

Proopiomelanocortin Peptide Immunocytochemistry in Rhesus Monkey Brain

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Received 14 December 1984

KHACHATURIAN, H., M. E. LEWIS, S. N. HABER, H. AKIL AND S. J. WATSON. *Proopiomelanocortin peptide immunocytochemistry in rhesus monkey brain*. BRAIN RES BULL 13(6) 785-800, 1984.—The immunocytochemical distribution of proopiomelanocortin (POMC) peptides (β -endorphin, ACTH, α -MSH, 16K fragment) was studied in the brain of the rhesus monkey (*Macaca mulatta*). Some animals were administered colchicine intracerebroventricularly prior to sacrifice to enhance the visualization of perikaryal immunoreactivity. Immunoreactive perikarya are localized to hypothalamic infundibular nucleus, giving rise to several distinct projections. *Rostral projections* extend through midline diencephalic and preoptic areas, and enter the telencephalon. Along this course, immunoreactive fibers are seen in midline hypothalamic and preoptic nuclei, nucleus of the diagonal band, olfactory tubercle, nucleus accumbens, bed nucleus of stria terminalis, septum, and other limbic structures in telencephalon. Caudal to the anterior commissure, some fibers ascend dorsally to enter the midline thalamus, which they innervate. *Lateral projections* of the infundibular perikarya course through the medial-basal hypothalamus, dorsal to the optic tracts, and enter the amygdala region where they innervate more medially situated amygdaloid nuclei. Caudal projections of the POMC neurons also extend through midline diencephalon, some coursing along a periventricular path to innervate midline hypothalamic and thalamic nuclei. This projection extends into the mesencephalic substantia grisea centralis and may also contribute to the innervation of more dorsally situated nuclei in the pons and medulla, such as the parabrachial nuclei and nucleus tractus solitarius. Other caudal projections originating in the hypothalamus course through the ventral tegmentum of mesencephalon and pons and may contribute to the innervation of midline raphe and other ventrally situated nuclei in the pons and medulla. The distribution of immunoreactive perikarya and fibers in the brain of rhesus monkey is strikingly similar to that found in the rat brain. However, subtle differences appear to exist in the innervation patterns of particular brain regions.

Proopiomelanocortin	β -Endorphin	ACTH	16K fragment	Rhesus monkey	Brain
Immunocytochemistry					

PROOPIOMELANOCORTIN (POMC) and its derivative peptides have recently been the subject of much intensive investigation (see [3, 4, 23, 34, 48]). The interest in POMC derives from the fact that it contains several known bioactive peptides (i.e., β -endorphin, ACTH, α -MSH), that it is produced in both pituitary and brain (hence the potential for both hormone-like and transmitter-like actions of its products), and also that its end products have possible physiological roles in the modulation of pain, stress, neuroendocrine, cardiovascular, respiratory, and several other autonomic processes [23].

Most of our current knowledge of POMC and its processing into bioactive peptides derives from work on the pituitary gland [14, 17, 54]. The processing of POMC in brain neurons is less well understood, but appears to be dissimilar to that occurring in the pituitary (see [50]). Neurons that synthesize POMC are located in the arcuate nucleus of rat hypothalamus. This nucleus is the equivalent of the primate infundibular nucleus. Immunocytochemical studies in both rat and human have demonstrated the colocalization of POMC-

derived peptides within the same arcuate (infundibular) neurons [5, 37, 42, 49]. In rat, the arcuate POMC neurons project to many telencephalic, diencephalic, and brainstem sites; these projections have been extensively mapped in the rodent central nervous system [6, 7, 16, 19, 23, 32, 49, 51, 52, 55]. Some preliminary observations have also been made in the pigtail monkey, *Macaca nemestrina* [1]. The latter investigators have described ACTH immunoreactivity in infundibular perikarya and in fibers located in the preoptic area, hypothalamus, paraventricular thalamus, substantia grisea centralis (periaqueductal gray), and midline reticular formation.

Most of our knowledge of the anatomy of POMC systems derives from work on the rat central nervous system [3]. However, there are known interspecies differences in the central nervous system distribution of neurotransmitter substances [18, 40, 43]. An example which is most pertinent to the present study is the work by Haber and Elde [18] on the distribution of Met-enkephalin immunoreactivity in the brain of the African green monkey (*Cercopithecus aethiops*), in

LIST OF ABBREVIATIONS

3	oculomotor n.	mgn	medial geniculate n.
3v	third ventricle	mif	medial longitudinal fasciculus
4v	fourth ventricle	mrf	mesencephalic reticular formation
aa	anterior amygdala	ms	medial septum
ab	basal n. of amygdala	mtn	motor trigeminal n.
ac	anterior commissure	mv	medial-ventral n. of thalamus
acb	n. accumbens	ncs	n. centralis superior (raphe)
ace	central n. of amygdala	ncu	n. cuneatus
aco	cortical n. of amygdala	ndb	n. of diagonal band
aha	anterior hypothalamic area	ngr	n. gracilis
al	lateral n. of amygdala	nrm	n. reticularis magnocellularis
am	medial n. of amygdala	nrp	n. reticularis parvocellularis
amb	n. ambiguus	nts	n. tractus solitarius
aq	cerebral aqueduct	oc	optic chiasm
at	anterior n. of thalamus	on	oculomotor nerve
bc	brachium conjunctivum	ot	optic tract
bp	brachium pontis	otu	olfactory tubercle
bst	bed n. of stria terminalis	pbn	parabrachial n.
cau	caudate	pc	posterior commissure
cbl	cerebellum	pf	parafascicular n. of thalamus
cc	corpus callosum	pha	posterior hypothalamic area
ce	central canal	pir	piriform cortex
ci	internal capsule	pl	posterior-lateral n. of thalamus
cl	claustrum	pn	pons
cm	centrum medianum n. of thalamus	poa	preoptic area
cp	cerebral peduncle	pta	pretectal area
dbc	decussation of brachium conjunctivum	pul	pulvinar
dg	dentate gyrus	put	putamen
dha	dorsal hypothalamic area	pv	periventricular n. of thalamus
dm	dorsomedial n. of hypothalamus	pvn	paraventricular n.
dpy	decussation of pyramidal tract	pyr	pyramidal tract
ds	dorsal septum	rd	raphe dorsalis
fn	facial n.	re	reuniens n. of thalamus
fx	formix	rm	raphe magnus
gp	globus pallidus	rn	red n.
hl	lateral habenula	rt	reticular n. of thalamus
hm	medial habenula	sc	superior colliculus
hpc	hippocampus	sgc	substantia grisea centralis
ic	inferior colliculus	si	substantia innominata
inf	infundibular n. of hypothalamus	sn	substantia nigra
io	inferior olivary n.	son	supraoptic n.
lc	locus coeruleus	st	subthalamic n.
lgn	lateral geniculate n.	stn	spinal trigeminal n.
lha	lateral hypothalamic area	va	ventral-anterior n. of thalamus
lrn	lateral reticular n.	vl	ventral-lateral n. of thalamus
ls	lateral septum	vm	ventromedial n. of hypothalamus
lv	lateral ventricle	vpl	ventral-posterolateral n. of thalamus
ma	mammillary n.	vpm	ventral-posteromedial n. of thalamus
md	medial-dorsal n. of thalamus	vst	vestibular n.
me	median eminence	vta	ventral tegmental area
met	mesencephalic trigeminal n.	zi	zona incerta

which several marked differences were noted between monkey and rat. In the present study, we describe the POMC system of the rhesus monkey brain using antisera raised against ACTH, β -endorphin, α -MSH, and 16K fragment, and compare these findings to those obtained using the same antisera in rat.

METHOD

Immunocytochemistry

Adult male and female rhesus monkeys (*Macaca mulatta*) were obtained for the present study. The monkeys were either kept under normal conditions (one) or were treated with colchicine (four, see below). Prior to sacrifice, each

animal was anesthetized with sodium pentobarbital (30 mg/kg) injected into the saphenous vein. Once completely anesthetized, the animal was placed on a surgical table, an endotracheal tube was inserted and attached to a respirator, and the chest cavity was exposed. The pericardium was excised and the apex of the ventricles was cut open. A plastic tube (connected to the perfusion apparatus) was inserted through the left ventricle into the aorta and was tied in place with a suture. After cutting the right atrium, the cardiovascular system was flushed with 2 liters of normal saline through the inserted tube. Following the completion of the saline flush, 16 liters of neutral-buffered 4% formaldehyde were pumped through the vasculature for 30 minutes. The tube was then removed and the head was severed from the trunk

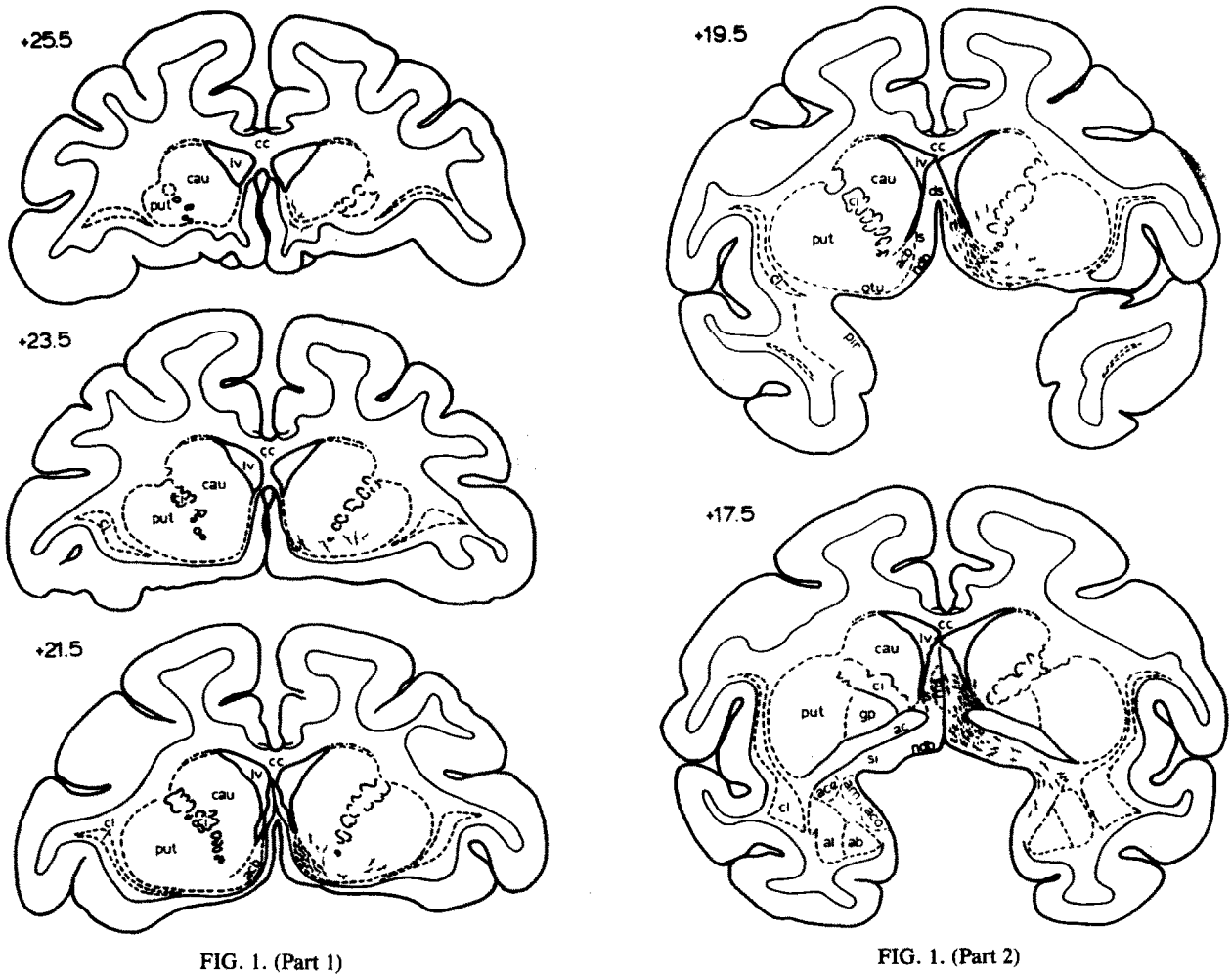


FIG. 1. (Part 1)

FIG. 1. (Part 2)

FIG. 1 parts 3-9 on following pages.

FIG. 1. Frontal atlas of the rhesus monkey brain through 21 levels. Coordinates are in reference to the interaural line. Structures are identified on the left, while POMC fibers (short curved lines and dots) and perikarya (in the infundibular nucleus) are shown on the right side. See list of abbreviations.

and placed in a stereotaxic apparatus. The skull was exposed, the top of the calvarium removed, and the brain was cut into 1 cm thick slices in the frontal or parasagittal planes using a Kopf stereotaxic device with blades attached. The skull was then removed from the stereotaxic apparatus and the brain slices were removed and postfixed in buffered 4% formaldehyde for 2-4 hours. The tissue was transferred into 15% sucrose in phosphate-buffered saline (PBS) and stored overnight at 4°C. The tissue was then frozen in -50°C isopentane or liquid nitrogen, and stored at -80°C.

For peroxidase-antiperoxidase immunocytochemistry [24], the tissue was sectioned at -20°C in a Bright cryostat and the sections were mounted onto subbed slides and were either stored at -80°C or allowed to dry at room tempera-

ture. Frontal and parasagittal sections were used in order to facilitate identification of long tract POMC projections which had also been identified in rat. The dried sections were incubated at 37°C with normal goat serum (NGS, from GIBCO) for 10 minutes, followed by primary rabbit antiserum raised against ACTH, α -MSH, β -endorphin, or 16K fragment. The antisera were diluted with 0.02 M PBS containing 0.3% Triton X-100. Incubation was continued for an additional hour after which the slides were transferred onto wet foam rubber in a moist box and stored at 4°C overnight. The next day, the tissue was washed thoroughly in PBS for 30 minutes with several solution changes. The slides were again incubated at 37°C with NGS for 10 minutes, followed by goat anti-rabbit serum (Arnel) at 1/1000 dilution for an additional 30 minutes.

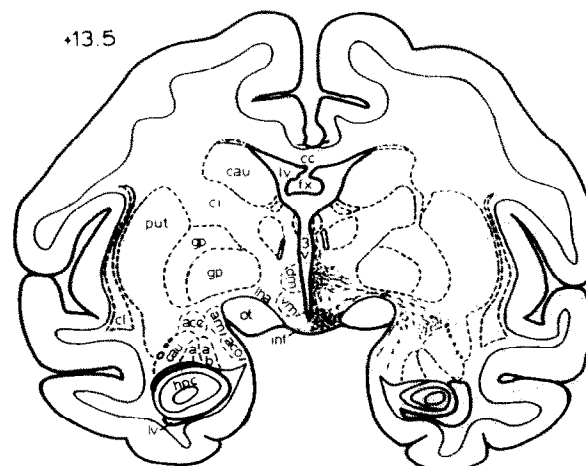
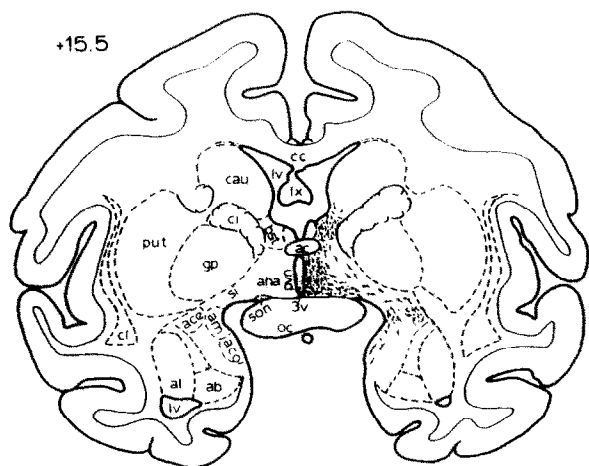
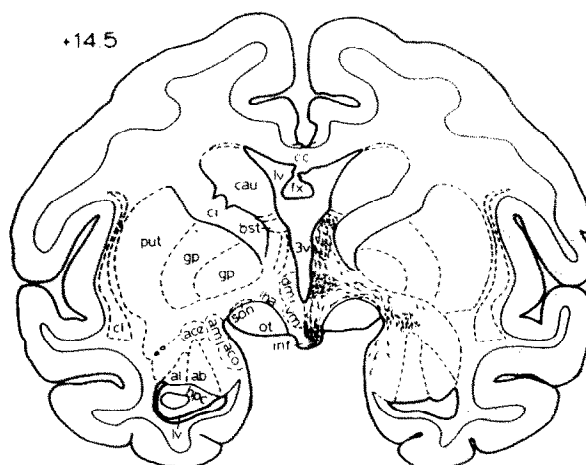
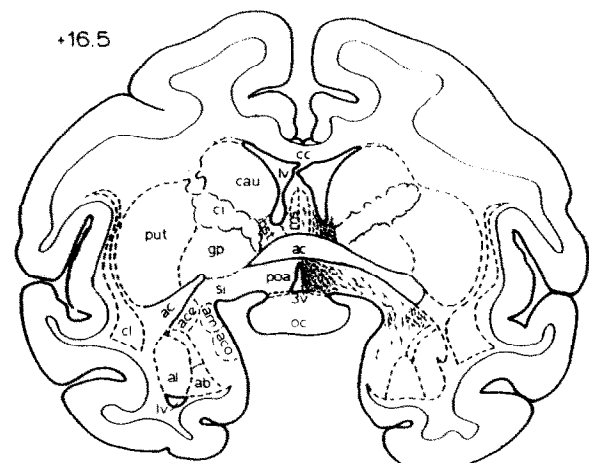


FIG. 1. (Part 3)

FIG. 1. (Part 4)

They were then transferred into the moist box and stored overnight at 4°C. On the third day, the tissue was washed in PBS for 30 minutes, incubated at 37°C with NGS for 10 minutes, and subsequently with rabbit anti-horseradish peroxidase (HRP) serum at 1/200 dilution for 40 minutes. The slides were then washed for 30 minutes in PBS and incubated directly with 4 µg/ml HRP enzyme (Sigma, type VI) at 37°C for 40 minutes. After completion of this step and PBS wash, the slides were immersed in a solution of 0.125 mg/ml diaminobenzidine tetrahydrochloride (Sigma) and 0.03% H₂O₂ for 15 minutes with constant stirring. The tissue was then washed for 30 minutes in distilled water, briefly osmicated with 0.01% OsO₄, dehydrated through graded alcohols and xylenes, and mounted in Permount. Observations and photomicrographs were made using a Leitz Orthoplan microscope.

Colchicine Treatment

To enhance the visualization of peptide immunoreactivity in neuronal perikarya, four animals were treated with colchicine prior to sacrifice [25]. Colchicine is a neurotoxin, one action of which is the inhibition of microtubule formation [33]. Since axonal transport requires microtubular (or

neurotubular) action, colchicine administration results in a loss of transport of synthesized materials (proteins, peptides) to axonal terminals, and an accumulation of this material in the neuronal perikarya and truncal axons.

For colchicine administration, the animal was anesthetized with sodium pentobarbital injected into the saphenous vein, and the head was placed in a stereotaxic apparatus. The skull was exposed and a small hole was drilled into the skull at a stereotaxic coordinate overlying the widest region of the right lateral ventricle (8.0 mm anterior, 4.0 mm lateral, with reference to bregma). A cannula (attached to a volume-calibrated plastic tube) was introduced through the hole and inserted into the ventricle (19.6 mm deep). The successful insertion into the ventricular lumen was judged by a sudden drop in the column of fluid (colchicine solution) in the plastic tube which was attached vertically above the cannula. Colchicine (75, 150, 300, 600 µg per kg body weight) was thus allowed to diffuse into the cerebrospinal fluid. Pressure was allowed to reach an equilibrium, after which the cannula was retrieved, the hole was blocked with bone wax and the scalp was sutured. The animal was allowed to recover from the anesthesia and was kept under normal conditions for 24 to 48 hours prior to sacrifice and fixation as described above.

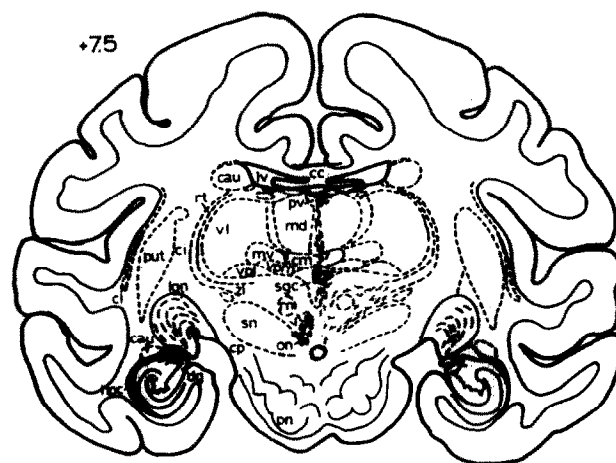
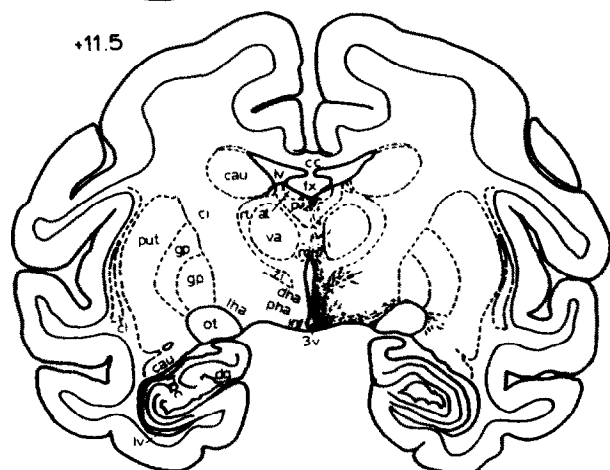
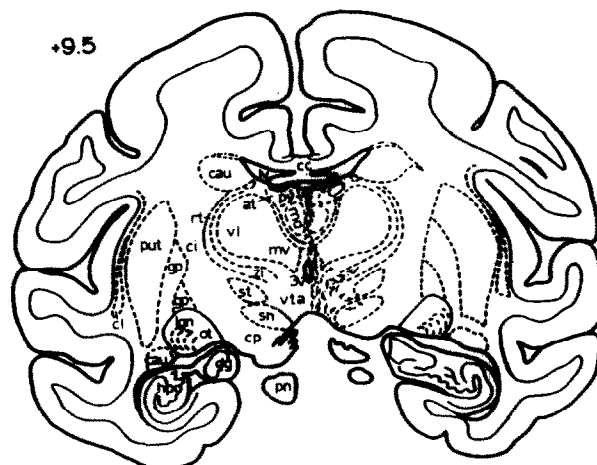
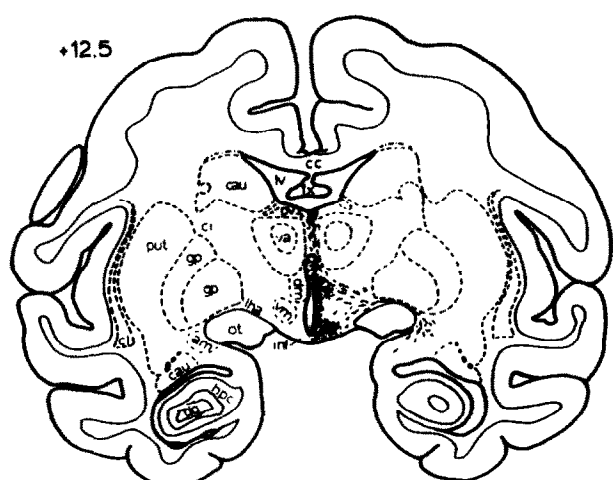


FIG. 1. (Part 5)

FIG. 1. (Part 6)

RESULTS

The antisera used in the present study have been characterized extensively (see [50]). All immunoreactivity seen using each antiserum was blockable by the preabsorption of the primary antibody by 1 μ M concentration of peptide against which the serum was raised. Extensive cross-blocking studies also confirmed the specificity of each antiserum. Peptides with amino acid sequences homologous to the peptide antigen did not block the immunoreactivity when added to the primary antiserum in up to 50 μ M concentration. The specificity of the ACTH antiserum is toward the midregion of ACTH, i.e., ACTH(11-24). The immunoreactivity observed using this antiserum could be blocked by the preabsorption of the primary antibody by 1 μ M concentrations of ACTH(1-24), ACTH(1-39), ACTH(11-24) and ACTH(11-39), but not by up to 50 μ M concentrations of β -lipotropin, β -endorphin or α -MSH. β -Endorphin immunoreactivity could not be blocked by Met- or Leu-enkephalin, ACTH, or α -MSH. The α -MSH immunoreactivity was not blocked by ACTH(1-24), ACTH(1-39), or β -endorphin.

No perikaryal immunoreactivity could be detected in the normal animal; however, increasingly intense perikaryal

staining was noted with successively higher doses of colchicine. Neuronal perikarya immunoreactive to POMC peptides are localized to the hypothalamic infundibular nucleus throughout its rostral-caudal extent, as well as scattered in the medial-basal hypothalamus (Fig. 1: +14.5 to +11.5; Figs. 2, 3 and 8). These parvocellular perikarya appear to be multipolar, exhibiting numerous processes (Fig. 3C,D). Perikaryal and fiber immunoreactivity was best demonstrated by ACTH and 16K antisera, with decreasing intensity of staining observed using the β -endorphin and α -MSH antisera.

Immunoreactive POMC peptide-containing fibers are localized to many brain regions (Figs. 1 and 4-7), including the diencephalon as well as the limbic forebrain and the brain stem (see Fig. 8 for summary). Areas which appear devoid of immunoreactive fibers include the cerebral cortex, hippocampus, most of striatum, and the cerebellum. Like their counterparts in the arcuate nucleus of rat brain, the infundibular POMC perikarya of the monkey brain appear to contribute to several major projectional systems.

1. *Rostral projections* of the POMC perikarya can be traced through much of the anterior hypothalamic and preoptic areas (Fig. 1: +15.5, +16.5; Figs. 4D, 5A and 7), which will be described further. These projections extend into the telencephalon, innervating several major limbic-

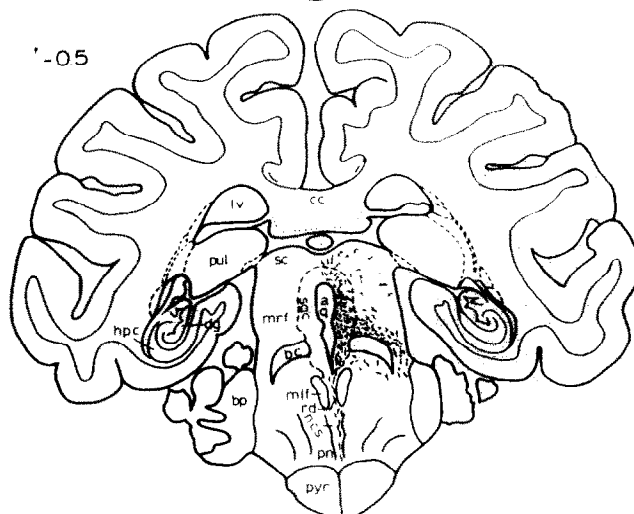
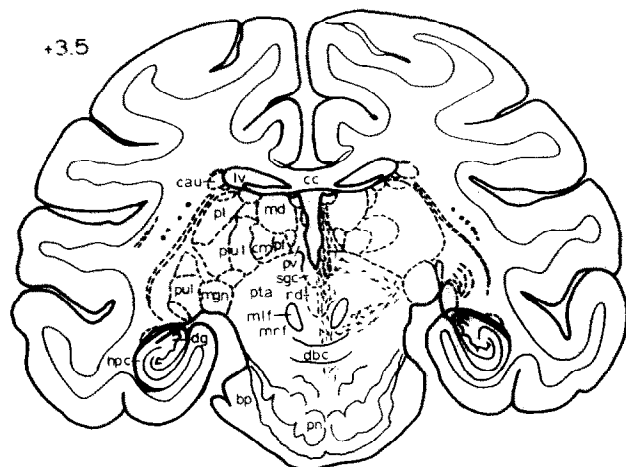
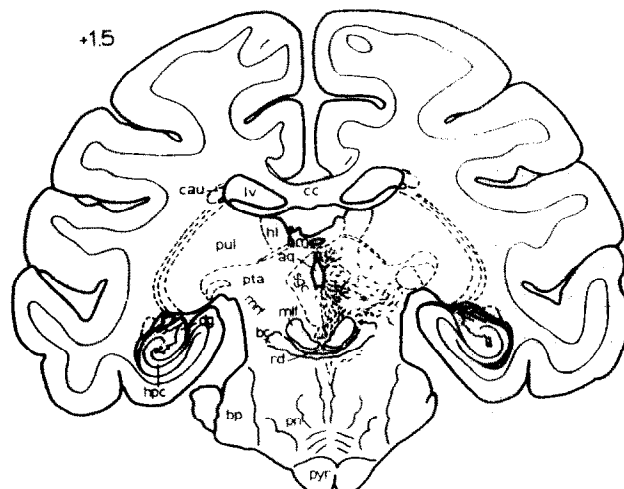
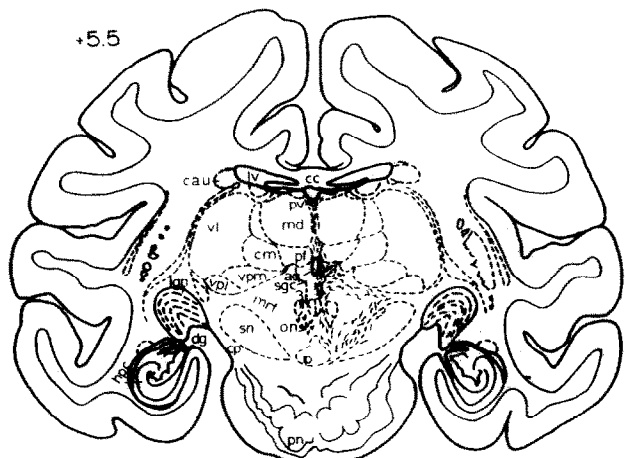


FIG. 1. (Part 7)

FIG. 1. (Part 8)

associated nuclei. Some fibers continue in a rostral direction to enter nucleus accumbens and nucleus of the diagonal band (Fig. 1: +21.5 to +17.5; Fig. 4B). From the diagonal band, scattered fibers are seen to enter olfactory-associated areas. These fibers are generally sparse, and are occasionally seen in the olfactory tubercle (Fig. 1: +19.5). Other fibers in the diagonal band follow a dorsal course and appear to enter the septal nuclei (Fig. 1: +19.5 to +16.5). Scattered fibers are also seen in the head of the caudate and putamen at more ventral and medial aspects (Fig. 1: +25.5 to +17.5).

A distinct bundle of immunoreactive fibers circumvents the anterior commissure, mainly from its rostral aspect, and enters into the septum (Fig. 8). Other fibers innervate the bed nucleus of the anterior commissure (Fig. 4C). Immunoreactive fibers which course caudal to the anterior commissure contribute to the innervation of the bed nucleus of stria terminalis (Fig. 1: +16.5 to +14.5). More caudally, fibers are seen to enter the periventricular thalamic nucleus which exhibits immunoreactivity throughout its rostral-caudal extent (Fig. 1: +12.5 to +3.5; Fig. 5D). Therefore, this component of the rostral projection system becomes a caudally projecting bundle within the periventricular nucleus of the thalamus. A continuation of these midline fibers ap-

pears to enter the mesencephalon near the posterior commissure, and thus may contribute to the innervation of the substantia grisea centralis (Fig. 1: +7.5 to -0.5; Fig. 6A and B) and other midline brainstem regions. These projections are further described below.

In the preoptic area and the hypothalamus, a general medial-to-lateral gradient of immunoreactive fiber density is seen. The more midline structures, such as the medial preoptic, anterior hypothalamic and infundibular nuclei, exhibit a relatively dense accumulation of fibers (Figs. 4D, 5A and 7), whereas the lateral preoptic and lateral hypothalamic areas appear to be innervated lightly (Fig. 5B). Other hypothalamic nuclei which appear to be densely innervated include periventricular and paraventricular nuclei and the dorsomedial nucleus (Figs. 5C and 7). Scattered immunoreactive fibers are also seen in the supraoptic nucleus as well as in the ventromedial nucleus (Figs. 5C and 7). In addition, there appears to be a heavy density of fibers in the external layer of the median eminence (Fig. 7).

2. *Lateral projections* from the infundibular POMC perikarya can be seen throughout the entire medial-basal hypothalamus. The density of these processes diminishes gradually in a medial-to-lateral gradient (Fig. 1: +15.5 to

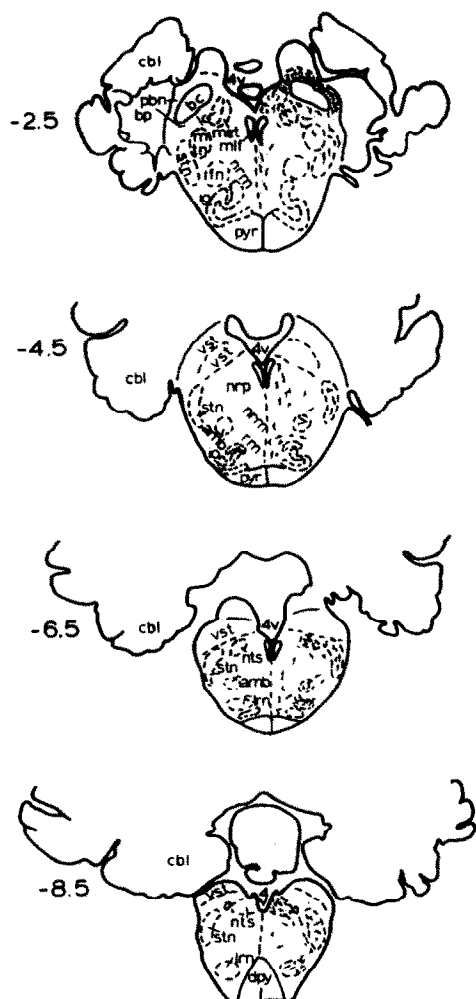


FIG. 1. (Part 9)

+11.5). A distinct band of immunoreactive fibers is seen to course laterally over the optic tracts and into the amygdala. At the level of the anterior hypothalamic area, some of these fibers course dorsal to the supraoptic nucleus, *en route* to the amygdala (Fig. 1: +14.5). These fibers perhaps contribute to the innervation of the supraoptic nucleus. More caudally, at the level of the ventromedial nucleus, the laterally projecting fibers are sandwiched between the optic tract ventrally and the globus pallidus dorsally (Fig. 1: +13.5). In amygdala, the immunoreactive fibers are seen to innervate the more medial nuclei, specifically the medial and central nuclei (Fig. 1: +17.5 to +12.5; Fig. 4A). Scattered fibers are also seen in the cortical nucleus, but the basal and lateral nuclei appear to be devoid of fiber immunoreactivity. Another probable source of amygdaloid fibers may be the stria terminalis, which exhibits immunoreactive fibers.

3. *Caudal projections* of the POMC neurons extend through the posterior and dorsal hypothalamic areas, and continue caudally to enter the mesencephalon (Fig. 1: +11.5 to +7.5). This projection bifurcates into two distinct bundles. Some fibers continue in a more dorsocaudal direction and join the periventricular thalamic fibers in the mesencephalon (Fig. 1: +5.5, +3.5; Fig. 8). These fibers contribute to

the innervation of the substantia grisea centralis (Fig. 6A and B). Other fibers project laterally and caudally through the ventral tegmental area, coursing dorsal to the substantia nigra to enter the pons (Fig. 1: +9.5 to +3.5). Scattered fibers are seen to penetrate the substantia nigra pars compacta. Other mesencephalic areas which exhibit fiber immunoreactivity include the superior and inferior colliculi (Fig. 1: -0.5), as well as a distinct band of fibers extending laterally from the substantia grisea centralis into the mesencephalic reticular formation (Fig. 1: +1.5). These dorso-laterally projecting fibers are joined by the ventrolateral projections at the level of the brachium conjunctivum, and contribute to the innervation of the parabrachial nucleus (Fig. 1: -2.5; Fig. 6C). In the pons and medulla, fiber immunoreactivity is more sparse, such that no distinct projections can be discerned (see Fig. 8). However, fibers are seen in such diverse brainstem nuclei as the raphe dorsalis and raphe magnus, all sensory and motor trigeminal nuclei, nuclei reticularis parvocellularis and magnocellularis, nucleus ambiguus, nucleus tractus solitarius, and lateral reticular nucleus (Fig. 1: +1.5 to -8.5; Fig. 6D).

DISCUSSION

The present study demonstrates POMC peptide immunoreactivity to be distributed widely throughout the rhesus monkey central nervous system, including diencephalic, limbic telencephalic and midline brainstem regions. This immunoreactivity represents β -endorphin, ACTH, α -MSH and 16K fragment distribution, consistent with extensive studies which have unequivocally demonstrated the co-synthesis of these peptides from a single precursor, POMC [30, 31, 39] as well as their colocalization within the same arcuate (infundibular) perikarya in both the rat and the human [5, 32, 42, 46, 47, 49]. To date, one immunocytochemical study has been carried out in the monkey brain demonstrating the distribution of ACTH cells and processes [1]. These authors have also described immunoreactive perikarya in the infundibular nucleus of the hypothalamus, and have generally noted fiber immunoreactivity surrounding the anterior commissure ventrally, preoptic area, nucleus accumbens, caudal paraventricular, periventricular, dorsomedial, infundibular, posterior and lateral hypothalamic nuclei and regions, periventricular nucleus of the thalamus, substantia grisea centralis (periaqueductal gray), and amygdala. Furthermore, Abrams *et al.* [1] did not observe ACTH fiber immunoreactivity in the cerebral cortex, hippocampus, substantia nigra, cerebellum, or striatum. For the most part, the results of the present study are in good agreement with the latter. We have further described additional fiber immunoreactivity in many telencephalic, diencephalic and brainstem areas. Other immunocytochemical studies, particularly in the human brain, have dealt with the localization of POMC peptide immunoreactivity only in the hypothalamus [5, 8, 38]. Thus the present study provides the first full descriptive account of the anatomical distribution of POMC-derived peptides in a primate brain.

The distribution of POMC-peptide immunoreactivity in neuronal structures of rhesus monkey brain is similar in many respects to that seen in rat brain [5-7, 16, 23, 49, 51, 52, 55]. The major pathways in rat also appear to be present in monkey brain. In both species, a prominent rostrally projecting bundle courses through midline diencephalic and telencephalic structures, innervating various hypothalamic,

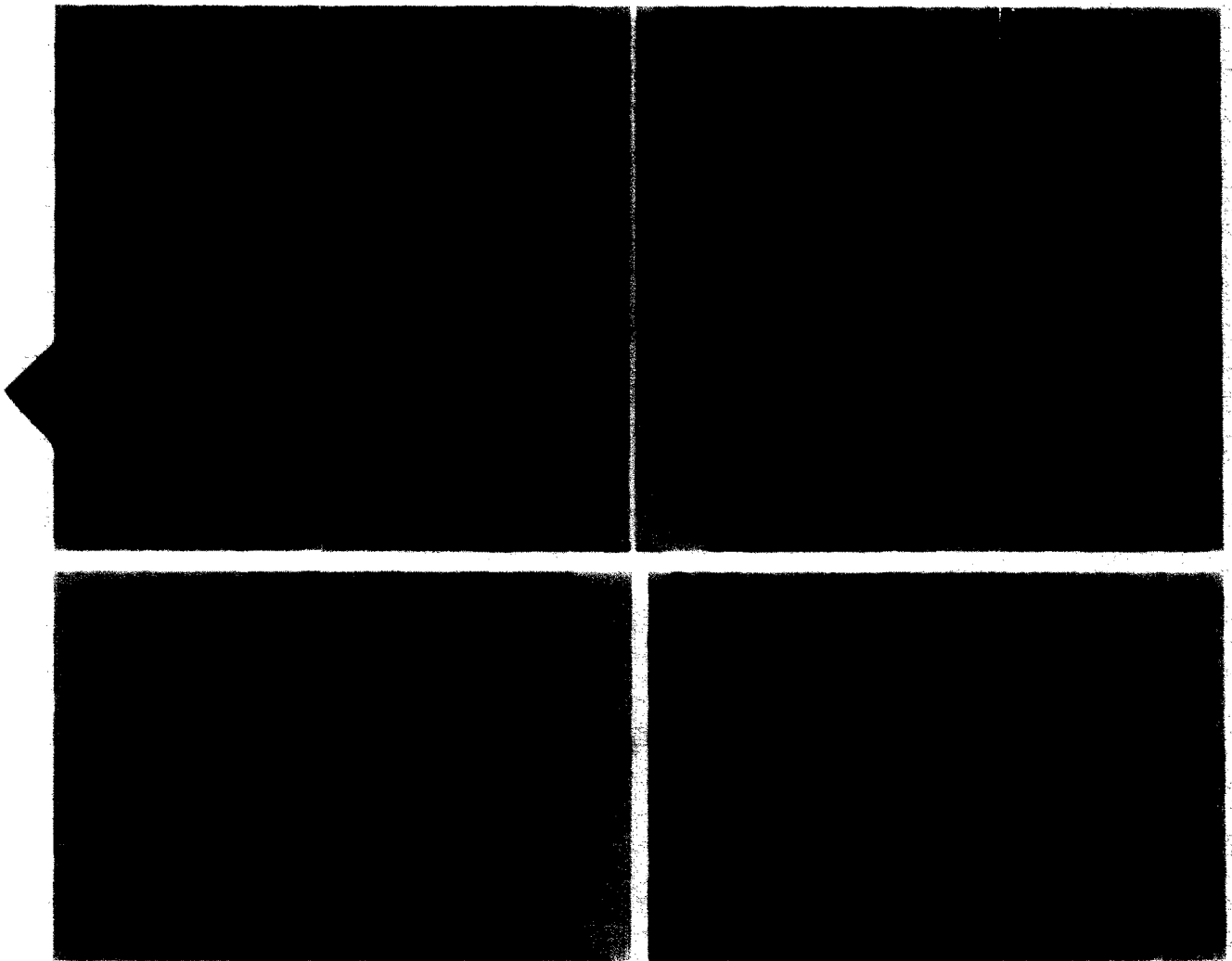


FIG. 2. A is a photomontage of the rhesus monkey infundibular nucleus showing the dense accumulation of 16K immunoreactive perikarya in a $32\ \mu\text{m}$ thick section. In B, β -endorphin immunoreactive perikarya are seen in a section $32\ \mu\text{m}$ caudal to A. Note that while these two immunoreactivities are distributed very similarly, the β -endorphin staining is less pronounced. A region of panel A outlined by the box is magnified in C. Bar (A,B)=0.5 mm. Bar (C)= $100\ \mu\text{m}$.

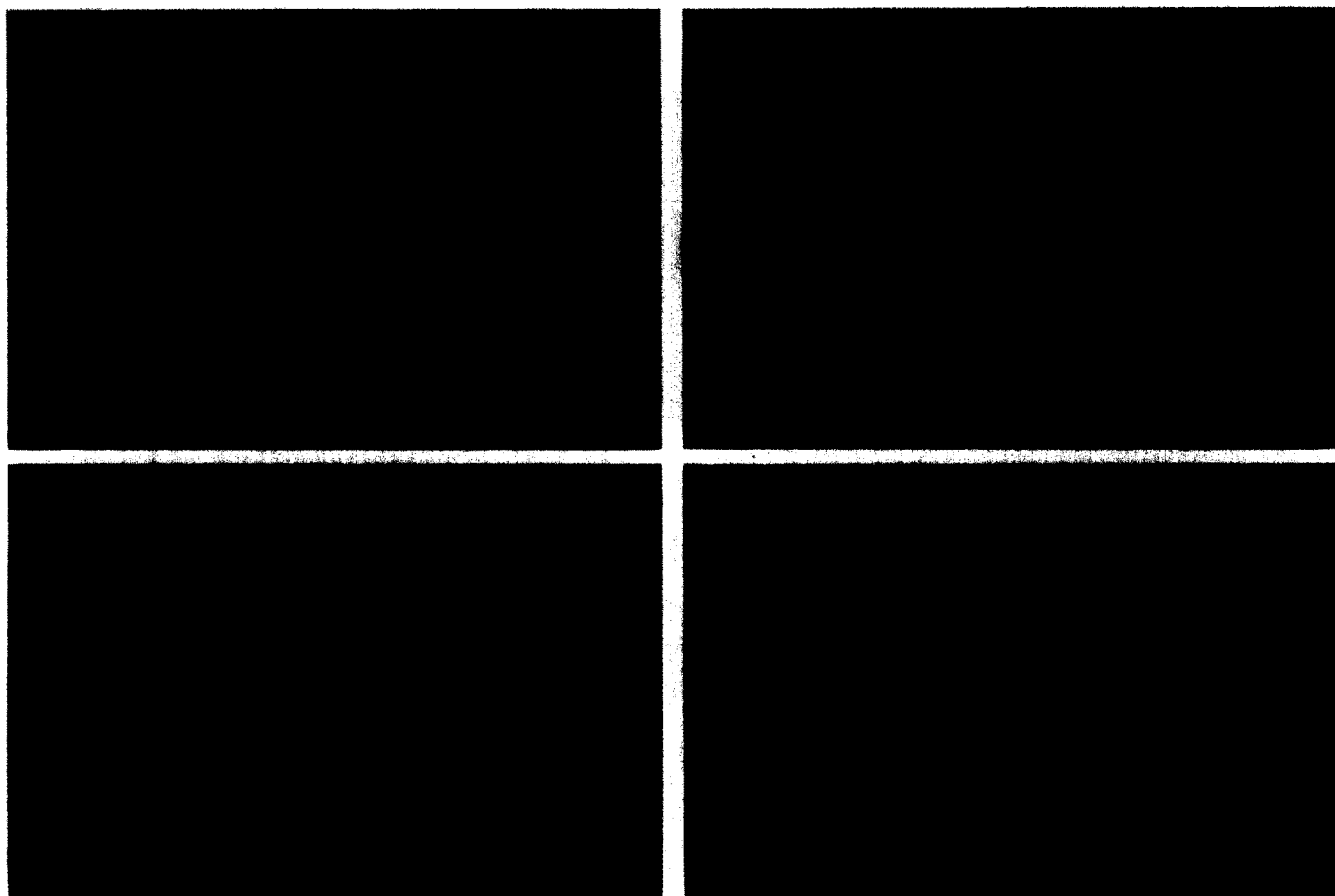


FIG. 3. A and B are adjacent $32\ \mu\text{m}$ thick horizontal sections through the infundibular nucleus showing the identical distribution of 16K (A) and ACTH (B) immunoreactive perikarya. Note that both antisera stain the POMC neurons with similar intensity. In C and D, numerous ACTH-containing perikarya and beaded varicosities are magnified. Bar (A,B)= $200\ \mu\text{m}$. Bar (C,D)= $25\ \mu\text{m}$.

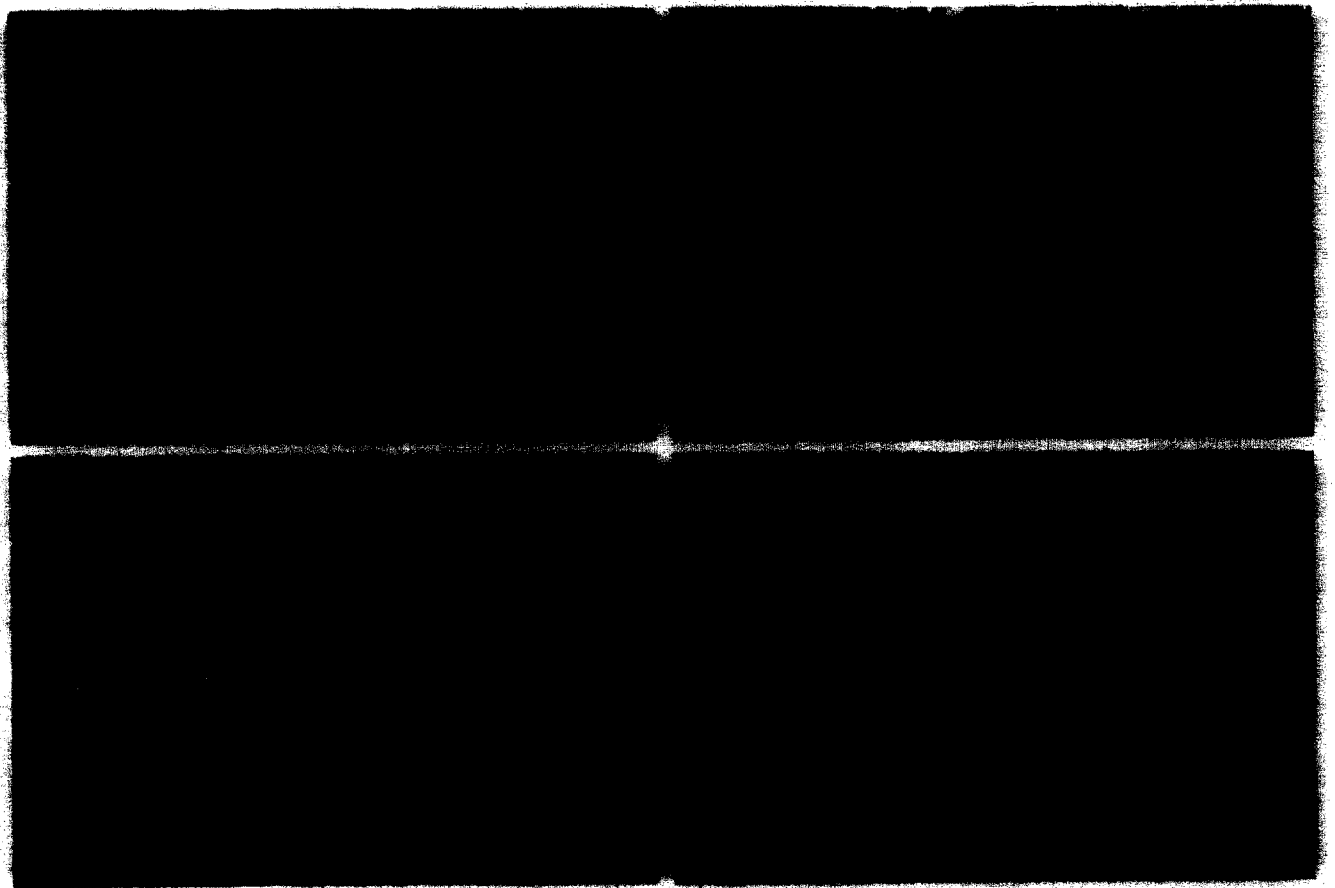


FIG. 4. 16K immunoreactive fibers are shown in darkfield photomicrographs through four telencephalic regions: the central nucleus of amygdala (A), nucleus accumbens (B), bed nucleus of the anterior commissure (C), and the medial preoptic area (D). Bar (A-D) = 200 μ m.

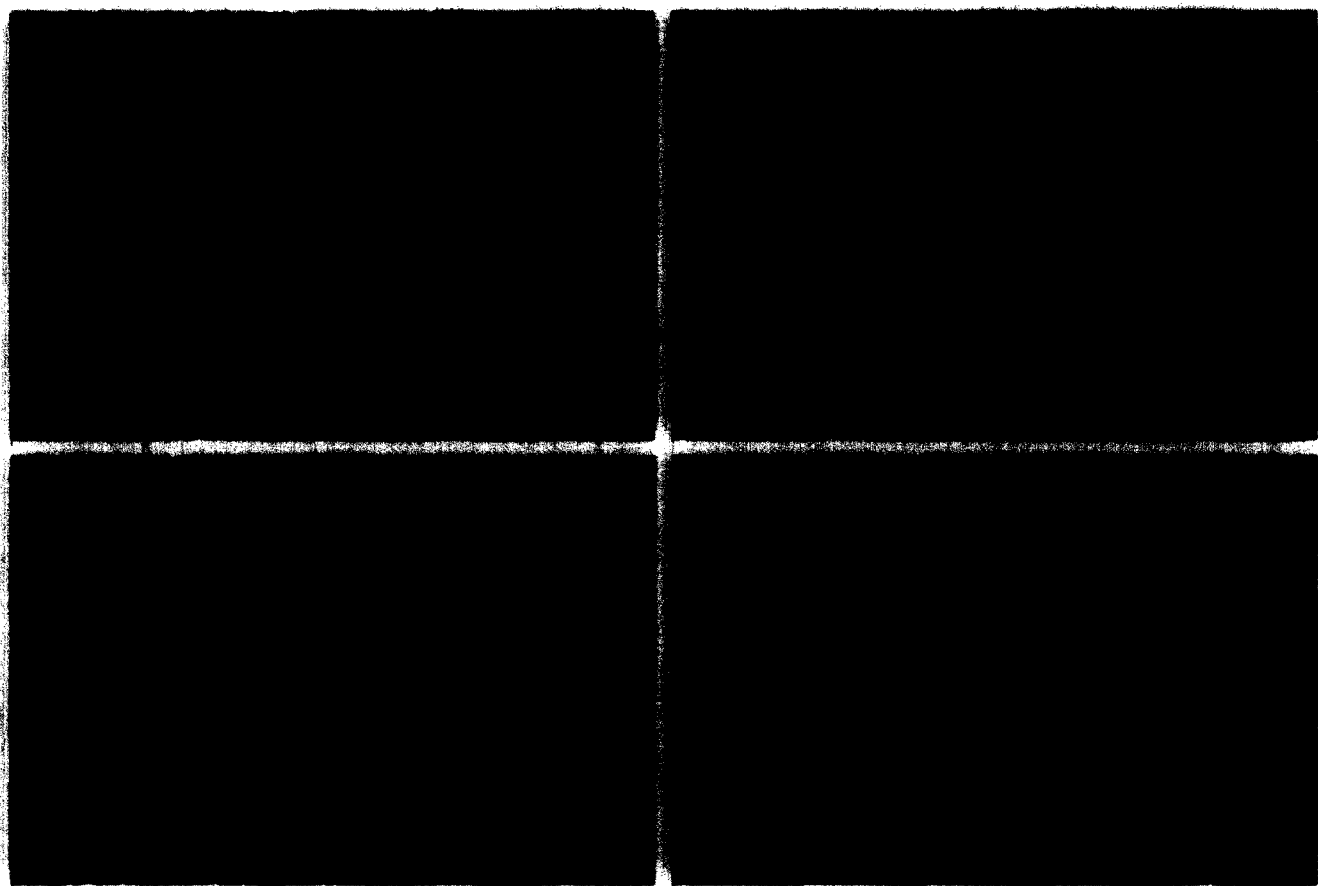


FIG. 5. Darkfield photomicrographs showing 16K immunoreactive fibers in four regions of the diencephalon. **A:** anterior hypothalamic area, **B:** lateral hypothalamic area, **C:** dorsomedial and ventromedial (scattered fibers) nuclei, **D:** periventricular nucleus of thalamus (arrows indicate the midline). Bar (A-D)=200 μ m.

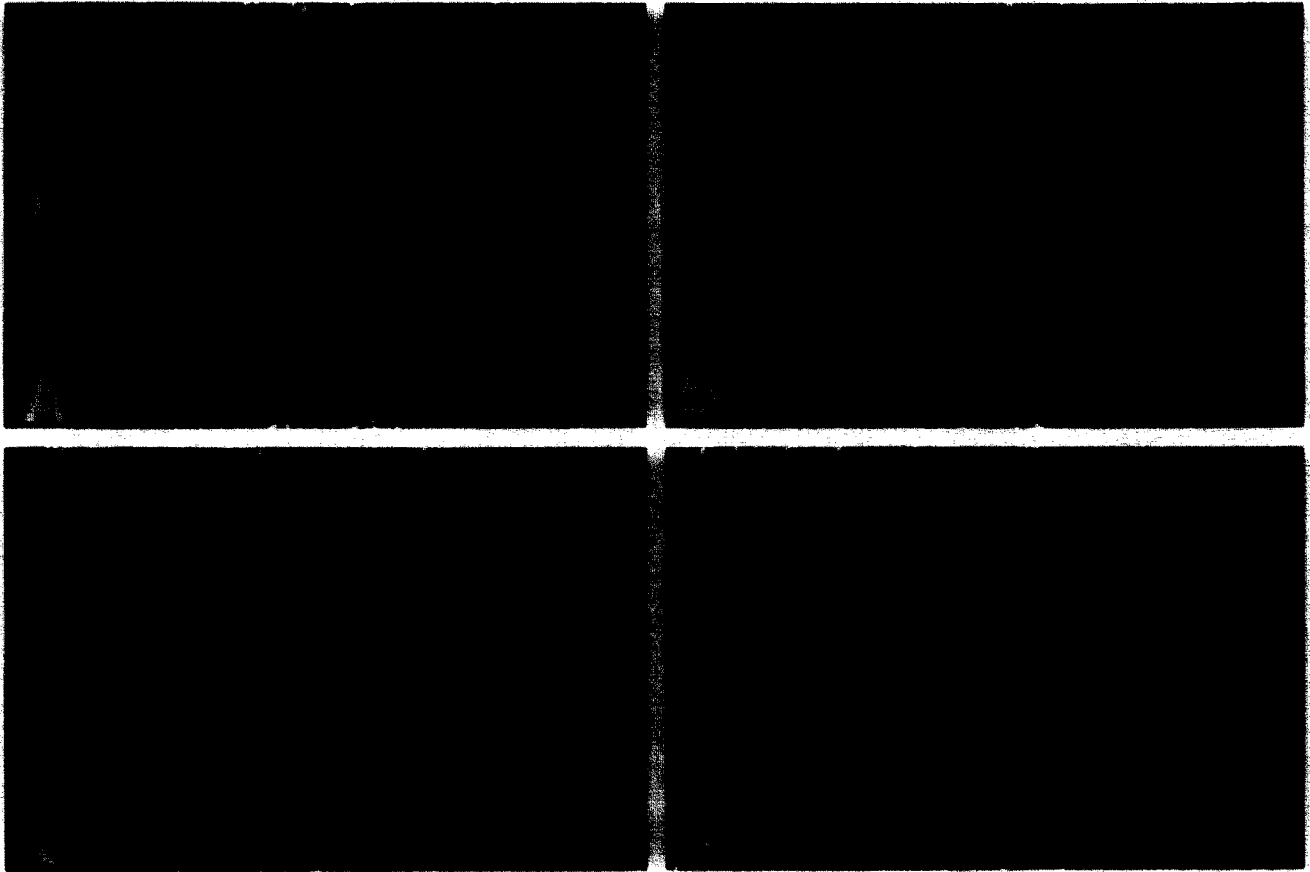


FIG. 6. Brainstem. A and B show 16K immunoreactive fibers (darkfield) in the lateral (A) and ventral (B) regions of the substantia grisea centralis. In C, fibers are seen in the dorsal parabrachial nucleus, and in D, 16K immunoreactivity is shown in the nucleus tractus solitarius (arrows indicate the midline). Bar (A-D)=200 μ m.

thalamic, and limbic forebrain structures. As is the case in rat brain, the bed nucleus of stria terminalis in monkey contains a dense distribution of immunoreactive fibers and terminals. In contrast, the monkey nucleus accumbens is more prominently innervated compared to the scattered fibers seen in the medial aspects of the rat nucleus accumbens. The scattered immunoreactive fibers seen in the head of the caudate and putamen in monkey are virtually non-existent in rat caudate-putamen. Furthermore, the septal nuclei of monkey do not appear to be as heavily innervated as their counterparts in rat brain; the main concentration of septal fibers in monkey is restricted to the ventrolateral septal nucleus. In the diencephalon, again very similar fiber projection patterns are noted in monkey and rat. The major pathways appear similar and the nuclear innervations virtually identical.

Many elements of the lateral and caudal projections of the POMC containing neurons of monkey brain are also similar to those observed in rat brain. A major laterally projecting system appears to traverse the floor of the monkey hypothalamus, coursing dorsal to the optic tract to enter the amygdala. However, the amygdaloid innervation appears to be restricted to the more medially situated areas such as the medial and central nuclei. By contrast, in rat brain, a particularly rich lateral projection penetrates deeply into the temporal region to include almost all regions of the amygdala and even the periamygdaloid cortex, which exhibits scattered fi-

bers. As in rat, the central nucleus of amygdala in monkey shows a dense accumulation of immunoreactive fibers and terminals. Also in both species, the stria terminalis, which connects the medially situated amygdaloid nuclei to other forebrain limbic structures, exhibits distinct fiber immunoreactivity. The direction of immunoreactive fiber flow in this tract has yet to be determined, since both the bed nucleus of stria terminalis, as well as the medial amygdala, exhibit immunoreactive fibers and terminals. Furthermore, both regions appear to be innervated independently at least by two separate projectional systems: the bed nucleus of stria terminalis via the rostral projections, and the amygdala through the lateral projections. Thus, fibers in the stria terminalis could conceivably arise from either of these sources, although their actual origin will remain unclear until appropriate tract-tracing studies can be performed.

As far as the caudal projections are concerned, the innervation of the substantia grisea centralis via the different caudal projections appears to be identical in monkey and rat brain. Further caudally, in the dorsal pons, the locus coeruleus also exhibits some fiber immunoreactivity; however, most of the fibers are localized to the periphery of this nucleus. This feature appears similar to the distribution of immunoreactive fibers in the vicinity of rat locus coeruleus. Caudal to this point, a relative decline in fiber immunoreactivity is seen in the monkey pons, with a few fibers seen



FIG. 7. This darkfield montage of the rhesus monkey hypothalamus and preoptic area shows the distribution of ACTH immunoreactive fibers in a parasagittal view 0.5 mm lateral to the midline. Note the intense immunoreactivity in the median eminence. Bar=1 mm.

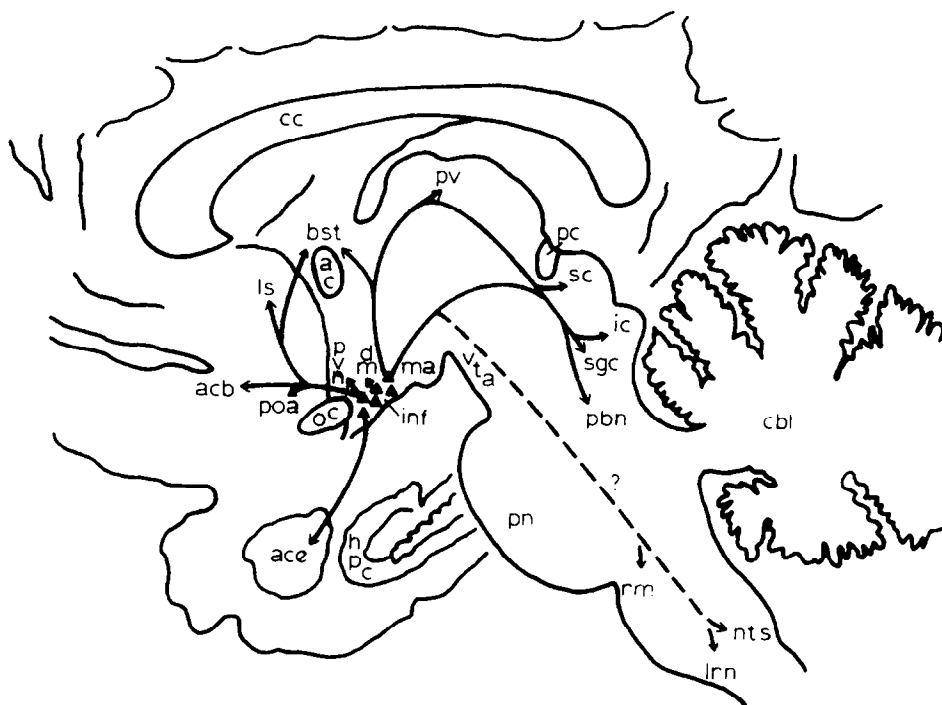


FIG. 8. Summary of POMC fiber pathways in the rhesus monkey brain in a schematic sagittal view. Rostral and lateral projections of infundibular neurons contribute to the innervation of various telencephalic limbic structures, e.g., the nucleus accumbens and central nucleus of amygdala. Various caudal projections innervate nuclei in the diencephalon, midbrain, and brainstem, such as the periventricular nucleus of thalamus and substantia grisea centralis. The exact course of other caudal projections which traverse the ventral tegmental area and brainstem is not clear. However, these projections apparently contribute to the innervation of various medullary raphe and reticular nuclei.

dorsally throughout the midline periventricular region as far caudal as the nucleus tractus solitarius. In the rat brain, a more pronounced bundle courses through the dorsal pons as does a more ventral pontine-medullary pathway which apparently contributes to the innervation of other brainstem reticular structures.

The relatively sparse nature of immunoreactive POMC fibers caudal to the substantia grisea centralis raises the question of the origin of fibers in brainstem regions such as the nucleus tractus solitarius. In monkey, with the doses of colchicine used in the present study, we have been able to visualize POMC immunoreactive perikarya only in the infundibular nucleus of the hypothalamus. However, we and other investigators have recently noted perikarya in the caudal nucleus tractus solitarius pars commissuralis of rat which exhibit immunoreactivity for ACTH, β -endorphin, and 16K fragment [20, 21, 41]. These perikarya apparently contribute to the innervation of this nucleus as well as projecting ventrally to the lateral reticular nucleus and perhaps rostrally to the parabrachial nuclei. As in monkey, the POMC-immunoreactive system of fibers seen in rat also appears relatively sparse caudal to the mesencephalon and rostral to the nucleus tractus solitarius, although this apparent separation is more pronounced in the monkey. Therefore, it is possible that the immunoreactive fibers in caudal medulla arise from similar POMC perikarya in monkey nucleus tractus solitarius, although we have not yet been able to demonstrate this system.

The findings presented here indicate a striking similarity

between the POMC systems in the rodent and primate species examined, suggesting the possibility of a similarity and thus conservation of the functional roles of the POMC peptides in these species (see [3, 4, 23, 34, 48] for review). Certain major components of this system appear to have been conserved through evolution while others have not, since we have recently shown major similarities and differences in the distribution of POMC peptide immunoreactivity in the brain of Anolis and rat [22]. The similarity of hypothalamic POMC systems in rat and monkey, for example, may point to a conservation of neuroendocrine regulatory mechanisms which are essential for survival and reproduction. However, there are also noteworthy species differences in the distribution of the POMC systems, which may be indicative of more specialized roles of the POMC peptides in brain function. For example, the modest innervation of caudate and putamen in monkey, which is not present in rat, raises the possibility that POMC peptides might influence extrapyramidal motor output in primates, particularly under conditions (e.g., stress) which activate POMC systems [3,4].

In addition to species differences in the distribution of the POMC systems, there are clear variations in the regional localization of opioid receptors in rat and primate brain [28,45]. This combination of species differences in the distribution of opioid pathways as well as receptors indicates the need to carefully examine pathway-receptor relationships. Combined immunocytochemical and receptor autoradiographic studies have been carried out in both rodent [26,29] and primate [27,28] species, and have indicated complex re-

relationships between multiple opioid pathways and receptors. In addition to this complexity, there is the issue of the relationship between POMC systems and receptors for the non-opioid POMC peptides (e.g., ACTH and α -MSH). Unfortunately, binding sites for these peptides have been very difficult to characterize [3]. Nevertheless, it is clear from studies of analgesia [44] and other behavioral measures [36] that ACTH and MSH related peptides are active through a non-opioid receptor. Therefore, it seems likely that the physiological actions of released POMC peptides will be due to both opioid and non-opioid receptor interactions. These actions will be a function of the particular peptide forms released, since these forms have quantitatively or qualitatively different biological activities (see [3, 4, 34] for review). Since there are regional differences in the processing of POMC in brain [2, 10, 54], it is likely that the consequences of neurotransmission at POMC synapses will be a function of both the ratio of peptide forms released and the relative abundance of receptors for these different peptides. It is also worth noting, in the context of the present study, that the processing of POMC also appears to differ between rodent and primate species [9,35], indicating the possibility that

POMC systems which are conserved anatomically may still differ in their biosynthetic output. Recent studies in reptiles have pointed clearly to major species differences in the post-translational processing of POMC [11-13]. Thus, in the consideration of the significance of species differences (or similarities) in the anatomy of the POMC systems, careful attention should be given to the existence of regional and species differences in POMC processing, for in these differences may lie important clues to the physiological functions of POMC peptides.

ACKNOWLEDGEMENTS

This work was supported by NIDA Center Grant DA00154 to H.A. and S.J.W., NIMH Training Grant MH15794 to M.E.L., and the Theophile Raphael Fund. We wish to thank Dr. Elizabeth Eipper and Dr. Richard Mains for providing the 16K fragment antiserum. We also wish to acknowledge the laboratory of neuropsychology, NIMH, for the original atlas plates from which our atlas figures were prepared. We are grateful to S. Burke for expert technical assistance, and A. Henry for manuscript preparation.

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