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The Vasopressinergic Innervation of the Brain in Normal and Castrated Rats

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ABSTRACT

A detailed description is given of the distribution of vasopressin-immunoreactive structures in the brain of intact adult male rats. By application of a modified immunocytochemical procedure, vasopressin- immunoreactive fibers were detected in many new areas.

In adult male rats which were castrated 15 weeks before death, vasopressin-immunoreactive cell bodies had disappeared from the bed nucleus of the stria terminalis and the medial amygdaloid nucleus. No obvious changes were found in vasopressin-immunoreactive cell bodies in other areas. Furthermore, a very strong reduction was seen in the density of vasopressin-immunoreactive fibers in the olfactory tubercle, nucleus of the diagonal band and its immediate surroundings, ventral pallidum, basal nucleus of Meynert, lateral septum, septofimbrial nucleus, ventral hippocampal formation, amygdaloid area, pre- and supramammillary nucleus, supramammillary decussation, (inter)dorsomedial, parafascicular, and ventral aspect of paraventricular thalamic nuclei, zona incerta, lateral habenular nucleus, ventral tegmental area, substantia nigra, periventricular gray, dorsal and median raphe nucleus, and locus coeruleus. No changes were observed in other areas containing vasopressin-immunoreactive fibers. These changes following gonadectomy were not observed in castrated rats which had been treated with testosterone.

The results suggest that vasopressin projections from the bed nucleus of the stria terminalis and possibly from the medial amygdaloid nucleus require the presence of gonadal hormones for their normal appearance. This is in contrast to pathways arising from the hypothalamic vasopressin-producing nuclei, which fail to show obvious changes following castration.

Key words: gonadectomy, bed nucleus of the stria terminalis, amygdala, paraventricular nucleus, suprachiasmatic nucleus

In 1951 Bargmann and Scharrer concluded, largely on the basis of their Gomori staining of the hypothalamus, that the secretory material of the posterior pituitary, containing the vasopressor, antiduretic, and oxytocic principles, was probably synthesized in neurons of the supraoptic (SON) and paraventricular nucleus (PVN). From these sites it would be transported within axons projecting to the neurohypophysis in order to be released into the bloodstream. More than two decades later, immunocytochemical techniques using antisera to vasopressin (VP) or oxytocin demonstrated that separate neurons in the PVN and SON produce VP and oxytocin (Swaab et al., '75a,b; Vandesande et al., '75a). The same technique also revealed that the suprachiasmatic nucleus (SCN) contains VP-immunoreactive cell bodies (Swaab and Pool, '75; Vandescande et al., '75b). More recently, pretreatment of rats with colchicine enabled the detection of large groups of VP-immunopositive cell bodies within the bed nucleus of the stria terminalis (BST), the medial amygdaloid nucleus, and the locus coeruleus, while some scattered cells were found within the lateral septum and dorsomedial hypothalamic nucleus (Caffé and Van Leeuwen, '83; Van Leeuwen and Caffé, '83). Furthermore, in addition to the neurosecretory pathway from the SON and PVN to the neurohypophysis, VP- and oxytocin-immunoreactive fibers were found to reach many other areas of the brain (Swanson, '77; Buijs, '78; Buijs et al., '78; Sofroniew and Weindl, '78a,b), where they terminate syn-

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aptically on other neuronal structures (Buijs and Swaab, '79; Voorn and Buijs, '83). VP fibers appear to terminate preferentially in limbic brain areas, while oxytocin fibers predominate in the medulla and spinal cord (Buijs, '78; Swanson and McKellar, '79).

Whereas most of the oxytocinergic fiber pathways in the brain appear to be derived from the PVN, multiple sites of origin exist for VP innervation (De Vries and Buijs, '83). That portion of the VP innervation which appears to be derived from the BST is sexually dimorphic; the plexus of VP-immunoreactive fibers in the lateral septum and lateral habenular nucleus is denser in male than in female rats (De Vries et al., '81). This difference arises during development under influence of gonadal hormones (De Vries et al., '83). However, gonadal hormones also affect these VP fibers in adulthood (De Vries et al., '84a), since in long-term gonadectomized adult rats these fibers could not be stained unless the rats had been treated with testosterone. Similar effects of endocrine manipulations were not observed in the nucleus of the solitary tract, nor in the paraventricular thalamic nucleus (referred to in the previous studies as the periventricular nucleus) and the dorsomedial hypothalamic nucleus, the VP innervation of which is probably derived from, respectively, the PVN and SCN (Sofroniew and Schrell, '81; Sawchenko and Swanson, '82; Hoorneman and Buijs, '82).

Before we could extend these observations to all areas where central VP fibers terminate, we first had to reconsider the overall distribution of VP-immunoreactive structures in the brain, since recent improvements in the immunocytochemical procedure had revealed many new anatomical details. In this paper we present a map of the VP distribution in the rat brain, including all new areas where VP-immunoreactive structures are found, and indicating the changes in VP distribution detected in long-term castrated rats.

MATERIALS AND METHODS

Male Wistar rats, WU:Cpb (3 months old), were obtained from TNO, Zeist (The Netherlands). During the course of

Abbreviations

10	dorsal motor nucleus of the vagus
3n	root of oculomotor nerve
3V	third ventricle
AA	anterior amygdaloid area
ac	anterior commissure
Acb	accumbens nucleus
AHi	amygdalohippocampal area
AHy	anterior hypothalamic area
Amb	ambiguus nucleus
AP	area postrema
Aq	cerebral aqueduct
Arc	arcuate nucleus
B	cells of the basal nucleus of Meynert
BL	basolateral amygdaloid nucleus
BST	bed nucleus of the stria terminalis
BSTL	bed nucleus of the stria terminalis, lateral part
BSTM	bed nucleus of the stria terminalis, medial part
CA	central amygdaloid nucleus
CA1	CA1 field of the hippocampus
CA3	CA3 field of the hippocampus
cc	corpus callosum
CG	central gray
CGD	central gray, dorsal part
Cl	claustrum
cp	cerebral peduncle, basal part
CPu	caudate putamen
cu	cuneate facilculus
CxA	cortex-amygdala transition zone
DG	dentate gyrus
DMH	dorsomedial hypothalamic nucleus
DPB	dorsal parabrachial nucleus
DR	dorsal raphe nucleus
DVC	dorsal vagal complex
En	endopiriform nucleus
Ent	entorhinal cortex
f	fornix
fi	fimbria hippocampus
gcc	genu corpus callosum
GP	globus pallidus
HDB	nucleus of the horizontal limb of the diagonal band
Hip	Hippocampal formation
ic	internal capsule
ICjM	Islands of Calleja, major island
IMD	intermediodorsal thalamic nucleus
Inf	infundibulum
IO	inferior olive
IP	interpeduncular nucleus
LA	lateral amygdaloid nucleus
LC	locus coeruleus
LH	lateral habenular nucleus
lo	lateral olfactory tract
LRt	lateral reticular nucleus
LS	lateral septum
LV	lateral ventricle
MA	medial amygdaloid nucleus
MB	mammillary body
MD	mediodorsal thalamic nucleus
ME	median eminence
mfb	medial forebrain bundle
MGN	medial geniculate nucleus
MHY	medial habenular nucleus
ml	medial lemniscus
MnR	median raphe nucleus
MPO	medial preoptic area
MS	medial septum
mt	mammillothalamic tract
oc	optic chiasm
ot	optic tract
pc	posterior commissure
Pe	periventricular hypothalamic nucleus
PMD	premammillary nucleus, dorsal part
PV	paraventricular thalamic nucleus
PVN	paraventricular nucleus
py	pyramidal tract
Re	reuniens thalamic nucleus
Rh	rhomboid thalamic nucleus
S	subiculum
SCN	suprachiasmatic nucleus
SFi	septofimbrial nucleus
SFO	subfornical organ
sm	stria medullaris
SNC	substantia nigra, pars compacta
SNL	substantia nigra, pars lateralis
SNR	substantia nigra, pars reticularis
Sol	nucleus of the solitary tract
SON	supraoptic nucleus
SOR	supraoptic nucleus, pars retrochiasmaticus
sp5	spinal tract of the trigeminis nerve
st	stria terminalis
SubC	subcoeruleus nucleus
SuM	supramammillary nucleus
TS	triangular septal nucleus
Tu	olfactory tubercle
VDB	nucleus of the vertical limb of the diagonal band
VMH	ventromedial hypothalamic nucleus
VP	ventral pallidum
VTA	ventral tegmental area
ZI	zona incerta

the experiments the rats were fed ad libitum, and lights were on from 7:00 A.M. to 7:00 P.M. The rats were killed between 10:00 a.m. and 12 noon. Twenty untreated rats were used for immunocytochemical analysis of VP distribution in the brain. For this purpose the rats were anaesthetized with sodium pentobarbital (Nembutal; 0.1 mg/100 gm body weight) and perfused through the ascending aorta with 0.9% saline, followed by 5% glutaraldehyde (Merck) in 0.1 M Na-cacodylate buffer, pH 7.5. The brains were dissected and immersed in the same fixative for 2 hours at 4°C, after which they were stored at 4°C in Tris-HCl in 0.9% NaCl (pH 7.6). Subsequently, 50-μm-thick transverse sections were cut with a Lancer vibratome. The free-floating sections were incubated with the following solutions: (1) 0.05 M Tris-HC1 (pH 7.6) containing 0.9% NaC1 and 0.5% Triton X-100 (Tris-Triton), 30-minute rinse; (2) VP antiserum (#126 or W1) 1:1,000 in Tris-Triton, overnight at 4°C; (3) Tris-Triton, 30-minute rinse; (4) goat anti-rabbit-IgG serum (Betsy) 1:50 in Tris-Triton, 90 minutes; (5) Tris-Triton, 30-minute rinse; (6) peroxidase-antiperoxidase (PAP) 1:1,000 Tris-Triton, 90 minutes; (7) Tris-Triton, 20-minute rinse, followed by 0.5 M Tris-HC1 (pH 7.6), 0.9% NaCl (Tris-NaCl); (8) 0.05% 3-3-diaminobenzidine (Sigma) in Tris-NaCl with 0.01% H_2O_2, 20 minutes. Following a final rinse in Tris-NaCl, the sections were mounted on glass slides, air dried, and coverslipped. For details on the raising of the antisera used and for information about the tests performed to prove the specificity of the staining, see Buijs et al. ('78), De Vries et al. ('81), Pool et al ('83), and Caffé and Van Leeuwen ('83). In short, the specificity of the staining was checked by staining alternating sections with (1) VP antiserum preabsorbed with agarose beads coupled with VP, which eliminated immunoreactivity, (2) oxytocin (OXT) antiserum (O-1-V), to find out in which areas cross-reactivity with OXT could be expected, and (3) VP antiserum preabsorbed with agarose beads coupled with OXT, to exclude the possibility of such cross-reactivity. In addition, sections of VP-deficient Brattleboro rats were stained with VP antisera to see whether staining was absent in those areas where no cross-reaction with oxytocin could be expected.

The atlas of Paxinos and Watson ('82) was used for the identification and nomenclature of the brain areas. To enable a thorough mapping of the VP-containing structures the sections were studied not only with light-field, but also with dark-field illumination, which in our vibratome sections revealed additional cytoarchitectural details. In addition, some sections were counterstained with thionine.

To study the effects of castration and of testosterone substitution, 20 rats were castrated and ten were sham operated. After a survival period of 15 weeks (which had proven to be necessary for a virtually complete disappearance of VP fiber staining from the lateral septum; De Vries et al., '84a), ten castrated and ten sham-operated rats were processed for immunocytochemistry. Forty-eight hours prior to death, five rats of each group were anaesthetized with Hypnorm (Duphar; 0.1 ml/100 gm body weight), after which colchicine (30 μg in 20 μl 0.9% NaCl) was administered stereotaxically into the lateral ventricle. Eighteen weeks after castration, five rats received an implantation of silastic tubing (Talas SR3; 1.5 mm I.D., 2.5 mm O.D.) packed with testosterone (Roucel UCLAF), which releases testosterone in physiological quantities (De Vries et al., '84a). Five other castrated rats received empty tubes. Twenty-two weeks after castration, these rats too were processed for immunocytochemistry.

RESULTS

The distribution of VP-immunoreactive cells and fibers in untreated rats is indicated in the left part of Figures 1–5. A distinction is made between (1) VP-immunoreactive cell bodies, (2) fibers which are very thick and probably neurosecretory in nature (Fig. 8), (3) much thinner beaded fibers, which rarely ramify and probably are "fibers of passage," and which will be referred to as elongated fibers (Fig. 9), and (4) fibers which ramify extensively and form numerous perineuronal and bouton-like structures which will be referred to as terminals (Fig. 11B).

Vasopressin-immunoreactive cell bodies

Detailed descriptions of the localization and number of VP-immunoreactive cell bodies in the rat brain could be found in earlier studies (Swaab et al., '75b; Sofroniew and Weindl, '80; Kelly and Swanson, '80; Rhodes et al., '81a; Van Leeuwen and Caffé, '83; Caffé and Van Leeuwen, '83). The observations in the present study largely conform to these reports. VP-immunoreactive cell bodies are observed in the PVN and SON, including its retrochiasmatic part, in small islands scattered over the hypothalamus (Figs. 2, 3, 4A), in the SCN (Figs. 2B,C), BST (Figs. 2A,B, 6B), and in the medial amygdaloid nucleus (Figs. 3C, 4A, 7B). In the dorsomedial nucleus of the hypothalamus and the locus coeruleus, VP-immunoreactive neurons could only be visualized in rats which were pretreated with colchicine (Figs. 4A, 5C). However, no VP-immunoreactive cells were found in the lateral septum of any of the rats studied, which is in contrast to Van Leeuwen and Caffé ('83).

Vasopressin immunoreactive fibers

In the *telencephalon*, scattered elongated fibers are seen in the external plexiform layer of the olfactory bulb and in the cingulate and prefrontal cortex. Dense accumulations of VP-immunoreactive terminals are visible in the ventral part of the vertical limb of the diagonal band of Broca, where the innervation extends laterally into the olfactory tubercle and into the ventral aspect of the ventral pallidum (Figs. 1, 12B). A similar network is found in the perimeter of the transitional zone of the medial septum and the diagonal band (Fig. 1B). This network extends laterally to the borders of the accumbens nucleus. Elongated fibers seem to run from the dorsal parts of the diagonal band to the medial septum (Fig. 9) and from the area lateral to the diagonal band via the septohypothalamic tract (König and Klippel, '63) to the lateral septum (Fig. 1). The most conspicuous VP-innervated area of the entire brain is found in the caudal part of the lateral septum (Figs. 1C, 2A, 11B). More caudally, a small number of elongated fibers and some scattered terminals are visible in the septofimbrial nucleus (Fig. 2B). The subfornical organ also contains a few fibers

Fig. 1–5. Line drawings representing frontal sections of the rat brain, indicating on the left side the distribution of vasopressin-stained cell groups (large dots), very thick fibers (fat curved lines), thin elongated fibers (thin curved lines), and terminals (small dots). The symbols do not represent the actual number of vasopressin-immunoreactive structures but indicate their relative density. Hatched areas on the right side indicate where vasopressin terminals disappear following castration; triangles indicate the disappearance of cell staining. The frontal planes were modified from the atlas of Paxinos and Watson ('82). Their anterior-posterior positions relative to Bregma are: 1A, +1.2; 1B, +0.7; 1C, +0.2; 2A, −0.3; 2B, −0.8; 2C, −1.3; 3A, −1.8; 3B, −2.3; 3C, −2.8; 4A, −3.3; 4B, −3.8; 4C, −4.8; 5A, −5.8; 5B, −7.8; 5C, −9.3; 5D, −11.8; 5E, −13.8.

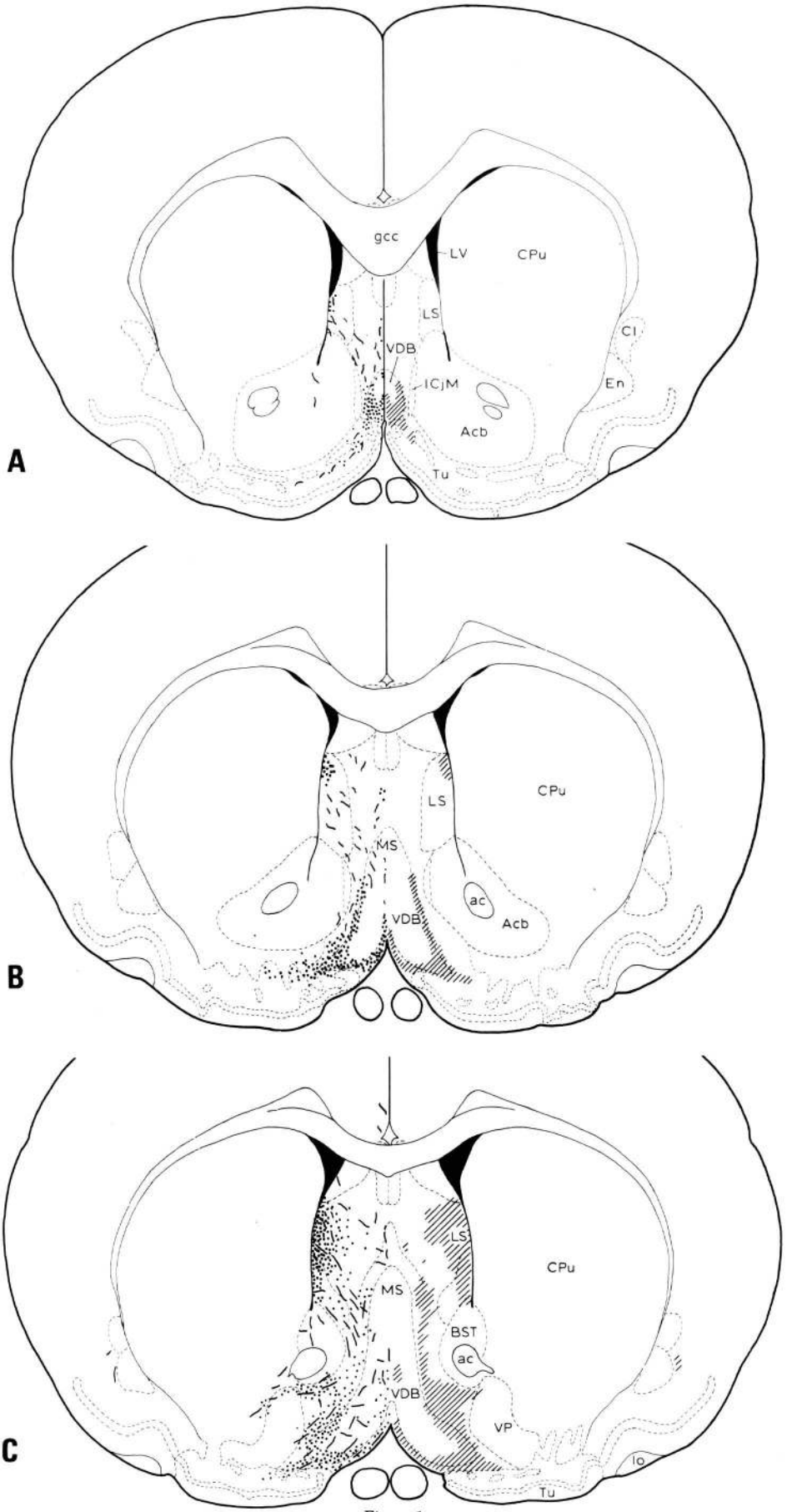
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Figure 1

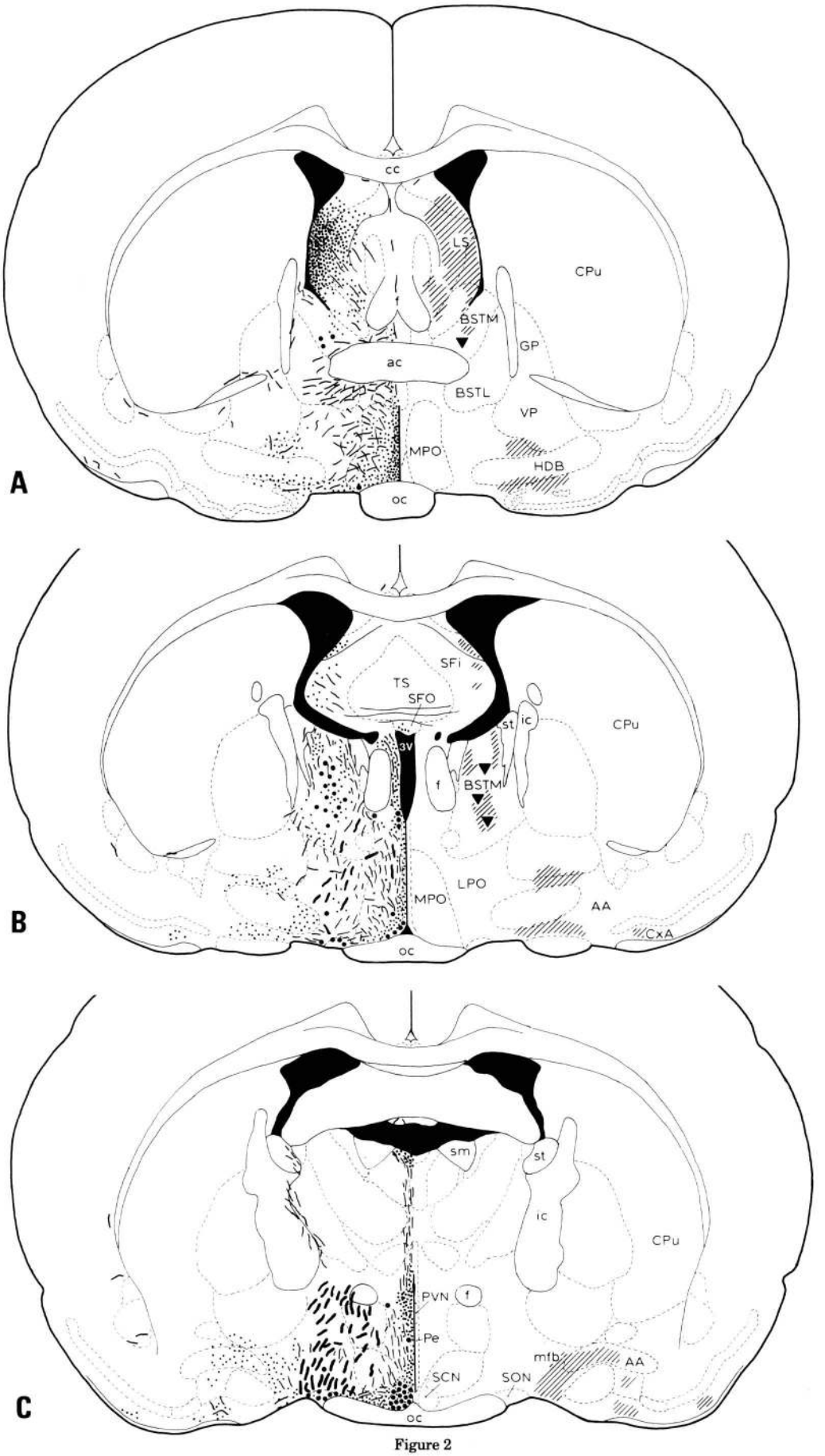
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Figure 2

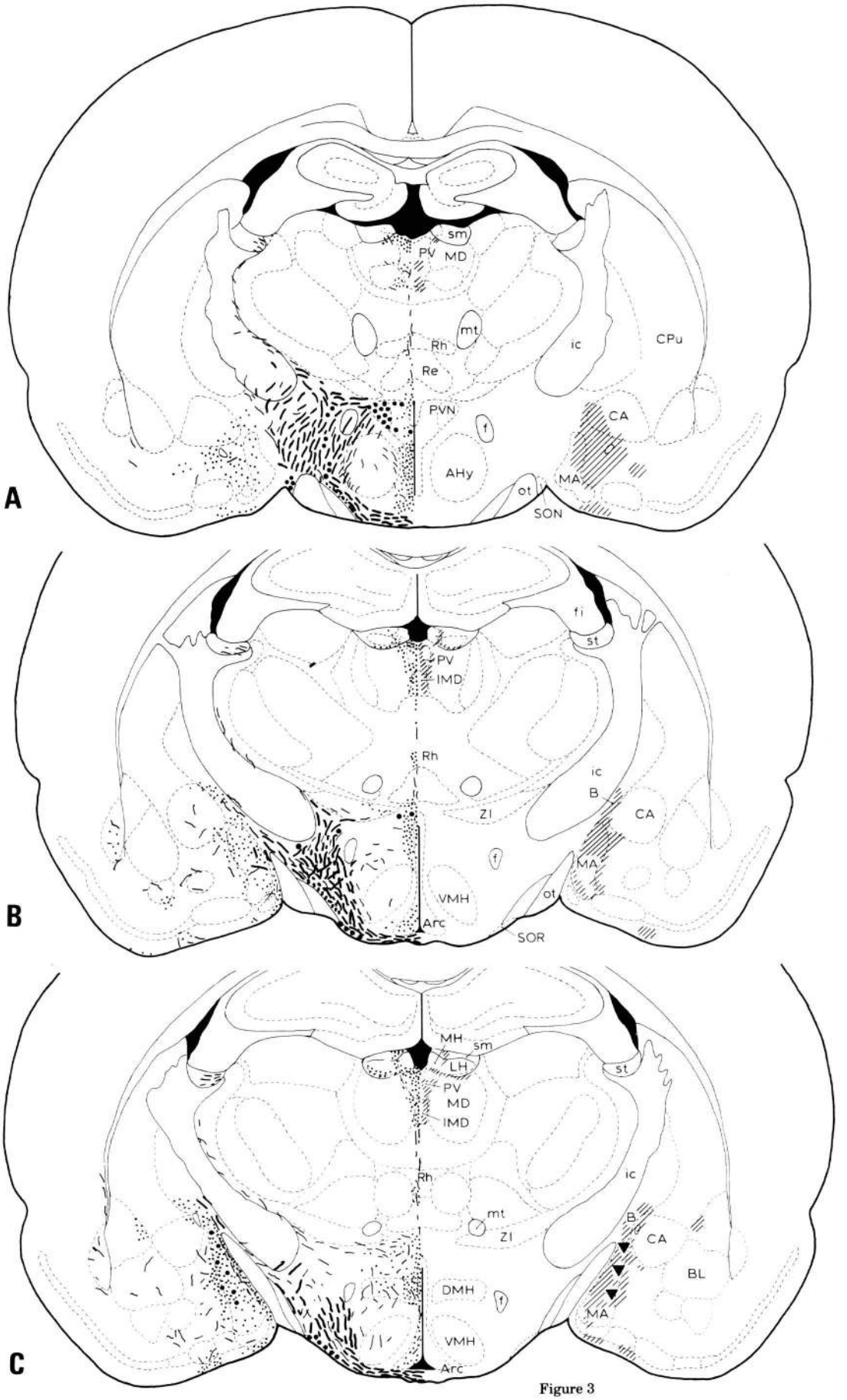
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Figure 3

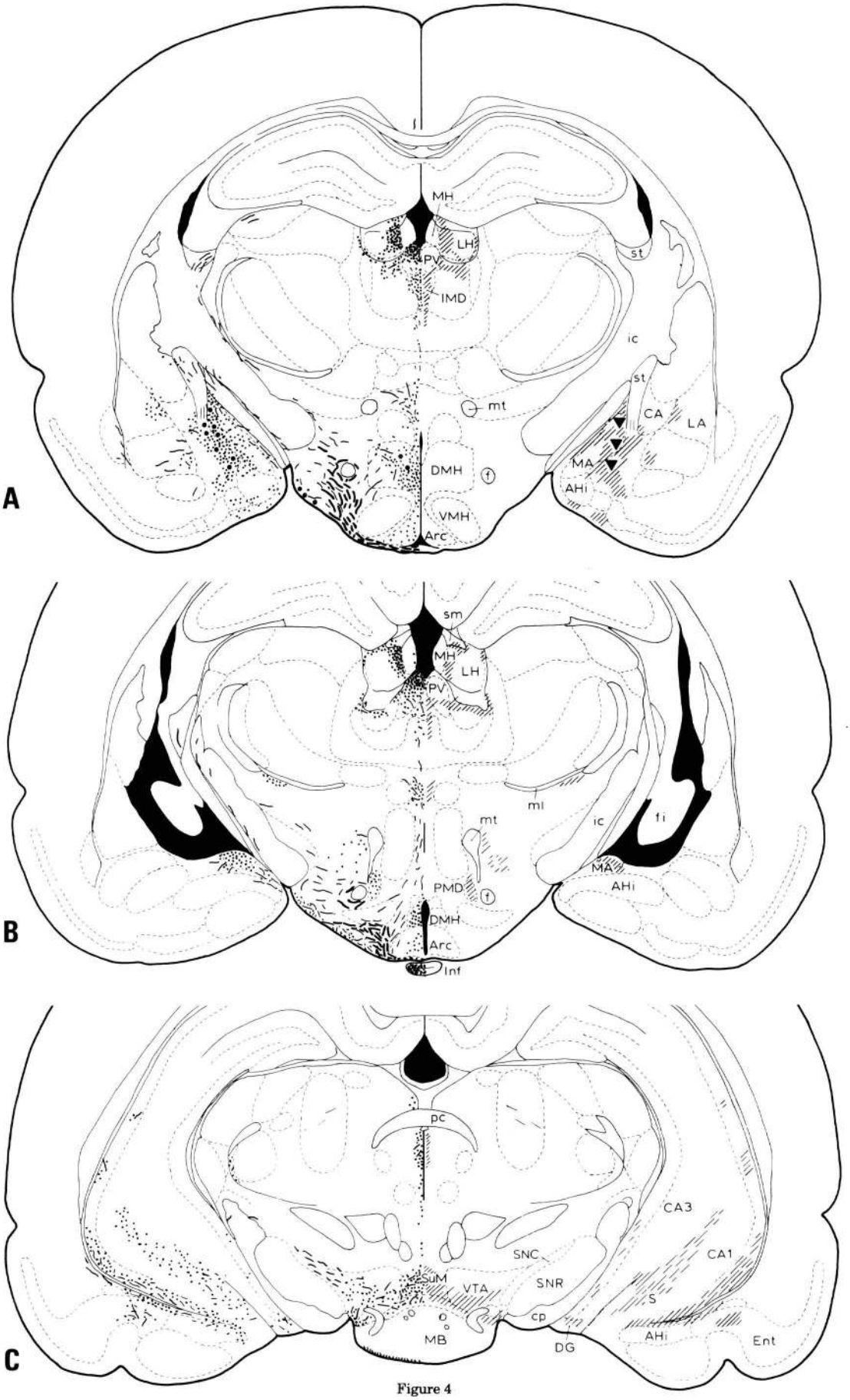
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Figure 4

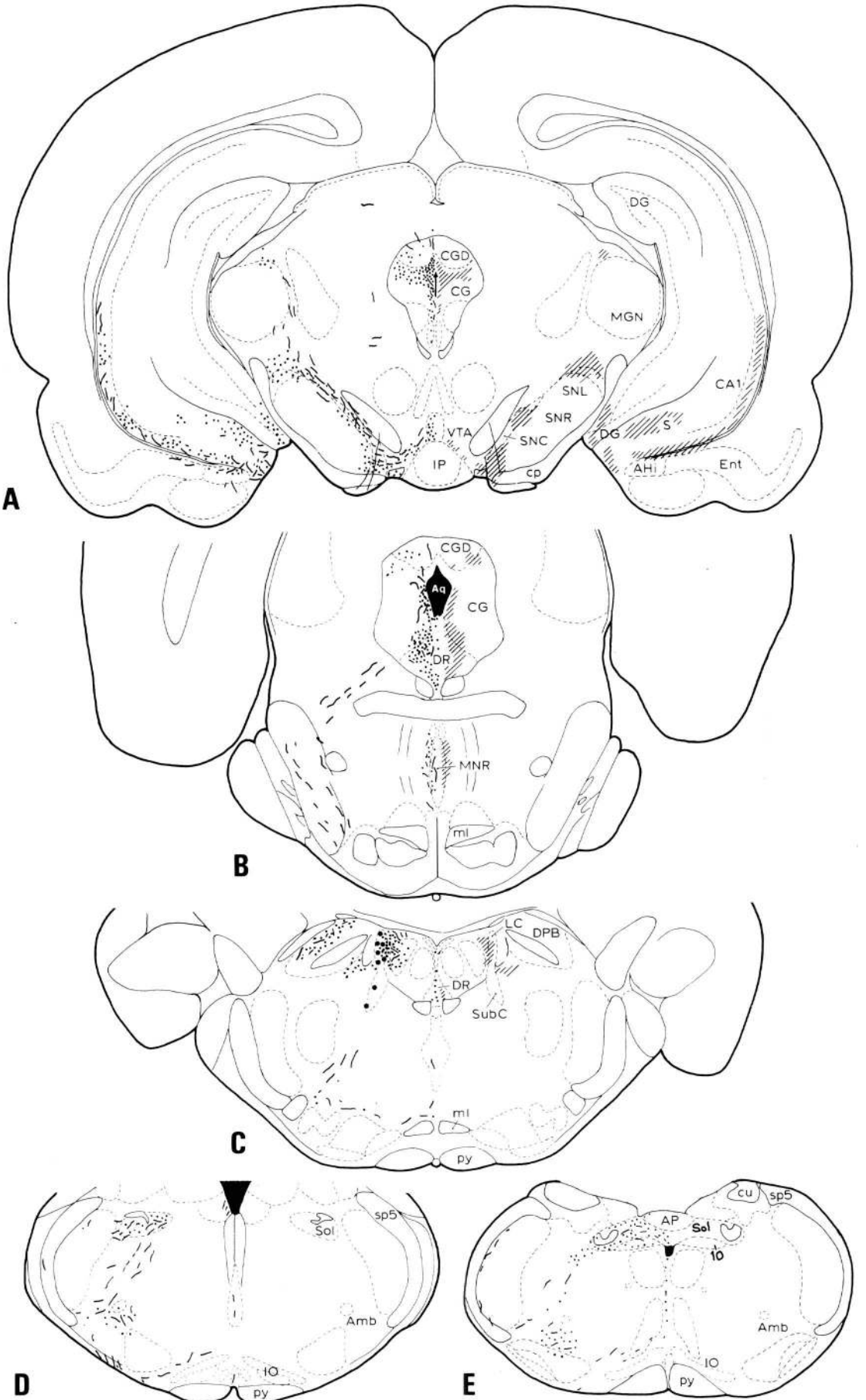
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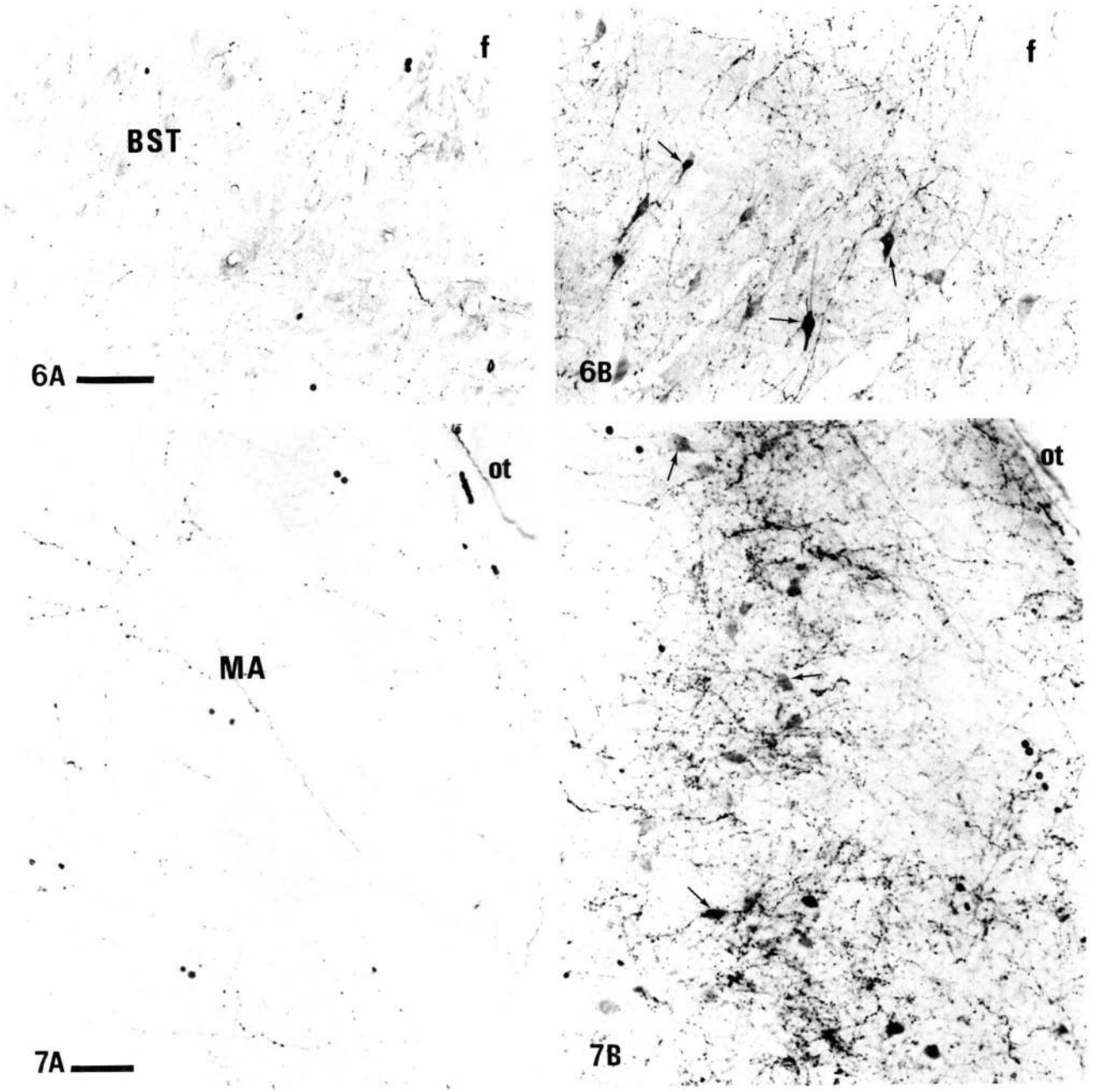
Figure 5



Figs. 6, 7. Photomicrographs indicating vasopressin immunoreactivity in the bed nucleus of the stria terminalis (BST; 6) and medial amygdaloid nucleus (MA; 7) of rats which were not pretreated with colchicine. Arrows in B indicate vasopressin-stained cells in an intact rat. Note that in a castrated rat (A) vasopressin cells and terminals have virtually disappeared, although elongated fibers remain visible. ot, optic tract. Bar in Figure 6 = 100 μm, in Figure 7 = 50 μm.

(Fig. 2B). A small area of densely packed VP-immunoreactive terminals appears just ventral to the most rostral part of the BST (Fig. 1C). More caudally in this structure, a moderate number of VP terminals can be observed in its medial part at the same level of the BST cells (Figs. 2A, 6B). This area further contains elongated VP fibers which seem to enter the stria terminalis, probably to reach the amygdaloid area (Figs. 2B,C). Here, low densities of terminals are present in the anterior amygdaloid area, the cortex-amygdala transition zone, the basolateral and central amygdaloid nucleus, and the area just around the stria terminalis (Figs. 2B,C, 3, 4A). Dense accumulations of terminals are found in a small medial part of the lateral amygdaloid nucleus and in the dorsolateral aspect of the medial amygdaloid nucleus (Figs. 3, 4A,B, 7B). Dorsally, the latter fiber plexus is continuous with the innervation of the area containing the cells of the basal nucleus of Meynert (Fig. 3B). Elongated fibers are visible throughout the

amygdala, especially in its most medial parts, where they run parallel to the optic tract (Fig. 7B). Scattered elongated fibers are seen in the endopiriform nucleus. More caudally a moderate terminal density is present in the ventral part of the hippocampal formation, i.e., in the stratum oriens and stratum moleculare of the CA1, CA3, and subiculum and in the most ventral part of the dentate gyrus (Figs. 4C, 5A, 13B). Scattered terminals and elongated fibers are present in the entorhinal cortex. Many elongated fibers can been seen to run parallel to the alveus and perpendicular to the hippocampal axis.

In the *diencephalon*, very thick (probably neurosecretory) fibers emerge from the magnocellular neurons of the PVN and SON and the additional hypothalamic sites containing magnocellular VP-immunoreactive neurons. Most of these fibers follow the hypothalamo-neurohypophyseal tract toward the median eminence (Figs. 2B, C, 3, 4A,B, 8). However, some of them seem to course dorsolaterally in the direction of the stria terminals at both sides of the internal capsule. From the SCN, elongated fibers are seen to course in rostral directions. These fibers appear to be continuous with a plexus of terminals in the area of the organum vasculosum laminae terminalis. Other fibers leave the area of the SCN in lateral, dorsocaudal, and dorsal directions (Fig. 3). The dorsally directed fibers appear to course via the periventricular hypothalamic nucleus, which contains many terminals (Figs. 3, 14), toward the paraventricular thalamic nucleus. Many elongated fibers and terminals are visible in the medial preoptic area (Figs. 2A, 14), while some fibers are situated around the medial part of the anterior commissure (Fig. 2A). Almost no fibers are present in the ventromedial hypothalamic nucleus, in contrast to the dorsomedial hypothalamic nucleus, which contains a very dense accumulation of terminals (Figs. 3B,C, 4A,B). The arcuate and premammillary nuclei contain a moderate number of VP-immunoreactive terminals (Fig. 4A,B). At the base of the lateral hypothalamus elongated fibers seem to course caudally in such a way as to pass the mamillary body both ventrally and dorsally to reach the ventral tegmental area. These fibers seem to be continuous with fibers that run lateral to the medial leminiscus, some of which bend more caudally in mediodorsal directions to enter the area of the periventricular gray, while others appear to follow the lateral tegmentum, to reach the medulla oblongata. Over the entire length of the thalamus, elongated fibers are seen to course from the hypothalamic ventricular area dorsally toward the paraventricular thalamic nucleus. These fibers are restricted to the most medial part of the thalamus (Figs. 2C, 3, 4A,B). At the level of the rhomboid thalamic nucleus, scattered terminals are noted (Fig. 3B,C). In addition, moderate densities of VP-immunoreative terminals can be seen in the intermediodorsal thalamic nucleus, the dorsal part of the mediodorsal thalamic nucleus (Fig. 15B), the ventral part of the paraventricular thalamic nucleus, the area surrounding the fasciculus retroflexus, and the dorsal part of the zona incerta (Figs. 3, 4A,B). More dorsally, the paraventricular thalamic nucleus contains a dense accumulation of VP terminals over its entire dorsal aspect (Figs. 3, 4A,B, 15B). A similar band of VP terminals is present in the medial part of the lateral habenular nucleus, whereas only some scattered terminals are visible in the lateral part of this structure (Figs. 3B,C, 4A,B).

In the *mesencephalon*, a moderate density of terminals is visible in the supramammillary nucleus and supramammillary decussation (Figs. 4C, 16B), while many fibers are present in the central gray, especially in the part immediately dorsolateral to the aquaduct (Figs. 4C, 5A,B, 17B). In this region, many elongated fibers appear to run caudally along both sides of the ependyma parallel to the aquaduct. Some of these fibers seem to course dorsocaudally to reach the superior colliculi, while others continue caudally to reach the parabrachial and raphe nuclei and the locus coeruleus. Moderate densities of VP terminals are seen in the ventral tegmental area, the zona lateralis, and the ventral aspect of the zona compacta of the substantia nigra (Figs. 4C, 5A, 18B).

In the *metencephalon*, the innervation of the central gray forms a continuum with a field of terminals in the dorsal raphe nucleus (Figs. 5B, 19B). Other areas in the metencephalon which contain moderate numbers of VP-immunoreactive terminals are the median raphe nucleus, locus coeruleus, and dorsal parabrachial nucleus (Fig. 5B,C). Some scattered fibers are visible in the white matter of the cerebellum.

In the *myelencepahalon*, scattered VP terminals can be found within the raphe magnus. A moderate density of VP terminals is present in the entire area of the nucleus of the solitary tract and in the dorsal motor nucleus of the vagus (Fig. 5D,E). Ventrolaterally oriented elongated fibers can be found between these areas and the region containing the ambiguus nucleus and the lateral reticular nucleus, where a few VP terminals are present (Fig. 5D,E). Scattered terminals and elongated fibers can be seen in the most lateral part of the nucleus of the spinal trigeminis nerve (Fig. 5E). The spinal cord, which contains a very low amount of VP-immunoreactive fibers (Swanson and McKellar '79: Buijs, '80; Nilaver et al., '80), and the pineal gland, where scattered VP fibers have also been found (Buijs and Pevét, '80), were not included in the present study.

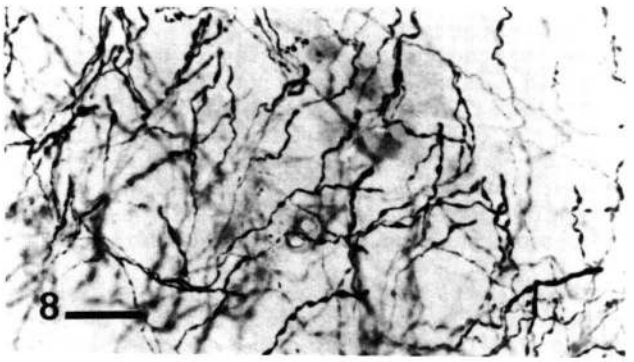
Although most of the VP fibers are clearly embedded in the neuropil, at some places the fibers seem to cross the ependyma and make direct contact with the ventricular space. This is most notable in the ependyma lining the rostral tip of the third ventricle, the part bordering the arcuate nucleus, the lateral ventricles at the level of the stria medullaris, the aquaduct, and finally, the ependyma covering the fourth ventricle at the cerebellar side (Fig. 10).

Effects of castration

In the right half of Figures 1–5 are indicated the areas from which VP-immunoreactive terminals and/or cell bodies are seen to disappear after castration. The distribution of VP immunoreactivity in sham-operated rats appears to be similar to that in untreated rats.

No obvious changes following castration are seen in the distribution of VP-immunoreactive cells in the SCN, PVN, and SON, nor in the locus coeruleus and dorsomedial hypothalamic nucleus. In the latter two areas, VP-immunoreactive cell bodies are only observed in castrated or sham-operated rats, if they have been treated with colchicine. In contrast with these results, almost no VP-immunoreactive cells could be visualized in the bed nucleus of the stria terminalis (Fig. 6A) and medial amygdaloid nucleus (Fig. 7A) of castrated rats which were otherwise untreated or had received a colchicine pretreatment.

No effects of castration are noticeable with regard to staining of the very thick VP-immunoreactive fibers of the hypothalamo-neurohypophyseal tract. Neither are any changes detected in the appearance of elongated fibers, unless specified below. In many areas, however, a very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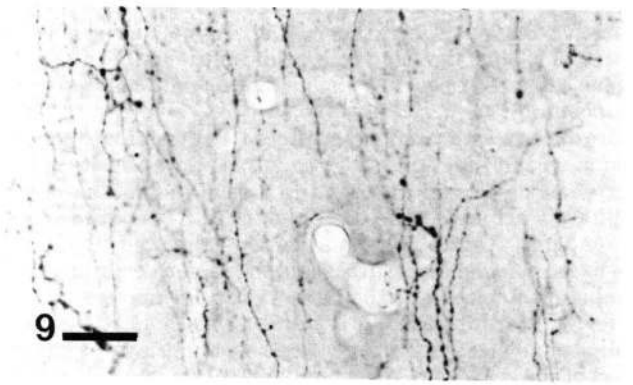
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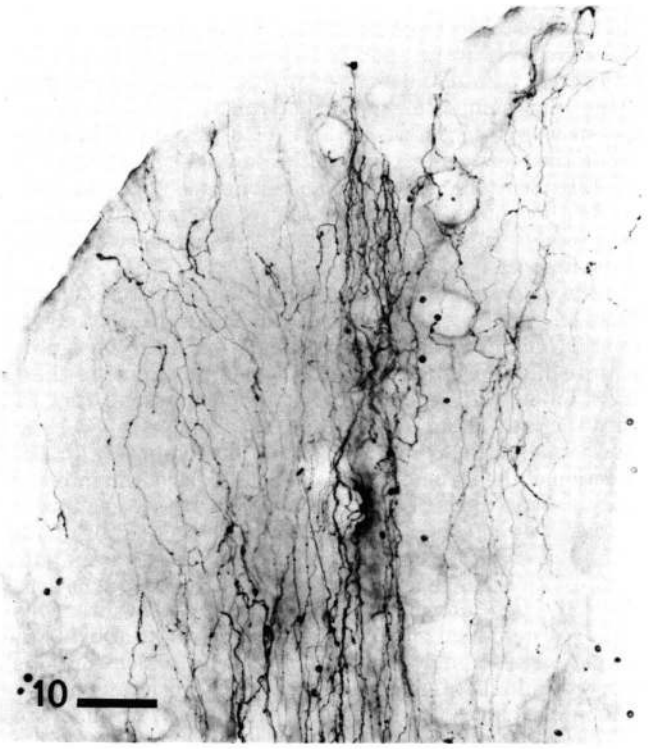
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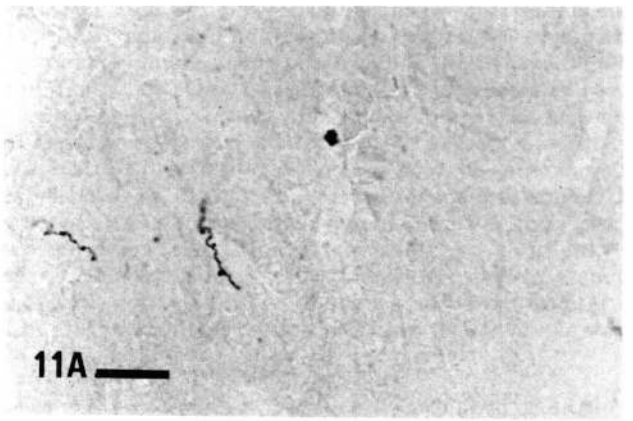
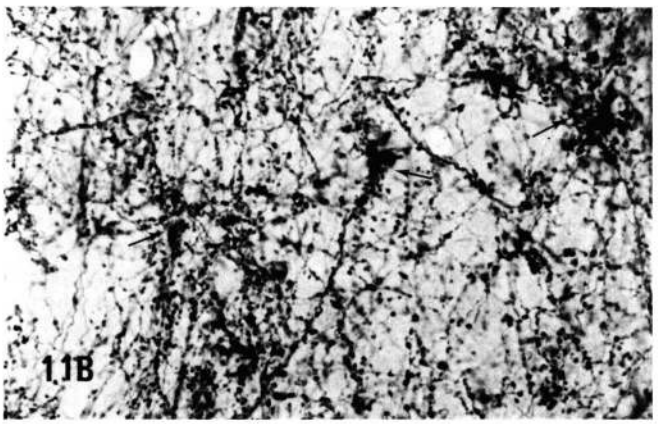
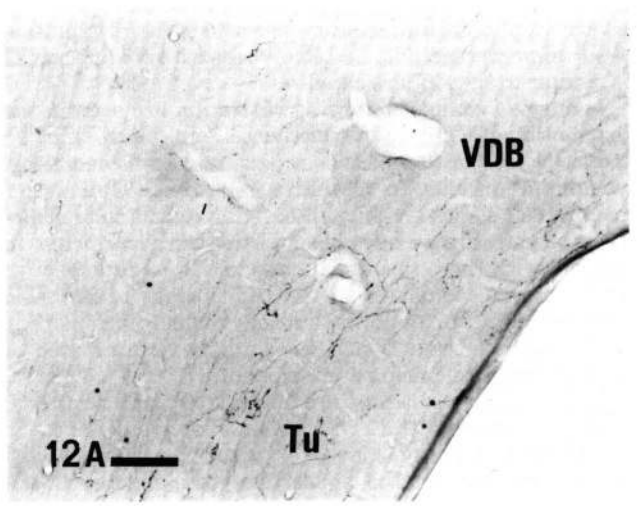
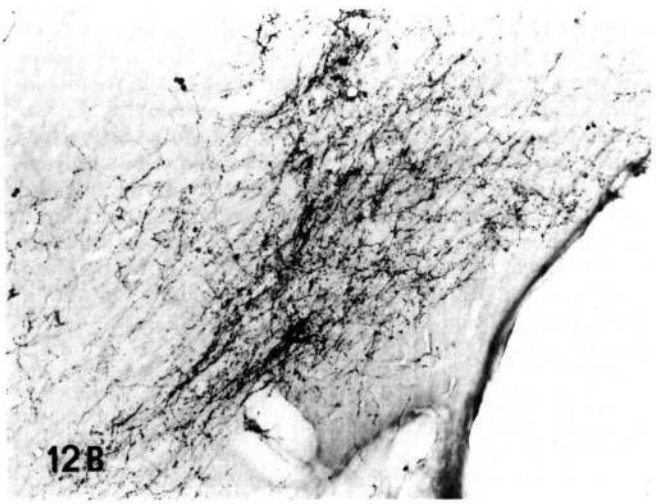

11A


11B


VDB
12A
Tu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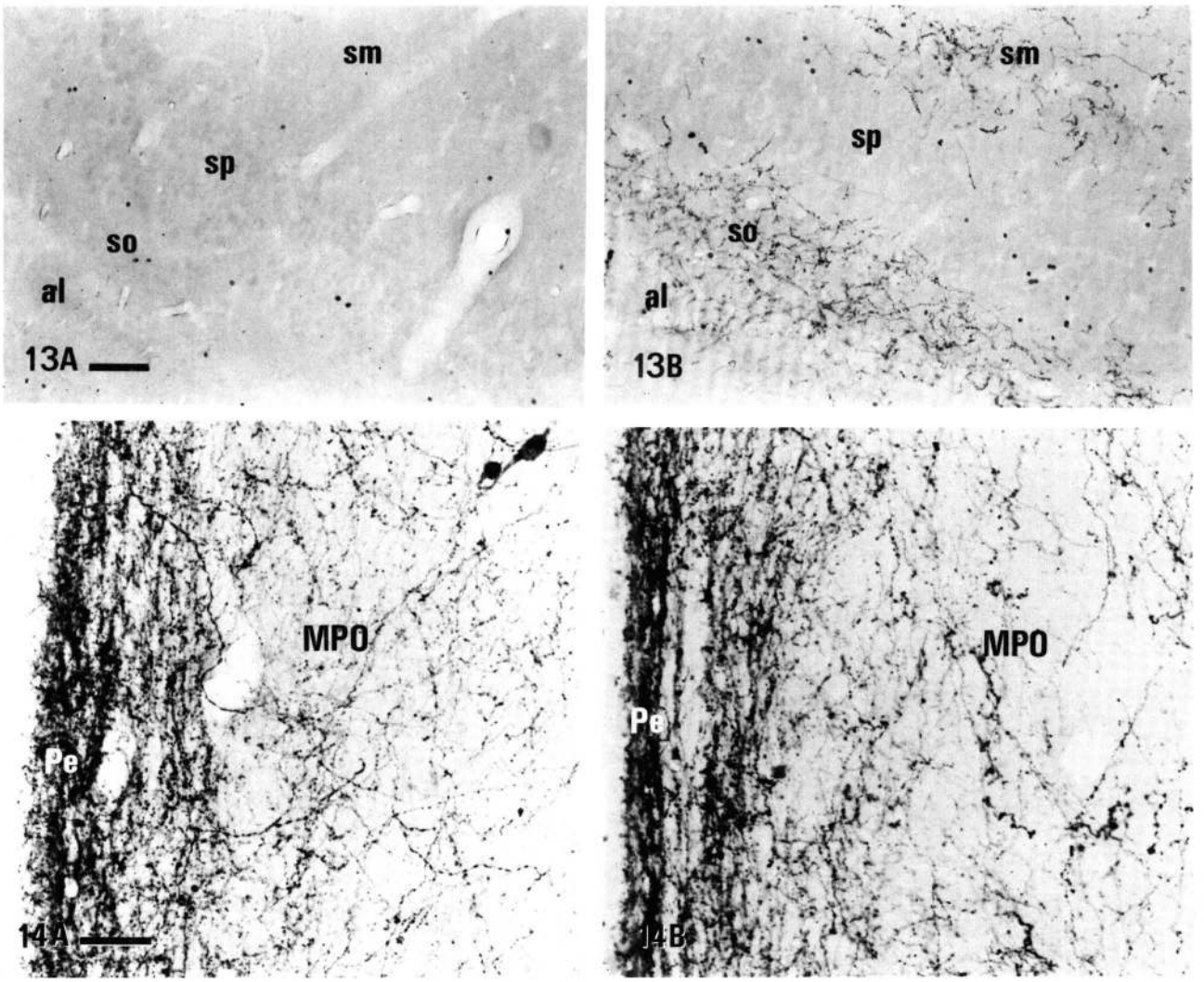


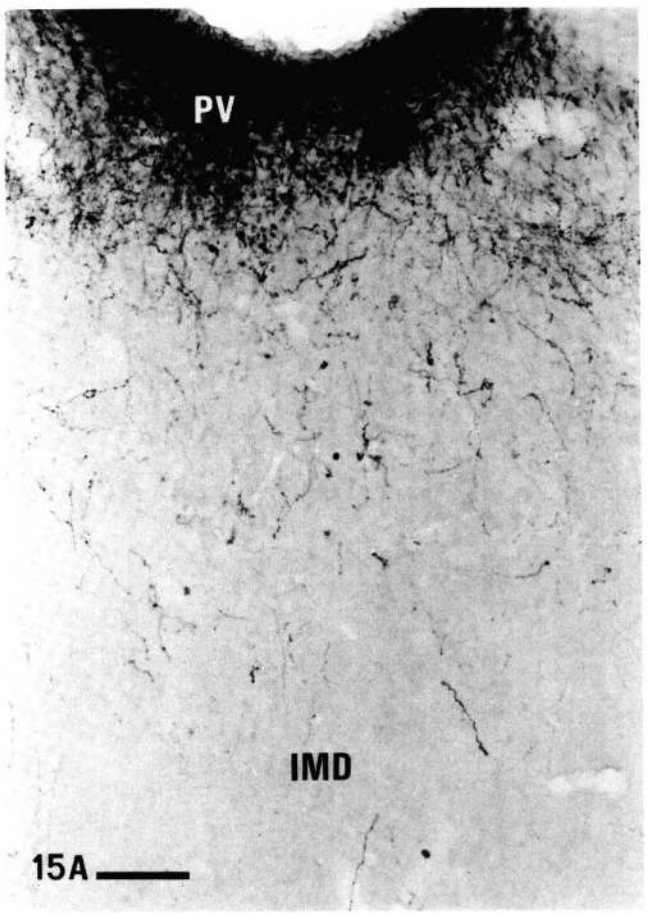
Figs. 13, 14. Photomicrographs of transverse sections of the brain of a castrated (A) and intact (B) rat stained for the presence of vasopressin. Figure 13 shows the distribution of vasopressin-immunoreactive fibers in the CA3 field of the hippocampus, which is clearly affected by castration. Similar effects are not visible in the periventricular region of the hypothalamus (14F). al, alveus; MPO, medial preoptic area; Pe, periventricular hypothalamic nucleus; so, stratum oriens; sp, stratum pyramidale, sm, stratum moleculare. Bar in Figure 13 = 75 μm, in Figure 14 = 50 μm.

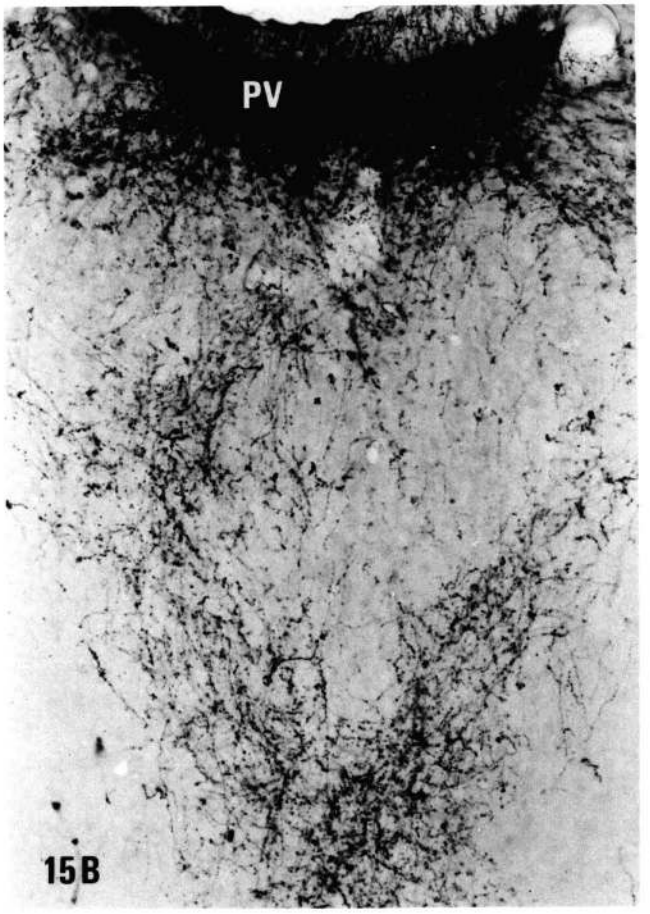
strong reduction is noted in the density of VP-immunoreactive terminals. These effects are present in all castrated rats studied.

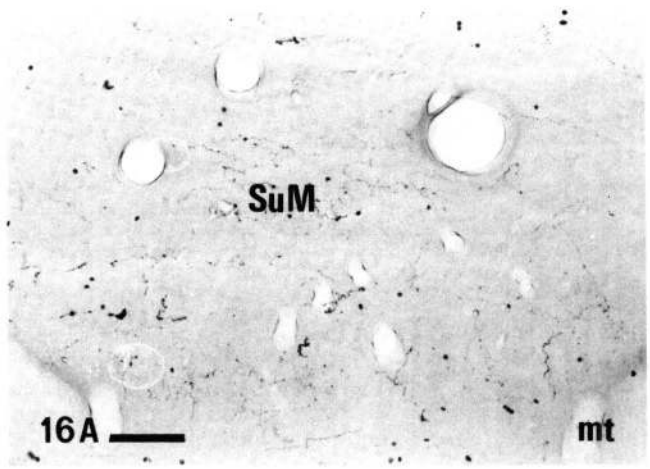
In the *telencephalon* of castrated rats, VP-immunoreactive terminals have almost completely disappeared from the olfactory tubercle and the rostral parts of the nucleus of the diagonal band and its immediate surroundings (Fig. 12A), as well as from the lateral septum (Fig. 11A), the septofimbrial nucleus, the bed nucleus of the stria terminalis (Fig. 6A), the area containing the basal cells of Meynert (cf. Paxinos and Watson, '82), all the innervated amygdaloid areas (Fig. 7), and all the innervated areas of the hippocampal formation (Fig. 13A). In the latter area, besides terminals, most of the elongated fibers have disappeared. In the *diencephalon* of castrated rats, virtually all VP-immunoreactive terminals have disappeared from the lateral habenular nucleus and the mediodorsal, intermediodorsal (Fig 15A), and parafascicular thalamic nuclei, as well as from the ventral part of the paraventricular thalamic nucleus (Fig. 15A), the premammillary nucleus, and the dorsal part of the zona incerta.

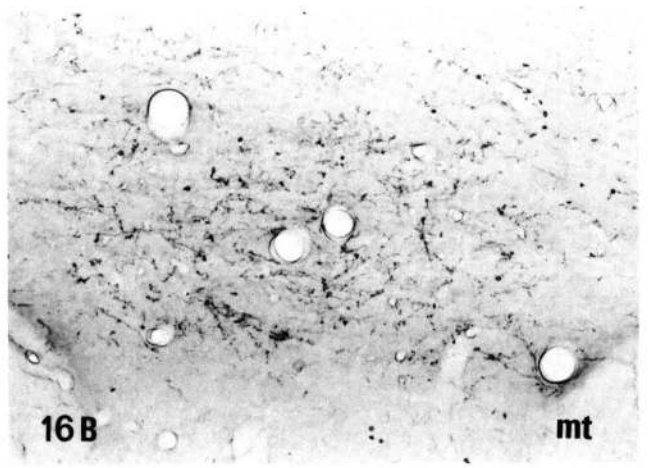
In the *mesencephalon*, almost all VP-immunoreactive terminals have disappeared from the supramammillary nu-

Figs. 8–12. Photomicrographs of transverse sections of the brain showing various types of vasopressin-immunoreactive fibers. Figure 8 shows very thick fibers in the hypothalamo-neurohypophyseal tract. Figure 9 demonstrates elongated fibers in the medial septum which are probably "of passage." In Figure 10 fibers can be seen running along the surface of the ependyma lining the fourth ventricle at the cerebellar side. Figure 11B shows terminals in the lateral septum. Arrows indicate basket-like perineuronal structures. These terminals are not visible in the lateral septum of a castrated rat (11A). Figure 12 indicates vasopressin immunoreactivity in the region of the diagonal band of Broca in a castrated (A) and intact rat (B). Note that in the castrated rat terminals have disappeared, while elongated fibers remain visible. VDB, ventral limb of the nucleus of the diagonal band of Broca; Tu, olfactory tubercle. Bars in Figures 8, 9, and 11 = 25 μm, in Figures 10 and 12 = 75 μm.



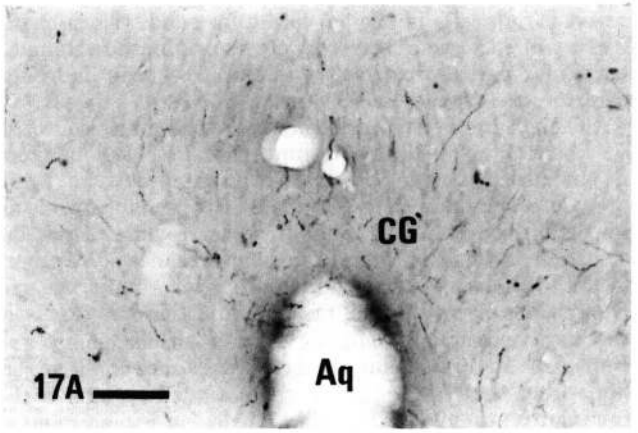






Figs. 15, 16. Photomicrographs of transverse sections of the brain of a castrated (A) and intact (B) rat stained for the presence of vasopressin. Figure 15 illustrates that whereas no effects of castration are noticeable in the dorsal aspect of the paraventricular thalamic nucleus (PV), vasopressin-stained terminals have disappeared from its ventral part and from the intermediodorsal thalamic nucleus (IMD). Elongated fibers, however, do not appear to be affected. Figure 16 shows the region of the supramammillary nucleus (SuM). mt, mamillothalamic tract. Bar in Figure 15 = 75 μm, in Figure 16 = 100 μm.

Figs. 17–19. Photomicrographs of vasopressin-stained transverse sections of the brain of a castrated (A) and intact (B) male rat. Vasopressin-stained terminals are seen to disappear following castration from the central gray (CG; 17), the ventral part of the pars compacta of the substantia nigra (SNC; 18), and the dorsal raphe nucleus (DR; 19). Arrows in 19 indicate the region where the highest terminal density is found in the intact rat. 3n, root of oculomotor nerve; Aq, cerebral aquaduct; ml, medial lemniscus. Bars = 100 μm.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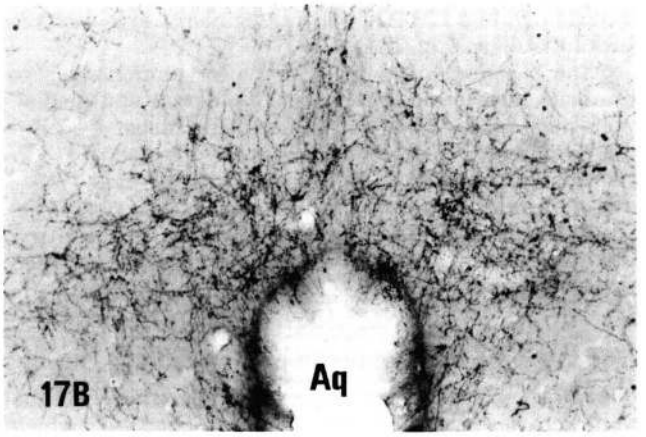
Aq
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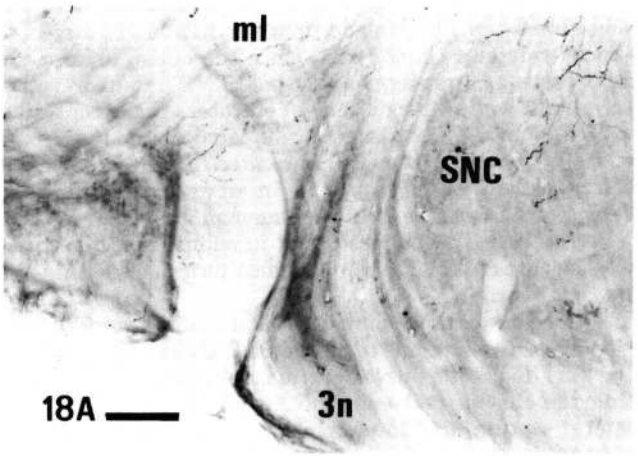
ml
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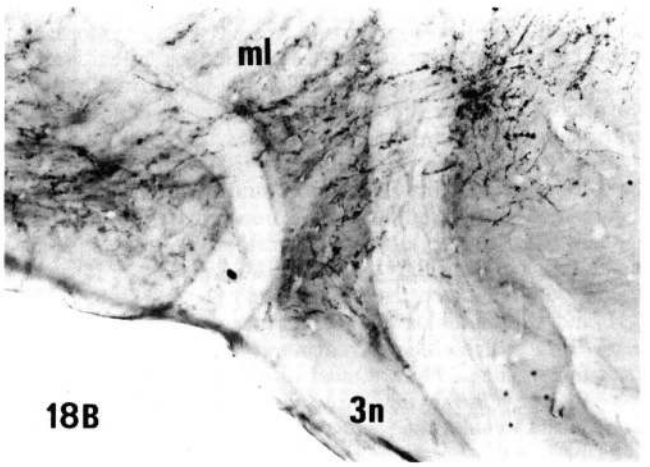
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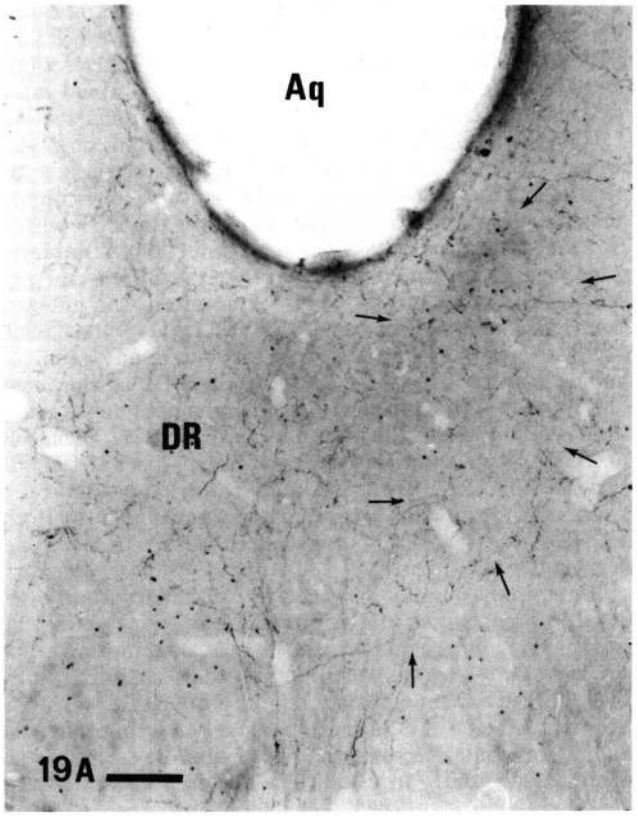
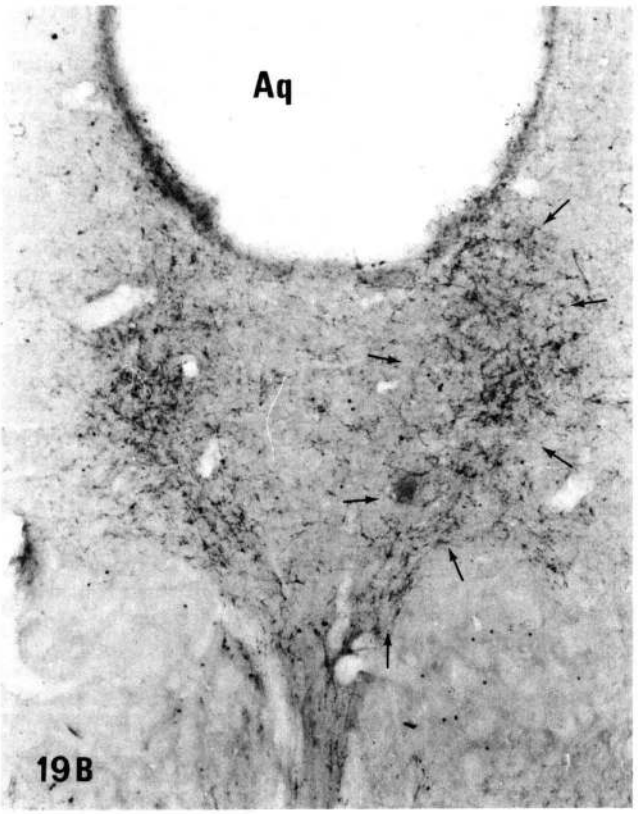

Aq
DR
19A


Aq
19B

cleus (Fig. 16A), the supramammillary decussation, the central gray (Fig. 17B), the ventral tegmental area, and the substantia nigra (Fig. 18A).

In the *metencephalon*, most of the VP-immunoreactive terminals have disappeared from the dorsal and median raphe nucleus (Fig. 19A) and the locus coeruleus.

In the *myelencephalon*, no changes are detected.

No changes following castration are observed in any other area of the brain containing VP-immunoreactive fibers (see, e.g., Figs. 14, 15). All the changes detected in the long-term castrated rats are not observed if those rats were treated with testosterone.

DISCUSSION

The anatomy of vasopressin pathways in the brain

The first reports on the vasopressinergic pathways in the brain were based on immunocytochemical studies in which use was made of paraffin-embedded sections (Buijs, '78; Buijs et al., '78; Sofroniew and Weindl '78a,b). More recently, the sensitivity of the immunocytochemical procedure has been considerably increased by using free-floating vibratome or frozen sections, which show a Golgi-like appearance of VP-containing structures after staining (Buijs et al., '80; Sofroniew and Glasmann, '81). Application of this procedure indicated that additional VP pathways could be detected (Buijs, '80). Another important improvement appears to be an increase of glutaraldehyde concentration in the fixative from 2.5 to 5% and the omission of paraformaldehyde, by which the VP-immunoreactive cells of the BST and the medial amygdaloid nucleus could be visualized without colchicine pretreatment. The use of these modifications in the procedure most likely explains why in the present study many new brain areas could be added to those found in the first reports to contain VP-immunoreactive fibers, e.g., the olfactory tubercle, the pre- and supramammillary nucleus, the basal nucleus of Meynert, and the rhomboid thalamic nucleus.

No complete picture has yet emerged concerning the sites or origin of the VP innervation. Recent studies have, however, added considerably to our knowledge about the anatomy of this system. This is summarized in Figure 20A. General agreement exists that about all the VP neurons of the SON, including those of the retrochiasmatic part, project to the neural lobe (e.g., Silverman and Zimmerman, '83; Swanson and Sawchenko, '83). There are, however, indications that these neurons might also send axon collaterals to the PVN (Silverman et al., '81) and lateral hypothalamus (Mason et al., '84).

In addition to its projection to the neural lobe, the PVN also sends VP fibers to the external layer of the median eminence (Vandesande et al., '77; Alonso and Assenmacher, '81; Zimmerman and Silvermann, '83). In addition, retrograde labeling studies combined with immunocytochemistry indicate that the PVN is the origin of the VP-immunoreactive fibers seen within the spinal cord and the dorsal vagal complex, i.e., the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (Sofroniew and Schrell, '81; Sawchenko and Swanson, '82). Consistent with these findings is the observation that destruction of the PVN results in the virtually complete disappearance of VP innervation from the dorsal vagal complex, the nucleus ambiguus, and the parabrachial nucleus (De Vries and Buijs, '83, and unpublished observations), while similar lesions led to the disappearance of the major part of the radioimmunoassayable VP from the spinal cord (Lang et al., '83). The descending pathways of the PVN are probably derived from cells in the parvocellular subdivisions of the PVN, while the neurosecretory fibers originate in its magnocellular part (Armstrong et al., '80; Swanson and Kuypers, '80; Sawchenko and Swanson, '82).

Although the PVN thus seems to be the major source of VP fibers of the medulla and spinal cord, PVN lesions cause no obvious changes in VP innervation of more rostral brain areas (De Vries and Buijs, '83). The SCN probably forms an important source for VP fibers in these regions. Thus, destruction of the SCN caused the disappearance of VP fibers from the organum vasculosum laminae terminalis, the periventricular hypothalamic nucleus, and the dorsal part of the paraventricular thalamic nucleus (Hoorneman and Buijs, '82). In further support for the existence of VP projections from the SCN to the aforementioned regions is the finding that they are all heavily labeled after placement of tritiated amino acids in the SCN (Stephan et al., '81; Berk and Finkelstein, '81). In addition, the SCN might project to its contralateral counterpart, since the entire area of the SCN contains large agglomerations of VP-immunoreactive terminals, while after placement of tracers in the contralateral SCN retrogradely labeled cells can be found in the part of the SCN where VP cells are located (Pickard, '82). Such a high density of VP terminals in an area containing VP cells seems to be a general phenomenon for all the areas of VP synthesis. This suggests that in addition to projections to other areas, VP fibers might also terminate within the nucleus of origin.

A third important source of VP-immunoreactive fibers within the brain seems to be the BST. Unilateral lesions of the BST led to an ipsilateral disappearance of VP fibers from the olfactory tubercle, the diagonal band of Broca, the lateral septum, the anterior amygdaloid area, the lateral habenular nucleus, the periventricular gray, the dorsal raphe nucleus, and the locus coeruleus (De Vries and Buijs, '83, and unpublished observations). As with VP projections of the SCN, final proof for the existence of VP projections from the BST to these areas can only be obtained after retrograde tracing in combination with the demonstration of VP in the labeled neuron. Several observations, however, argue in favor of the existence of such BST projections: (1) after injection of tracers in the lateral septum, retrogradely labeled cells were found in the regions of the BST where VP cells are located (De Vries and Buijs, '83); (2) anterograde tracing studies have shown that the BST sends projections to the areas where VP innervation was affected after BST lesions (Conrad and Pfaff, '76); and (3) the effects of gonadectomy described in the present study seem to provide another argument favoring the BST as the most likely source of this innervation. Whereas no changes could be detected either in the VP-immunoreactive cell bodies of the PVN and SCN or in their projections, most of the VP cells of the BST and their presumable projections could not be stained following long-term gonadectomy (Fig. 20B). Both cell and fiber staining reappeared after testosterone treatment.

Interestingly, similar changes in VP innervation were found in areas where the origin of the fibers is unknown, e.g., the medial amygdaloid nucleus, the ventral hippocampus, the ventral tegmental area, and the substantia nigra (Fig. 20B). This innervation might be derived from the recently detected VP-immunoreactive cell groups in the medial amygdala, dorsomedial hypothalamus, or locus coeruleus (Caffé and Van Leeuwen, '83). In contrast to the

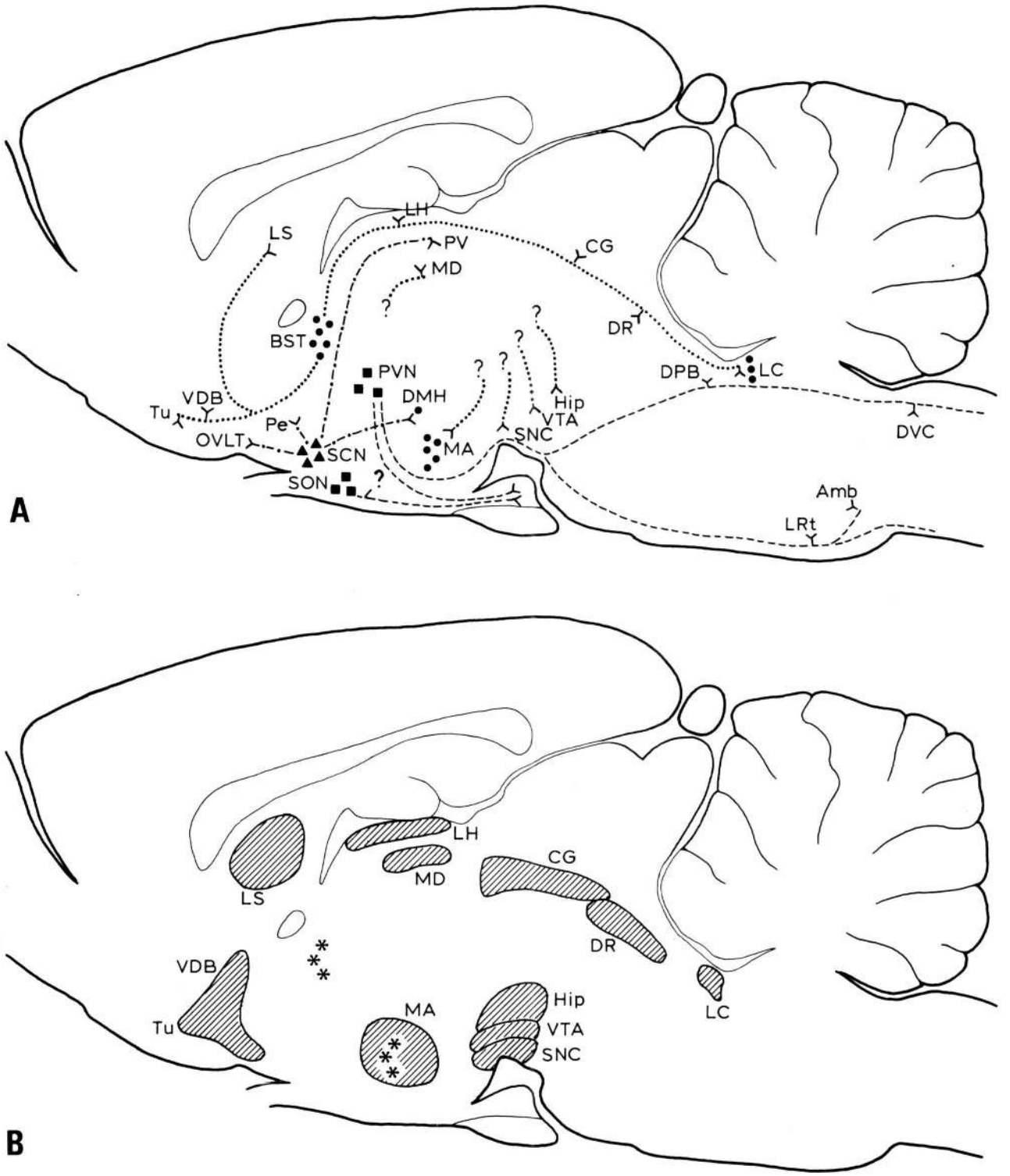


Fig. 20 Line drawings in 20A illustrate the major vasopressin (VP) pathways in the brain with their most likely origin (see text). Squares, triangles, and dots indicate, respectively, VP cell groups in the PVN, SCN, and the recently detected vasopressin cells in, e.g., the BST. (------), pathways arising from PVN and SON; (·····), pathways arising from the SCN; (·····), pathways arising from the BST; pathways with unknown origin indicated by question marks. The hatched areas in 20B indicate the major areas where VP terminals were seen to disappear after castration. Note that VP terminals disappear from all areas to which the BST presumably projects and from areas which contain VP fibers of unknown origin.

latter two areas, only in the medial amygdaloid nucleus were VP-immunoreactive cell bodies seen to disappear after castration, which might indicate that the medial amygdaloid nucleus contributes to this innervation. However, the possibility should not be excluded that besides the areas identified as containing VP-immunoreactive cells, VP-producing cells are present in other areas where they cannot be visualized with current methods. Such cells could theoretially constitute an additional source for the VP innervation described in the present study.

Influences of gonadal hormones

One should not conclude from the failure to detect changes in the neurosecretory pathways from the PVN and SON to the neurophypophysis and in the central VP projections from the PVN and SCN that these systems are not sensitive to gonadal hormones. In fact, high doses of estrogens have been demonstrated to stimulate VP neurosecretion and androgens have been found to inhibit it (Skowsky et al., '79). These effects could be caused by direct action of steroids upon the hypothalamic neurosecretory neurons. Using a combination of immunocytochemistry and steroid autoradiography, Sar and Stumpf ('80) demonstrated that VP-immunoreactive neurons of the mouse PVN and SON concentrate estrogens. In rats, however, almost no estradiol-concentrating cells could be found in the SON, while in the PVN many neurophysin-containing cells were found to concentrate estradiol (Rhodes et al., '81b). Because these cells were predominantly located in those subnuclei of the PVN, which contain oxytocin and VP cells projecting to the medulla and spinal cord rather than the neurohypophysis, the authors concluded that estrogens probably influence the neurosecretory activity of the hypothalamic VP neurons via indirect mechanisms. Interesting in this respect is the observation that gonadotropic hormones, rather than sex hormones, affect the neurosecretory activity of the cells of the PVN and SON (Swaab and Jongkind, '71; Swaab, '72). When studying the VP-deficient Brattleboro rat, which produces only the neurophysin asociated with oxytocin, Rhodes et al. ('82) found that not only neurophysin-containing (and thus oxytocinergic) neurons concentrate estradiol, but so do other elements which have the same appearance as do the VP neurons in Long Evans rats (Rhodes et al., '81a). From this finding they inferred that the VP projections of this nucleus to the medulla and spinal cord might also be directly affected by estrogens.

In contrast to the PVN, the SCN contains hardly any steroid-concentrating cells (Pfaff and Keiner, '73). It is possible, however, that steroids influence VP neurons of the SCN via other cells or via mechanisms not involving the concentration of steroids in the cell nucleus (see, e.g., Moss and Dudley, '84).

Although steroid effects on the central VP projections of the PVN and SCN are still questionable, the present results demonstrate beyond doubt that gonadal hormones influence the VP-immunoreactive cell bodies of the BST and medial amygdaloid nucleus and their presumable projections. Recent experiments in our laboratory suggest that these influences involve both estrogen and androgen mechanisms (De Vries et al., '84c), which is in line with the observation that the BST and the medial amygdaloid nucleus are among the most conspicuous sites of the brain where androgen- and estrogen-concentrating cells have been detected (Pfaff and Keiner, '73; Sheridan '79; Stumpf and Sar, '76). Thus, gonadal steroids might act directly on the BST and medial amygdaloid nucleus to influence their VP projections.

It is difficult to explain the disappearance of VP-immunoreactivity following castration in terms of activity, since the amount of VP in a given fiber is probably a balance between the rate of VP synthesis in the soma and release from the terminals. However, the observations in the colchicine-pretreated rats may be indicative in this respect. Because colchicine is supposed to block axonal transport (Kreutzberg, '69), one would expect that colchicine pretreatment would preferentially cause the appearance of cells with a high rate of VP synthesis. This, indeed, appears to be the case with hypothalamic neurons which are immunoreactive for luteinizing-hormone-releasing-hormone (LHRH). In male rats, castration leads to an increase in LHRH release into the hypophyseal portal blood (Eskay et al., '77), while under similar conditions the number of LHRH-immunoreactive cells is decreased (Shivers et al., '83). On the contrary, when castrated rats are pretreated with colchicine prior to sacrifice, an increase in the number of LHRH-immunoreactive cell bodies is observed (Barry et al., '73; Shivers et al., '83). Therefore, the failure to detect VP cells in the BST and the medial amygdaloid nucleus in castrated rats pretreated with colchicine in the present study suggests that in this case the absence of gonadal hormones is concomitant with a lower activity.

The results of the present study may be of help in assessing the physiological significance of VP innervation of the brain. They suggest that major part of VP innervation of the brain is implicated in functions which are influenced by gonadal steroids (cf. De Vries et al., '84b). Changes in the VP fiber pathways induced by fluctuations in gonadal hormone levels may in turn cause changes in other systems. It is noteworthy, in this respect, that VP terminals were seen to disappear from areas such as the ventral tegmental area, the substantia nigra, and the dorsal raphe nucleus. The activity of the dopaminergic and serotoninergic projections arising from these nuclei has clearly been demonstrated to be influenced by gonadal hormones (e.g., Di Paolo et al., '83, Long et al., '83; Alderson and Baum, '81). These projections seem to be implicated in functions such as gonadotropin regulation and reproductive behavior (Caggiula et al., '76; Arendash and Gallo, '78; Héry et al., '78; Crowley and Zemlan, '81). The fact that the cells in the aforementioned areas concentrate estrogen only to a very slight degree (Pfaff and Keiner, '73) suggests that their projections are influenced via indirect pathways. The results of the present study suggest that VP fibers might belong to those pathways.

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