removed, cleaned, and weighed. Brains were examined histologically to verify lesion size and location.

Histological verification of lesion sites. One-in-four series of 25 μm sections through the length of hippocampal lesions, cerebral cortical lesions, and cerebellar lesions were saved for analysis of lesion sites. Sections were postfixed for 1 hr in 4% buffered formaldehyde and stained with thionen.

In situ *hybridization.* Brains were sectioned at 10 μm in a Bright-Hacker cryostat (20°C), thaw-mounted onto polylysine-coated slides, and stored at −80°C until processed for *in situ* hybridization histochemistry. Tissue was sampled for *in situ* procedures at 50 μm intervals; an additional series was stained for Nissl substance with thionen to establish a cytoarchitectonic context for analysis. Tissue was processed in 2 separate experimental series: in *experiment 1,* SHAM, CTX, HPX, and DHPX brains were processed and analyzed simultaneously. In *experiment 2,* animals with control lesions of the cerebral cortex and cerebellum and appropriate sham-operated animals were processed and analyzed simultaneously.

The *in situ* protocol consisted of removing sections directly from the −80°C freezer and immersing them immediately in cold buffered 4% paraformaldehyde. Tissue was fixed for 1 hr and washed 3× in PBS (20 mM). Sections were deproteinated with proteinase K, 100 μl of a 1.0 μ/ml solution, for 1 hr at 37°C. After deproteination, slides were washed for 1 min in distilled H_2O, 1 min in 0.1 M triethanolamine, and 10 min in 0.1 M triethanolamine containing 2.5% acetic anhydride. The latter acetylation step reduces electrostatic binding of probe to tissue sections. Sections were then rinsed in distilled H_2O and dehydrated through graded alcohols.

^{35}S-labeled cRNA probes complementary to proAVP (subcloned in pGEM4, courtesy T. G. Sherman) and proCRF (subcloned in pSP64, R. Thompson and J. Douglass) cDNA clones were produced using the SP6 transcription system. Plasmids containing subcloned cDNAs were linearized with appropriate restriction enzymes to yield probes of desired length and C-G composition. The labeling reaction mixture contained 1 μg linearized plasmid, 1X SP6 transcription buffer (BRL), 125 μCi ^{35}S-UTP, 20–40 μM S-UTP, 150 μM NTPs-UTP, 12.5 mM dithiothreitol, 20 U RNAsin, and 6 U SP6 polymerase. Reaction was incubated for 90 min at 37°C, and labeled probe was separated from free nucleotide over a Sephadex G50-50 column. The proAVP probe was a 197 bp cRNA coding for the C-terminal region of the proAVP molecule, which bears little homology with proOxytocin. The proCRF probe was a 672 bp cRNA derived from a rat CRF cDNA clone, including the peptide coding region (exon 2) of the rat CRF gene. ^{35}S-labeled and unlabeled S-UTP was added to the transcription reaction in amounts calculated to yield specific activities estimated at 1.40 × 10^4 Ci/mmol probe and 2.10 × 10^4 Ci/mmol probe for proAVP and proCRF, respectively.

Probe was diluted in a standard hybridization buffer (75% formamide, 10% dextran sulfate, 3× SSC, 50 mM sodium phosphate buffer, pH 7.4, 1× Denhardt's, 0.1 mg/ml yeast tRNA, and 0.1 mg/ml sheared salmon sperm DNA) sufficient to yield 2,000,000 dpm/30 μl buffer. Then, 30 μl aliquots were applied to each slide, the slides coverslipped, and coverslips sealed with rubber cement. Slides were incubated in sealed plastic boxes containing moistened foam. Sections were incubated at 55°C for 48 hr, at which time the coverslips were removed, and the slides were rinsed in 2× SSC and immersed in fresh 2× SSC for 20 min. The tissue was then incubated with RNase A (200 μg/ml) at 37°C for 30 min to degrade any remaining single-stranded RNA. Sections were then washed successively in 2×, 1×, and 0.5× SSC for 10 min each, followed by a 1 hr wash in 0.5× SSC at the hybridization temperature. Sections were then dehydrated through alcohols, exposed to Kodak XAR x-ray film

overnight, and subsequently emulsion-dipped, along with slide-mounted 10 μm brain-paste standards containing known amounts of ^{35}S-labeled L-methionine in Kodak NTB2 nuclear emulsion. Emulsion-dipped sections and standards were exposed for 5 d (AVP) or 10 d (CRF); batch development was based on the signal strength and signal-to-noise ratio of test slides developed at regular intervals. For both *experiment 1* and *experiment 2*, all tissue hybridized with CRF or AVP cRNA probes were processed and developed simultaneously.

Quantitation of *in situ* hybridization autoradiographs was conducted utilizing Loats image-analysis software. Standards were constructed by thoroughly mixing brain paste (made by passing brain tissue from cortex and cerebellum through a 23 gauge syringe) with varying amounts of ^{35}S-labeled L-methionine in a small ultracentrifugation tube. Standards were centrifuged briefly to bring them to the bottom of the tubes, and the tubes were subsequently frozen on powdered dry ice. Standards were then sectioned at 10 μm, with adjacent sections reserved either for emulsion dipping or counting. All standards were placed in a vacuum desiccator and vapor fixed by heating 20 gm paraformaldehyde at 80°C under a vacuum for 2 hr. Radioactivity of each standard was determined by counting standard sections adjacent to those used in the image-analysis procedure in a Beckman liquid scintillation counter. Standard curves were derived by selecting the curve of best fit relating the optical density of digitized images of the ^{35}S-labeled brain-paste standards (exposed along with the experimental sections) with the amount of radioactivity per unit area of standard. Second-order curve fits were selected for both CRF and AVP analyses; in both cases, the curve of best fit accounted for over 90% of the variance. Emulsion-dipped sections were then digitized and subjected to analysis of optical density over the areas of interest in the images, those being the medial parvocellular and immediately adjacent posterior magnocellular divisions of the PVN. Areas of interest were defined on cytoarchitectonic criteria from adjacent Nissl-stained sections and by patterns of grain localization. In regard to analysis, one additional point bears mention: Scattered magnocellular neurons in the medial parvocellular PVN interfere with accurate determination of the relative amount of parvocellular AVP mRNA localized in this region. As this class of neuron is generally believed to be functionally distinct from parvocellular AVP neurons, we endeavored to exclude magnocellular AVP neurons from our analysis on the basis of size and relative grain density. Determination of disintegration values for areas selected was derived from the standard curve of best fit relating optical density values of the standards with their disintegration values. The optical density of all samples fell within the range of the standard values.

Hormone assays. For radioimmunoassay, pituitaries were dissected into anterior and neurointermediate lobes. Blood was collected in evacuated tubes containing EDTA, spun for 10 min at 1500 × *g*, and plasma acidified in 1 *N* HCl. Plasma samples were extracted prior to assay with Sep-Pak C_{18} cartridges as previously described (Cahill et al., 1983). Radioimmunoassay of β-endorphin was performed on pituitary and plasma samples utilizing a previously described procedure (Young and Akil, 1985). The antibody (Brenda) is a midportion antibody raised against β-endorphin 1–31. It was used at a final dilution of 1/40,000. ^{125}I-β-endorphin was used as the radiolabeled tracer. β-endorphin 1–31 was used for construction of a standard curve. Under these conditions, this antibody shows 100% cross-reactivity with β-lipotropin, 80% cross-reactivity with *N*-acetylated β-endorphin 1–31 and 50% cross-reactivity with *N*-acetylated β-endorphin 1–27, the primary species of β-endorphin secreted by rat intermediate lobe. Previous studies from our laboratory indicate that plasma β-endorphin values obtained using this antibody reliably parallel ACTH levels (E. A. Young and H. Akil, unpublished observations). Plasma corticosterone was measured using a standard competitive protein binding assay (Murphey, 1967), using ^{3}H-corticosterone as the radiolabeled tracer.

Results

Experiment 1: *effect of HPX on HPA function*

Diagrams illustrating the extent of hippocampal and cortical lesions in the HPX, DHPX, and CTX groups are shown in Figure 1. The HPX group sustained damage to the overwhelming majority of Ammon's horn and the dentate gyrus. In all but 2 cases there was some bilateral sparing of the ventral subiculum. Some animals in the HPX group retained a small amount of viable hippocampal tissue at the extreme posteroventral aspect of Ammon's horn. Secondary damage present in some animals included limited portions of the dorsal thalamus (lateral posterior nucleus, lateral geniculate nucleus), superior colliculus, medial geniculate nucleus, and temporal cortex. Animals in the DHPX group exhibited a complete loss of dorsal hippocampal tissue, dorsal subiculum, and presubicular cortex, with retention of a significant portion of ventral hippocampus. Secondary damage present in some animals included limited portions of the dorsal thalamus and super colliculus. Cortical control animals (CTX group) sustained damage to cortex overlying the dorsal hippocampus. This damage was, of course, sustained in both hippocampectomized groups as well. Regions of cortex damaged include portions of retrosplenial, frontal, and occipital cortices; some CTX animals suffered limited damage to portions of the dorsal subiculum and CA1 as well.

Results of PVN mRNA quantitation are illustrated in Figures 2–5. Emulsion-dipped autoradiographs indicate that following HPX, DHPX, or CTX, levels of CRF mRNA are markedly increased relative to SHAM animals (Fig. 2, *A–D*). The quantitative analysis based on optical density of target regions in digitized images through the PVN corroborates the observed increases, as demonstrated by a significant effect of group on detected disintegrations (expressed as percent control in Fig. 3) by 1-way ANOVA [$F(3,18) = 5.48, p < 0.001$]. Subsequent post hoc analysis revealed that the HPX and DHPX groups significantly differed from both operated controls and CTX animals ($p < 0.05$, Newman-Keuls test). The CTX group showed a 2-fold increase in CRF mRNA expression, compared with 4-fold increases seen in both the HPX and DHPX groups. However, post hoc analysis did not reveal a significant difference between the CTX and SHAM groups.

AVP mRNA data are shown in Figures 4 and 5. As is evident in Figure 4, cells showing positive signal for AVP mRNA could be observed in the CRF-rich dorsal aspect of the medial parvocellular PVN in both the HPX and DHPX groups. Particular attention should be paid to cells delineated by arrows in Figure 4*C* and in the inset of Figure 4*D*; these cells represent neurons that are considerably smaller and contain substantially fewer grains than identifiable magnocellular AVP neurons localized to the adjacent magnocellular subdivision and scattered throughout the parvocellular area. In contrast, labeling over parvocellular PVN regions in the CTX and SHAM groups was relatively diffuse and not readily associable to individual neurons. Quantitative analysis, however, failed to detect differences among the 4 groups by 1-way ANOVA, probably due to high variability in the intensity of AVP mRNA labeling in the HPX and DHPX groups. Analysis of the adjacent posterior magnocellular subdivision of the PVN, either qualitatively (Fig. 4) or quantitatively (Fig. 5*B*), reveal no differences among the groups in magnocellular AVP mRNA expression.

Table 1 illustrates pre- and postoperative body weights and endocrine organ weights for the respective groups. Groups did not differ in preoperative body weight. Overall analysis of variance revealed no significant effect of surgery on postoperative body weight ($p < 0.16$); however, 2-way ANOVA analyzing both pre- and postoperative body weights revealed a significant interaction effect, probably reflecting transient surgical effects in the HPX, DHPX, and perhaps CTX groups. No differences were observed in either thymus weight or adrenal weight. No surviving animals exhibited seizures during the postsurgical period.

Table 2 summarizes results of radioimmunoassay for β-en-

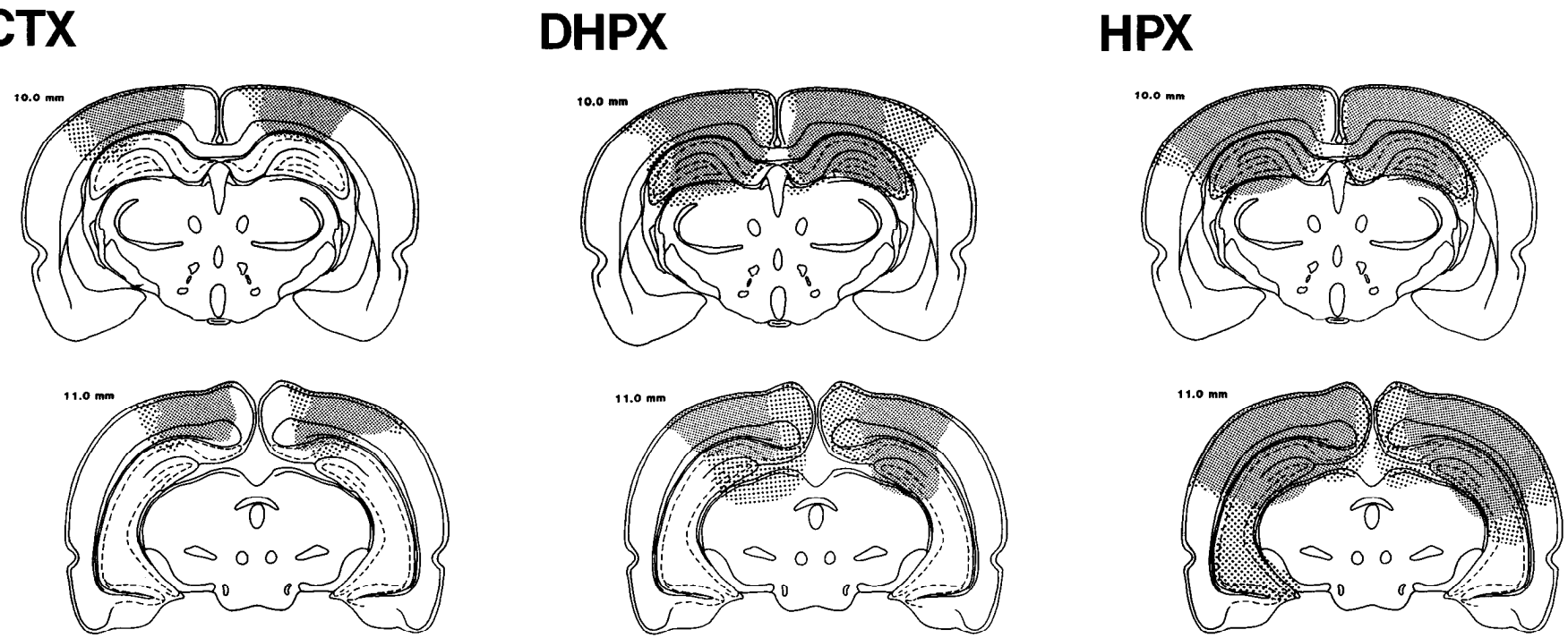


Figure 1. Diagram of cortical (*CTX*), dorsal hippocampal (*DHPX*), and large hippocampal (*HPX*) lesions. *Dark stippling* depicts the animal with the least extensive lesion in each group; *light stippling,* the animal with the most extensive lesion in each group. Cortical lesions compromised tissue overlying the dorsal hippocampus, including portions of retrosplenial, frontal, and occipital cortex. Limited damage to the dorsal subiculum and CA1 was observed in some animals. The DHPX lesion group manifested destruction of the vast majority of the dorsal hippocampus, with substantial sparing of ventral regions. The HPX lesion group sustained destruction of the dorsal hippocampus and the majority of the ventral hippocampus.

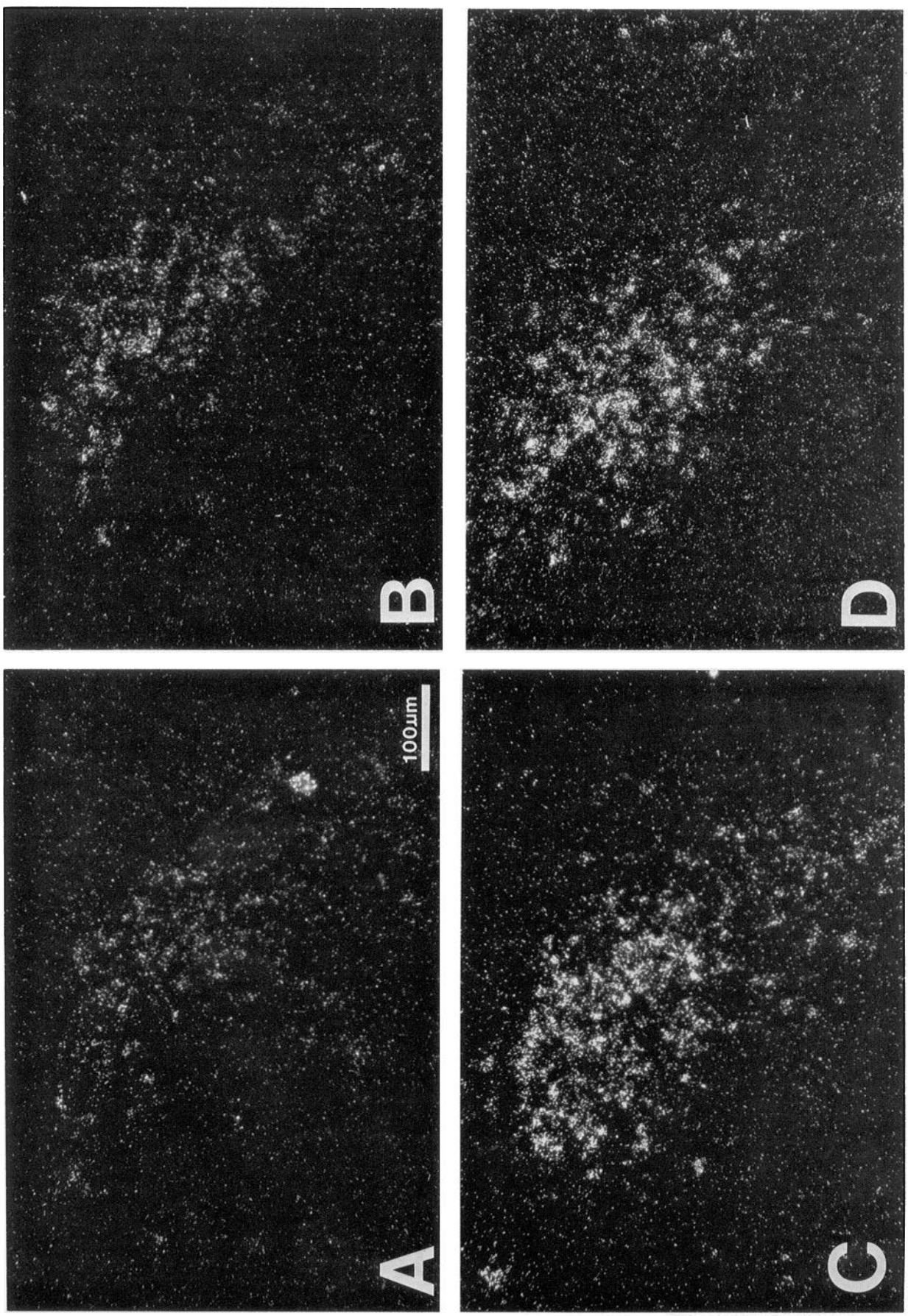


Figure 2. Photomicrographs of CRF mRNA localization in the PVN of SHAM (*A*), CTX (*B*), HPX (*C*), and DHPX (*D*) rats, visualized by *in situ* hybridization histochemistry. A marked increase in detected CRF mRNA was observed in HPX and DHPX rats relative to both the SHAM and CTX groups. The CTX group exhibited an increase in detected CRF message relative to SHAM animals.

dorphin in pituitary homogenates and plasma. No change in anterior pituitary content of β-endorphin was observed among the experimental and control groups. However, there was a significant effect of group on plasma β-endorphin levels [$F(3,82)$, $p < 0.03$]. Subsequent post hoc analysis reveals that the HPX, DHPX, and CTX all contained significantly higher levels of plasma β-endorphin than did operated controls ($p < 0.05$, Newman-Keuls test).

Corticosterone data are summarized in Table 3. Animals with hippocampal lesions (HPX), cortical lesions (CTX), or sham lesions (SHAM) were assayed for circulating corticosterone. There was a significant effect of group on plasma corticosterone [$F(2,14) = 4.67$, $p < 0.04$]. Post hoc analysis revealed that corticosterone is significantly elevated in the HPX group relative to SHAM and CTX rats.

Experiment 2: *effect of extrahippocampal lesion size and location on HPA function*

Lesions of the cerebral cortex (L-CTX) and cerebellum (CBM) are illustrated in Figure 6. L-CTX lesions were more extensive than the CTX lesions in *experiment 1*, both in terms of amount of cortex damaged at any given anterior–posterior level (cf. Figs. 1 and 6) and of overall anterior–posterior extent. CBM lesions involved the majority of the cerebellar cortex, with consistent sparing of cerebellar lobules 1–3 and portions of the flocculus.

The effects of CBM and L-CTX lesions on CRF and AVP mRNA content in the PVN are summarized in Figure 7. Overall effects of group on CRF and AVP mRNA expression did not reach the criterion value for statistical significance ($p < 0.05$). Results of *experiment 1* are also included for comparison of the relative magnitude of the effects of hippocampal and control lesions. In the case of CRF mRNA, the effects of HPX or DHPX greatly exceed that of any control lesion when expressed as a percentage of control (SHAM) values. Variation between total disintegration values of sham groups across the 2 experiments was 9 and 26% for CRF mRNA and parvocellular AVP mRNA analyses, respectively.

Results of plasma corticosterone assay revealed no differences among the CBM, L-CTX, and SHAM groups. Corticosterone values, defined as means ± SEM, were as follows: CBM, 4.69 ± 2.36 μg/dl; L-CTX, 3.18 ± 1.72 μg/dl; and sham operated, 3.67 ± 1.89 μg/dl.

Discussion

The results of this study strongly support the hypothesis that the hippocampus plays a role in tonic neuronal inhibition of the HPA axis. Upon damaging the hippocampus, expression of mRNA coding for ACTH secretagogues (CRF, AVP) is markedly increased in subdivisions of the PVN controlling CRF secretion into the hypophysial portal system. Up-regulation of CRF and AVP mRNA is accompanied by hypersecretion of the POMC product β-endorphin into the systemic circulation, suggesting a chronic activation of the HPA axis. Corticosterone levels are elevated in parallel groups of HPX animals relative to the SHAM and CTX control groups, further indicating increased adrenocortical glucocorticoid secretion in rats with hippocampal lesions. Up-regulation of β-endorphin and corticosterone secretion following hippocampal damage parallels previous reports demonstrating hypersecretion of ACTH and corticosterone following hippocampal removal (Fendler et al., 1961; Knigge, 1961; Moberg et al., 1971; Feldman and Confronti, 1980; Fischette et al., 1980; Wilson et al., 1980; Magariños et al., 1987).

The magnitude of the effect of HPX on CRF mRNA expression implicate this structure as an important modulator of neuroendocrine CRF neurons. The 4-fold change in CRF mRNA observed in the HPX and the DHPX groups is strikingly similar to that seen following removal of circulating glucocorticoids (bilateral adrenalectomy) in our laboratory (Schäfer et al., 1987; Watson et al., 1989) and others (Young et al., 1986). This qualitative similarity between hippocampal damage and adrenalectomy suggest that HPX neutralizes glucocorticoid-mediated in-

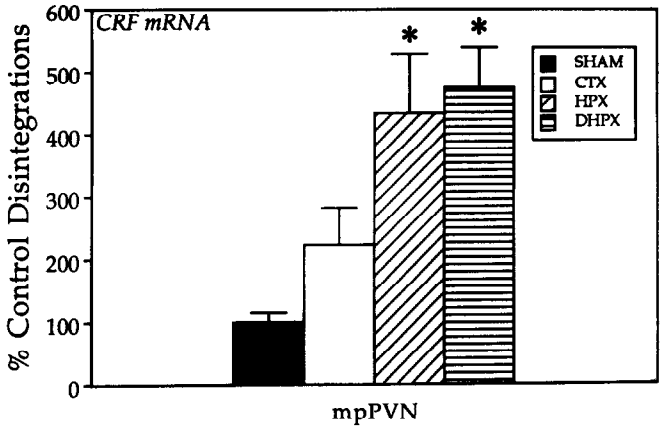


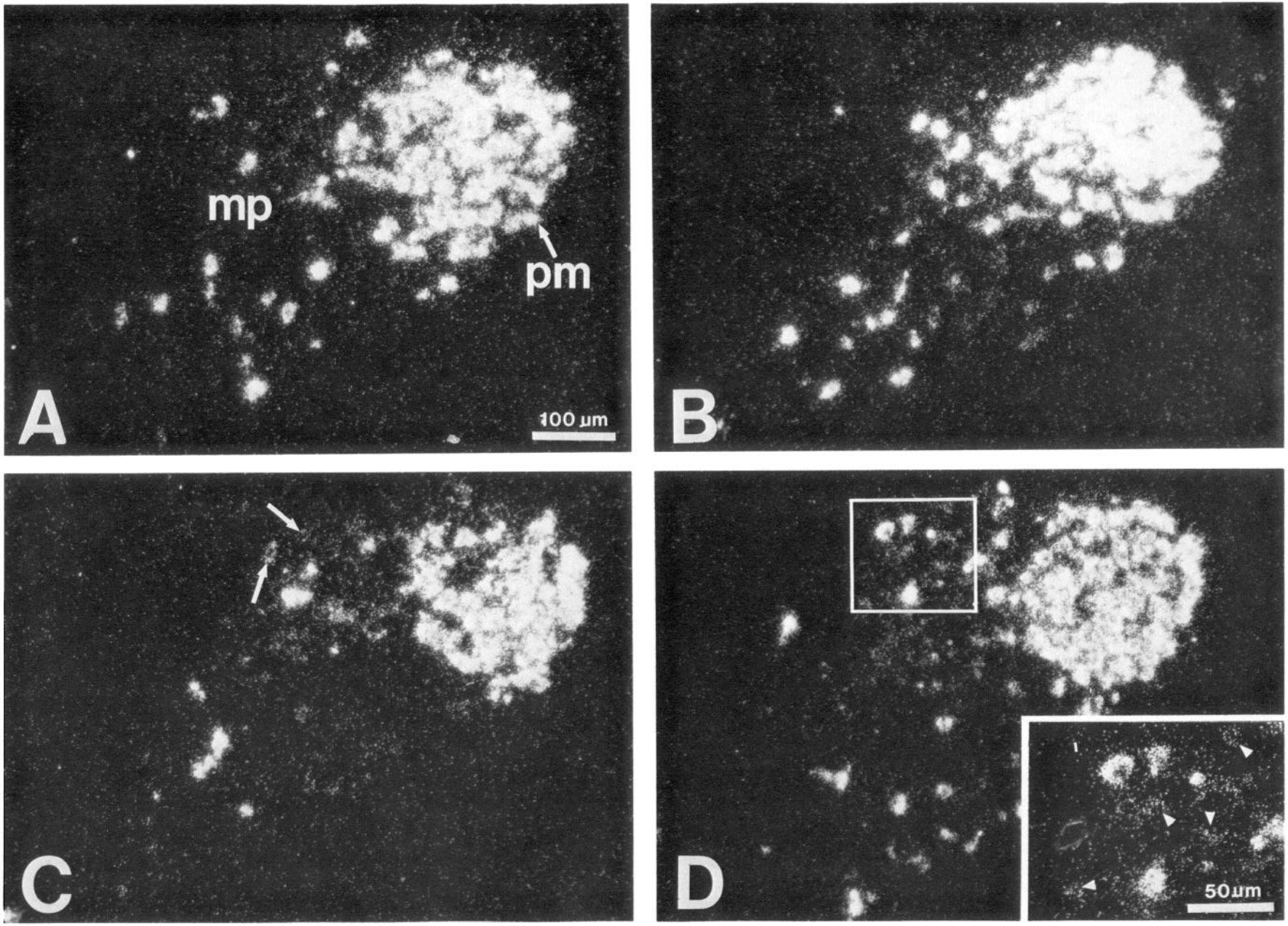
Figure 3. Semiquantitative *in situ* hybridization analysis of CRF mRNA in the medial parvocellular (mp) PVN of SHAM, CTX, HPX, and DHPX rats; values are derived from digitized images of emulsion-dipped autoradiographs. CRF mRNA was significantly increased in the HPX and DHPX groups relative to both CTX and SHAM animals [*single asterisks*: $p < 0.05$, Newman-Keuls test following significant 1-way ANOVA (see text)]. CTX animals manifested slightly increased CRF mRNA levels relative to SHAM controls, which did not attain statistical significance.

Table 1. Body and endocrine organ weights

Group	Preoperative body wt. (gm)	Postoperative body wt. (gm)	Adrenal weight (mg/100 gm body wt.)	Thymus weight (mg)
SHAM	285 ± 5 (11)[a,b]	354 ± 7 (11)	7.64 ± 0.65 (5)	504 ± 28 (6)
CTX	282 ± 6 (10)	338 ± 6 (11)	7.22 ± 0.41 (5)	448 ± 55 (6)
DHPX	274 ± 7 (9)	316 ± 26 (9)	7.55 ± 0.84 (4)	421 ± 63 (4)
HPX	281 ± 5 (10)	318 ± 16 (10)	7.70 ± 0.35 (5)	491 ± 50 (5)

[a] Number in parentheses represents subjects/group.

[b] Numbers represent mean ± SEM.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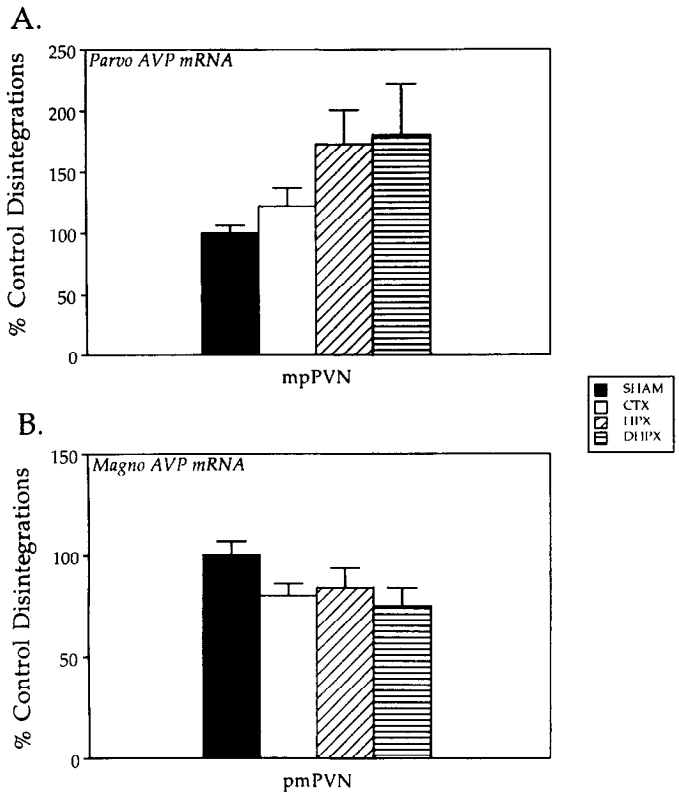

Figure 5. Semiquantitative *in situ* hybridization analysis of AVP mRNA in the medial parvocellular and posterior magnocellular PVN of SHAM, CTX, HPX, and DHPX rats; values are derived from digitized images of emulsion-dipped autoradiographs. AVP mRNA was slightly increased in the medial parvocellular (*mp*) PVN of HPX and DHPX rats relative to SHAM controls and CTX rats, although overall ANOVA did not yield a significant group effect. In contrast, there were no differences among the groups in AVP mRNA in the posterior magnocellular (*pm*) PVN.

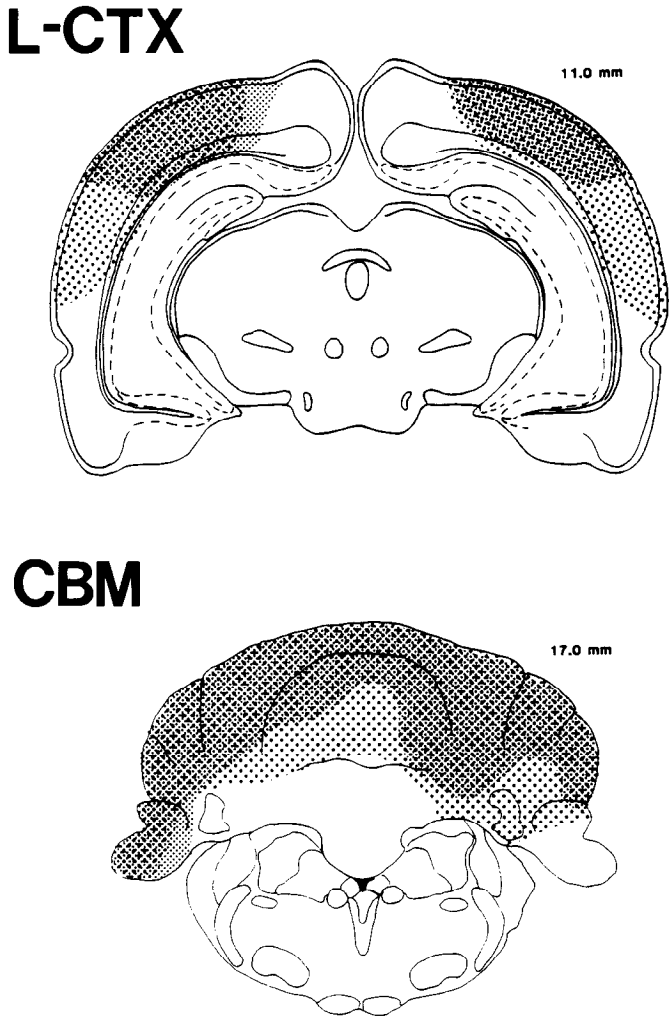

Figure 6. Diagram of large cortical (*L-CTX*) and cerebellar (*CBM*) lesions. *Dark stippling* depicts the animal with the least extensive lesion in each group; *light stippling*, the animal with the most extensive lesion in each group. L-CTX animals typically showed extensive damage to posterior parietal, occipital, and, to some degree, temporal cortices, which exceeded that of the CTX groups (see Fig. 1) in both size and anterior–posterior extent. CBM lesions involved large portions of the cerebellar cortex at all anterior–posterior levels, leaving only portions of lobules 1–3 and the flocculus intact.

hibition of CRF mRNA expression in parvocellular PVN neurons. On the other hand, the up-regulation of AVP mRNA in hippocampectomized rats is small relative to that seen following adrenalectomy. The dramatic increase in AVP gene expression (as well as peptide content) observed after adrenalectomy represents an induction of AVP synthesis in medial parvocellular CRF neurons in that these cells do not produce appreciable amounts of AVP under normal conditions (Davis et al., 1986; Schäfer et al., 1987; Watson et al., 1989). Typically, steroid removal results in a 7-fold increase in parvocellular AVP mRNA (Schäfer et al., 1987; Watson et al., 1989). In the present study, induction clearly occurs in some medial parvocellular PVN neurons in rats with hippocampal damage, resulting in detectable levels of AVP mRNA in this neuronal subpopulation (see Fig. 4, *C, D* inset). However, the amount of AVP message detected represents only a 2-fold change from control values. The induction of AVP mRNA seen in HPX and DHPX rats is therefore limited relative to that seen following adrenalectomy and indicates that cellular mechanisms governing expression of AVP message in the parvocellular PVN remain susceptible to steroid feedback despite the absence of hippocampal input.

Events occurring at the level of the anterior pituitary and adrenal in the experimental groups are worthy of mention. No differences in anterior pituitary content of β-endorphin were observed among the 4 groups. However, the presence of sig-

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Figure 4. Photomicrographs of AVP mRNA localization in the PVN of SHAM (*A*), CTX (*B*), HPX (*C*), and DHPX (*D*) rats visualized by *in situ* hybridization histochemistry. In *A*, *mp* represents the medial parvocellular subdivision of PVN, and *pm* the posterior magnocellular region. The pm is populated by large numbers of densely labeled cells. No visual change in AVP mRNA could be detected in magnocellular regions of the PVN in the respective groups. However, in the HPX and DHPX groups autoradiographic signals could be observed over cells in the parvocellular PVN (see *arrows* in *C* and *D*, inset). The grain density over these cells was limited relative to magnocellular neurons, yielding smaller cellular profiles and fewer grains per cell. The *inset* of *D* provides higher-magnification view of parvocellular elements in the medial parvocellular PVN.

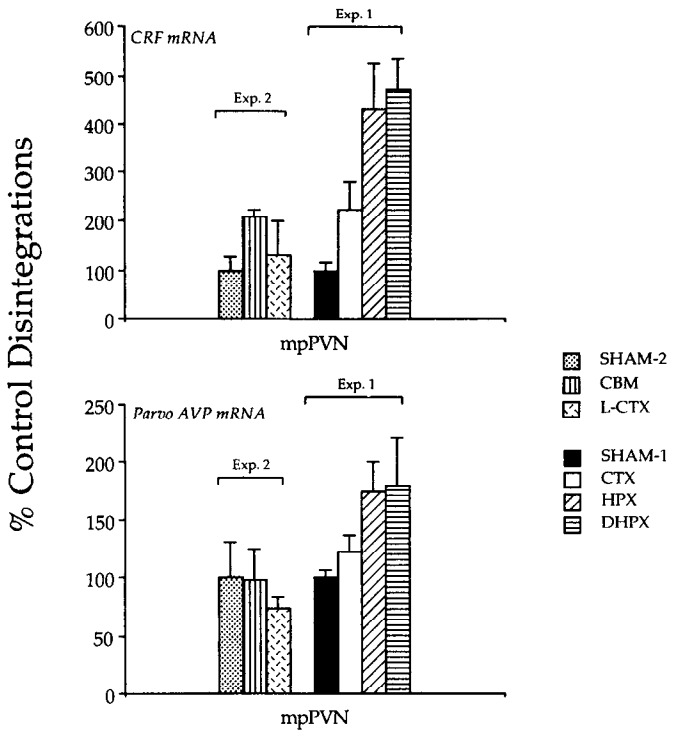

Figure 7. Semiquantitative *in situ* hybridization analysis of CRF and AVP mRNA in the medial parvocellular subdivision of the PVN; values are derived from digitized images of emulsion-dipped autoradiographs. Data from experiment 2 (*Exp. 2*) were analyzed separately from those of experiment 1 (*Exp. 1*) and can be seen as the *left 3* bars of both panels. No overall effect of group on either CRF or parvocellular AVP mRNA was evident from this analysis. The magnitude of effects of cerebellum (CBM) and large cortical (L-CTX) lesions on CRF and AVP mRNA can be contrasted with effects of hippocampectomy (HPX, DHPX) and restricted cortical lesion (CTX) by comparison with the results of experiment 1, presented in the *right 4* bars of both panels. Hippocampectomized animals show 4-fold changes in CRF mRNA, in contrast to (nonsignificant) 50–100% changes observed following surgical control operations.

nificantly elevated β-endorphin peptide of anterior pituitary origin in plasma suggests that animals bearing lesions are indeed in a state of chronic HPA drive. The presence of elevated plasma β-endorphin peptide in combination with normal pituitary content is suggestive of more rapid synthesis and release of peptide by the corticotrophs and, hence, more stimulation of adrenal glucocorticoid production. Stimulation of the adrenal is verified by elevated plasma corticosterone in the HPX group.

It has been recently noted that the medial parvocellular PVN contains a population of type 2 glucocorticoid receptors (Fuxe et al., 1985) and that CRF/AVP neurons residing in this region respond to local glucocorticoid administration following adrenalectomy with normalization of AVP mRNA and peptide expression (Kovacs et al., 1986; Sawchenko, 1987b). These reports provide evidence that the PVN is regulated to some degree by blood-borne glucocorticoids diffusing from the rich vasculature inherent to this region. However, elevated corticosterone levels in hippocampectomized rats are clearly unable to normalize parvocellular PVN CRF or, for that matter, AVP mRNA expression despite presumably intact, available type 2 glucocorticoid receptors in this region. These data suggest that the hippocampus exerts a powerful tonic inhibition of parvocellular PVN CRF/AVP neurons; glucocorticoid feedback via local type 2 receptors in PVN appears unable to fully suppress CRF and AVP mRNA expression in the face of hippocampal removal. It is thus apparent that the PVN CRF/AVP neuron must integrate both humoral and neuronal inputs for appropriate regulation of ACTH secretion.

The observation that HPX and DHPX groups could not be clearly differentiated suggests that the dorsal hippocampus may play the primary hippocampal role in regulation of PVN CRF/AVP neurons. However, it should be noted that both HPX and DHPX lesions involved extensive damage to subfields CA1 and CA2, the major projection neurons of the hippocampal formation, and the fornix, the major hippocampal outflow tract in the rat. Therefore, it cannot be ascertained at present whether dorsal hippocampal tissue or efferent outflow in the fornix is primarily responsible for increases in CRF and AVP mRNA.

In no cases were changes in AVP mRNA expression observed in magnocellular subdivisions of the PVN. This observation indicates that removal of the hippocampus does not result in altered AVP gene expression in neurohypophysial neurosecretory neurons involved in regulation of fluid and electrolyte balance and blood pressure (see Swanson and Sawchenko, 1983).

The observation that CRF mRNA was increased 2-fold in the PVN of CTX rats was somewhat disturbing. Although within the range of experimental error, this change was of sufficient magnitude to warrant further investigation, particularly in light of the fact that HPX and DHPX procedures involved more extensive brain damage than did the CTX protocol. Additional surgical control groups were thus included to determine whether (1) the surgical procedure employed produces nonspecific changes in PVN CRF mRNA related either to surgical stress or amount of tissue removed, or (2) the cortex is actually involved in transmitting information relevant to function of the HPA axis. Removal of large portions of cortex or cerebellum, the latter comparable in volume to the amount of tissue removed via HPX, did not significantly affect CRF or AVP mRNA expression. These data indicate that the results obtained under HPX were

Table 2. Plasma and anterior pituitary β-endorphin content

Group	Plasma β-endorphin (fmol/ml)	Anterior pituitary β-end (pmol/anterior pituitary)
SHAM	19.1 ± 4.1 (10)[a,b]	119 ± 17 (5)
CTX	35.0 ± 9.3 (9)[c]	118 ± 18 (5)
HPX	58.1 ± 14.1 (9)[c]	120 ± 10 (6)
DHPX	56.1 ± 13.4 (10)[c]	137 ± 7 (5)

[a] Number in parentheses represents subjects/group.
[b] Numbers represent mean ± SEM.
[c] Significantly different from SHAM group ($p < 0.05$).

Table 3. Plasma corticosterone levels

Group	Plasma corticosterone (μg/dl)
SHAM (5)[a]	3.6 ± 0.9[b]
CTX (5)	2.2 ± 0.5
HPX (7)	8.3 ± 2.0[c]

[a] Number in parentheses represents subjects/group.
[b] Numbers represent mean ± SEM.
[c] Significantly different from all other groups ($p < 0.05$).

not simply a consequence of volume of tissue damage and that the up-regulation seen in the CTX group was not a phenomenon related to cortical interactions with the HPA axis. Despite the fact that effects of L-CTX and CBM lesions on CRF message are not significantly different from control animals, it should be noted that CRF mRNA levels are consistently increased under conditions of limited cortical damage (CTX), more extensive cortical damage (L-CTX), and extensive cerebellar damage (CBM). The common deviation from sham-operated animals, observed across the various control groups, leaves open the possibility that surgical procedures may cause a slight up-regulation of CRF message in stress-sensitive neurons of the PVN, in a manner independent of lesion size or location. Comparison with HPX animals, however, reveals that any CRF mRNA up-regulation subsequent to extrahippocampal brain lesion is clearly less pronounced than that following HPX. In addition, in contrast to HPX and DHPX rats, plasma corticosterone is not significantly increased in any surgical control group. Combined with the observation that changes in CRF and AVP mRNA expression and plasma β-endorphin values were of approximately equal magnitude in animals with very large or fairly small hippocampal lesions, these data suggest that hippocampal damage, and not some artifact of surgery or brain tissue loss, is primarily involved in the observed HPX-induced changes in HPA function.

Analysis of the postoperative physiological status of the relevant experimental groups further suggests that up-regulation of the HPA axis following HPX and DHPX is independent of postoperative infection or chronic stress resulting from the surgical procedures. Overt postoperative infection (characterized by extensive tissue necrosis, secondary gliosis, and lymphocyte infiltration) was not observed in the lesion sites of HPX or DHPX animals. Chronic stress regimens commonly increase anterior pituitary β-endorphin content (Shiomi et al., 1986). No changes in anterior pituitary β-endorphin was observed among the groups included in this study, suggesting that drive of the HPA axis precipitated by hippocampal or cortical lesion was not due to a prolonged surgery-related stress response. In addition, extended periods of high levels of HPA activation commonly result in adrenal hypertrophy and atrophy of the thymus gland. Neither effect was observed in these studies. Finally, excessive weight loss in operated animals was not observed; indeed, all surgical groups gained weight following surgery. These data indicate that problems with surgical recovery are unlikely to account for our observed neuroendocrine changes.

While the results included in this study implicate the hippocampus as a regulatory influence on CRF/AVP neurons, it is generally acknowledged that with the possible exception of the ventral subicular area, this structure does not project directly to the medial parvocellular PVN (see the excellent tract tracing studies by Berk and Finkelstein, 1981; Silverman et al., 1981; Sawchenko and Swanson, 1983). Input to the PVN from hippocampus are apparently relayed through any of several intermediary structures that appear to interconnect these regions. The most logic circuits connect the hippocampus and PVN via (1) the lateral septum and bed nucleus of the stria terminalis, which receive afferents from the hippocampus via the pre- and postcommissural fornix (Swanson and Cowan, 1977) and project to the region of the PVN (Berk and Finkelstein, 1981; Silverman et al., 1981; Sawchenko and Swanson, 1983) and (2) the medial basal hypothalamus, which receives afferents from the ventral subiculum via the medial corticohypothalamic tract (Swanson and Cowan, 1976, 1977) and projects directly to the medial parvocellular PVN (Sawchenko and Swanson, 1983). The multisynaptic arrangement of the presumptive hippocampus–PVN circuit suggests that glucocorticoid-sensitive neurons may be (at least) one synapse removed from the PVN output neuron.

Last, the results of this study are consistent with the hypothesis that the hippocampus is a *potential* site for glucocorticoid feedback inhibition of the HPA axis. This supposition is supported by studies demonstrating localization of both high- (type 1) and low-affinity (type 2) glucocorticoid receptors in hippocampus (Reul and deKloet, 1985). The hippocampal lesion performed in this study does not address the issue of which receptor type, if any, is involved in hippocampal regulation of PVN CRF neurons. It is possible that the effect of HPX, in addition to eliminating both types of hippocampal glucocorticoid receptors, may by itself transcend the effects of removal of specific glucocorticoid-receptive cells. However, recent data from Dallman and colleagues suggest that type 1 glucocorticoid receptors play an important role in tonic inhibition of ACTH release by low levels of corticosterone in the rat (Dallman et al., 1987). Since the hippocampus is particularly rich in type 1 receptors, and since local type 2 receptors in the region of the PVN are clearly unable to normalize gene expression in CRF neurons following hippocampal removal, it is tempting to speculate that the apparent inhibitory influence of the hippocampus on the HPA axis may be mediated by the type 1 hippocampal receptor system. Further studies are required for a definitive assessment of the role of glucocorticoid receptor subtypes in hippocampal regulation of the HPA axis.

References

Antoni, F. A., M. Palkovits, G. B. Makara, E. A. Linton, P. J. Lowry, and J. Z. Kiss (1983) Immunoreactive corticotropin-releasing hormone (CRF) in the hypothalamo-infundibular tract. Neuroendocrinology *36:* 415–432.

Berk, M. L., and J. A. Finkelstein (1981) Afferent projections to the preoptic area and hypothalamic regions in the rat brain. Neuroscience *6:* 1601–1624.

Bruhn, T. O., P. M. Plotsky, and W. W. Vale (1984) Effect of paraventricular lesions on corticotropin-releasing factor-like immunoreactivity in the stalk-median eminence: Studies on the adrenocorticotropin response to ether stress and CRF. Endocrinology. *114:* 57–62.

Cahill, C. A., J. D. Matthews, and H. Akil (1983) Human plasma β-endorphin-like peptides: A rapid, high recovery extraction technique and validation of radioimmunoassay. J. Clin. Endocrinol. Metab. *56:* 992–997.

Dallman, M. F., G. B. Makara, J. L. Roberts, N. Levin, and M. Blum (1985) Corticotroph response to removal of releasing factors and corticosteroids *in vivo*. Endocrinology. *117:* 2190–2197.

Dallman, M. F., S. F. Akana, C. S. Cascio, D. N. Darlington, L. Jacobson, and N. Levin (1987) Regulation of ACTH secretion: Variations on a theme of B. Rec. Prog. Horm. Res. *43:* 113–171.

Davis, L. G., R. Arntzen, J. M. Reid, R. W. Manning, B. Wolfson, K. L. Lawrence, and F. Baldino, Jr. (1986) Glucocorticoid sensitivity of vasopressin mRNA levels in the paraventricular nucleus of the rat. Proc. Natl. Acad. Sci. USA *83:* 1145–1149.

Dunn, J. D., and S. E. Orr (1984) Differential plasma corticosterone responses to hippocampal stimulation. Exp. Brain Res. *54:* 1–6.

Feldman, S., and N. Confronti (1980) Participation of the dorsal hippocampus in the glucocorticoid feedback effect on adrenocortical activity. Neuroendocrinology *30:* 52–61.

Fendler, K., G. Karmos, and G. Telegdy (1961) The effect of hippocampal lesion on pituitary-adrenal function. Acta Physiol. (Budapest) *20:* 283–297.

Fischette, C. T., B. R. Komisaruk, H. M. Edinger, and A. Siegel (1980)

Differential fornix ablations and the circadian rhythmicity of adrenal corticosteroid secretion. Brain Res. *195:* 373–387.

Fuxe, K., A.-C. Wikström, S. Okret, L. A. Agnati, A. Härfstrand, Z.-Y. Yu, L. Granholm, M. Zoli, W. Vale, and J.-Å. Gustafsson (1985) Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. Endocrinology *117:* 1803–1812.

Gillies, G. E., E. A. Linton, and P. J. Lowry (1982) Corticotropin releasing activity of the new.CRF is potentiated several times by vasopressin. Nature *299:* 355–357.

Jennes, L., W. E. Stumpf, and P. W. Kalivas (1982) Neurotensin: Topographical distribution in rat brain by immunohistochemistry. J. Comp. Neurol. *210:* 211–224.

Keller-Wood, M. E., and M. F. Dallman (1984) Corticosteroid inhibition of ACTH release. Endocrin. Rev. *5:* 1–24.

Khachaturian, H., M. E. Lewis, and S. J. Watson (1983) Enkephalin systems in diencephalon and brainstem of the rat. J. Comp Neurol. *220:* 310–320.

Kiss, J. Z., E. Mezey, and L. Skirboll (1984a) Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. Proc. Natl. Acad. Sci. USA *81:* 1854–1858.

Kiss, J. Z., T. H. Williams, and M. Palkovits (1984b) Distribution and projections of cholecystokinin-immunoreactive neurons in the hypothalamic paraventricular nucleus of rat. J. Comp. Neurol. *227:* 173–181.

Knigge, K. M. (1961) Adrenocortical response to stress in rats with lesions in hippocampus and amygdala. Proc. Soc. Exp. Biol. Med. *108:* 67–69.

Kovacs, K., J. Z. Kiss, and G. B. Makara (1986) Glucocorticoid implants around the hypothalamic paraventricular nucleus prevent the increase of corticotropin-releasing factor and arginine vasopressin immunostaining induced by adrenalectomy. Neuroendocrinology *44:* 229–234.

Magariños, A. M., G. Somoza, and A. F. DeNicola (1987) Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. Horm. Metab. Res. *19:* 105–109.

Makara, G. B., E. Stark, M. Karteszi, M. Palkovits, and G. Rappay (1981) Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of rat. Am. J. Physiol. *240:* E441–E446.

McEwen, B. S., J. M. Weiss, and L. S. Schwartz (1968) Selective retention of corticosterone by limbic structures in rat brain. Nature *220:* 911–912.

Moberg, G. P., V. Scapagnini, J. deGroot, and W. F. Ganong (1971) Effect of sectioning the fornix on diurnal fluctuations in plasma corticosterone levels in the rat. Neuroendocrinology *7:* 11–15.

Murphey, B. E. P. (1967) Some studies of the protein binding of steroids and their application to the routine micro and ultra micro measurements of various steroids in body fluids by the competitive protein binding radioassay. J. Clin. Endocrinol. Metab. *27:* 973.

Olpe, H.-R., and B. S. McEwen (1976) Glucocorticoid binding to receptor-like proteins in rat brain and pituitary: Ontogenetic and experimentally-induced changes. Brain Res. *105:* 121–128.

Plotsky, P. M., and P. E. Sawchenko (1987) Hypophysial-portal plasma levels, median eminence content, and immunohistochemical staining of corticotropin-releasing factor, arginine vasopressin, and oxytocin after pharmacological adrenalectomy. Endocrinology *120:* 1361–1369.

Plotsky, P. M., S. Otto, and R. M. Sapolsky (1987) Inhibition of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation by delayed glucocorticoid feedback. Endocrinology *119:* 1126–1130.

Reul, J. H. M., and E. R. deKloet (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology *117:* 2505–2511.

Sapolsky, R. M., B. S. McEwen, and T. C. Rainbow (1983) Quantitative autoradiography of ^{3}H corticosterone in rat brain. Brain Res. *271:* 331–334.

Sapolsky, R. M., L. C. Krey, and B. S. McEwen (1984a) Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc. Natl. Acad. Sci. USA *81:* 6174–6177.

Sapolsky, R. M., L. C. Krey, and B. S. McEwen (1984b) Stress downregulates corticosterone receptors in a site-specific manner in the brain. Endocrinology *114:* 287–292.

Sawchenko, P. E. (1987a) Adrenalectomy-induced enhancement of CRF and vasopressin immunoreactivity in parvocellular neurosecretory neurons: Anatomic, peptide, and steroid specificity. J. Neurosci. *7:* 1093–1106.

Sawchenko, P. E. (1987b) Evidence for a local site of actions for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. Brain Res. *403:* 213–224.

Sawchenko, P. E., and L. W. Swanson (1985) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J. Comp. Neurol. *218:* 121–144.

Sawchenko, P. E., L. W. Swanson, and W. W. Vale (1984) Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. Proc. Natl. Acad. Sci. USA *81:* 1883–1887.

Schäfer, M. K.-H., J. P. Herman, E. Young, R. Thompson, J. Douglass, T. G. Sherman, H. Akil, and S. J. Watson (1987) Gene expression of neuropeptides related to CRF after adrenalectomy. Soc. Neurosci. Abstr. *13:* 583.

Shiomi, H., S. J. Watson, J. E. Kelsey, and H. Akil (1986). Pretranslational and postranslational mechanisms for regulating β-endorphin-adrenocorticotropin of the anterior pituitary lobe. Endocrinology *119:* 1793–1799.

Silverman, A. J., D. L. Hoffman, and E. A. Zimmerman (1981) The descending afferent connections of the paraventricular nucleus of the hypothalamus. Brain Res. Bull. *6:* 47–61.

Swanson, L. W., and W. M. Cowan (1976) Hippocampo-hypothalamic connections: Origins in subicular cortex, not Ammon's horn. Science *187:* 303–304.

Swanson, L. W., and W. M. Cowan (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J. Comp. Neurol. *172:* 49–84.

Swanson, L. W., and P. E. Sawchenko (1983) Hypothalamic integration: Organization of the paraventricular and supraoptic nuclei. Annu. Rev. Neurosci. *6:* 269–324.

Swanson, L. W., P. E. Sawchenko, R. W. Lind, and J.-H. Rho (1988) The CRH motoneuron: Differential peptide regulation in neurons with possible synaptic, paracrine and endocrine outputs. Annu. NY Acad. Sci. *512:* 12–23.

Tornello, S., E. Orti, A. F. DeNicola, T. C. Rainbow, and B. S. McEwen (1982) Regulation of glucocorticoid receptors in rat brain by corticosterone treatment of adrenalectomized rats. Neuroendocrinology *35:* 411–417.

Watson, S. J., T. G. Sherman, M. K.-H. Schäfer, P. Patel, J. P. Herman, and H. Akil (1989) Regulation of mRNA in peptidergic systems: Quantitative and *in situ* studies. In *Molecular Biology of Brain and Endocrine Peptidergic Systems,* K. W. McKerns and M. Chretien, eds., pp. 225–242, Plenum, New York.

Whitnall, M. H., D. Smyth, and H. Gainer (1987) Vasopressin coexists in half of the corticotropin-releasing factor axons present in the external zone of the median eminence in normal rats. Neuroendocrinology *45:* 420–424.

Wilson, M. M., S. E. Greer, M. A. Greer, and L. Roberts (1980) Hippocampal inhibition of pituitary-adrenocortical function in female rats. Brain Res. *197:* 433–441.

Wolfson, B., R. W. Manning, L. G. Davis, R. Arntzen, and F. Baldino, Jr. (1985) Co-localization of corticotropin releasing factor and vasopressin mRNA in neurons after adrenalectomy. Nature *315:* 59–61.

Young, E. A., and H. Akil (1985) Corticotropin-releasing factor stimulation of adrenocorticotropin and β-endorphin release: Effects of acute and chronic stress. Endocrinolgy *117:* 23–30.

Young, W. S., III, E. Mezey, and R. E. Siegel (1986) Quantitative *in situ* hybridization histochemistry reveals increased levels of corticotropin releasing factor mRNA after adrenalectomy in rats. Neurosci. Lett. *70:* 198–203.