

using preimmune serum, TH- and DBH-antiserum, serially.

Mesencephalon

One of the sagittal sections examined of the mesencephalon is shown by a montage of immunofluorescent pictures (Fig. 2) and its schematical drawing (Fig. 3). From the dorsal part of the nucleus interpeduncularis, dopaminergic neurons (TH-positive and DBH-negative) (A10 according to Dahlström and Fuxe (3)) continued rostral and dorsal to the pedunculus cerebellaris superior and crossed the fasciculus longitudinalis medialis and reached the substantia grisea centralis



FIG. 1. Locus coeruleus. DBH-positive cell bodies, terminals and fibers are observed in this sagittal section. The left side is rostral and the downside is ventral. $\times 111$

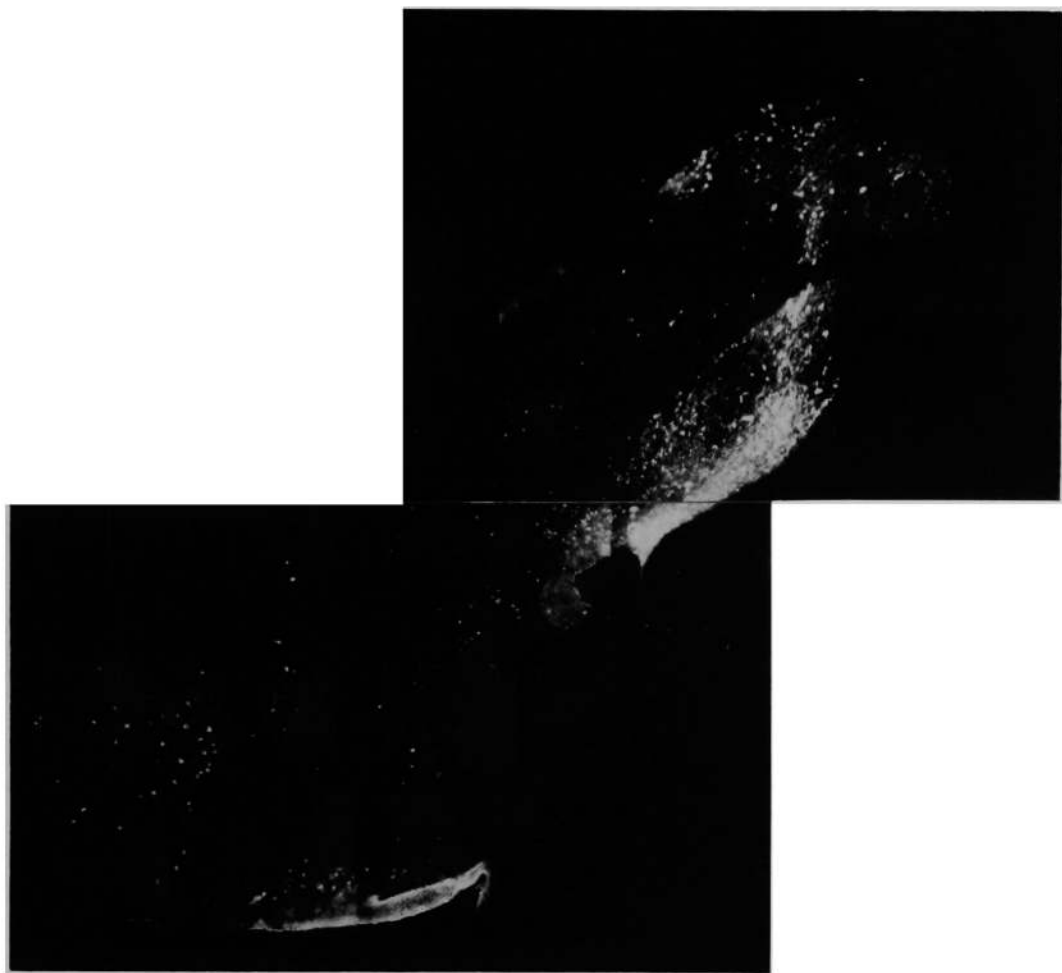


FIG. 2. Mount of the mesencephalon (the medial part, a TH-stained sagittal section). Unless otherwise stated all micrographs in the paper represent immunofluorescent micrographs of cryostat sections of rat brain incubated with TH or DBH antiserum, diluted 1 : 10, followed by FITC-conjugated serum diluted 1 : 30. $\times 17$

passing through nucleus raphe dorsalis. The dopaminergic neurons contained and followed the *fibrae periventriculares hypothalami* (A11 according to Dahlström and Fuxe (3)). The nucleus interpeduncularis and nucleus n. oculomotorius showed negative TH (Figs. 2, 3) and DBH.

As shown in Figs. 10 to 12, green immunofluorescence specific for TH was found in some cell bodies together with many serotonergic neurons of the nucleus raphe dorsalis. Especially in the part just above and medial to the *fasciculus longitudinalis medialis* (Fig. 9, a toluidine blue-stained frontal section), small to medium-sized immunofluorescent cells (Figs. 10 and 11, TH-stained frontal section) were scattered in the central gray ventral to the aqueduct of the mesencephalon. The

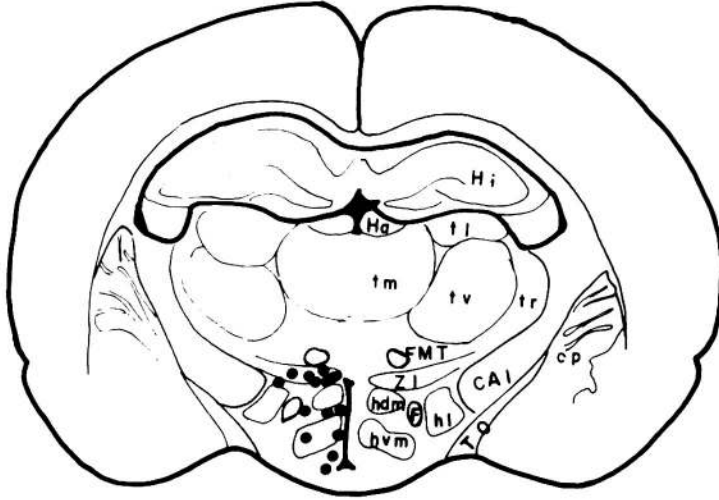


FIG. 7. Mesencephalon (a frontal section). TH-positive cell bodies are schematically drawn.

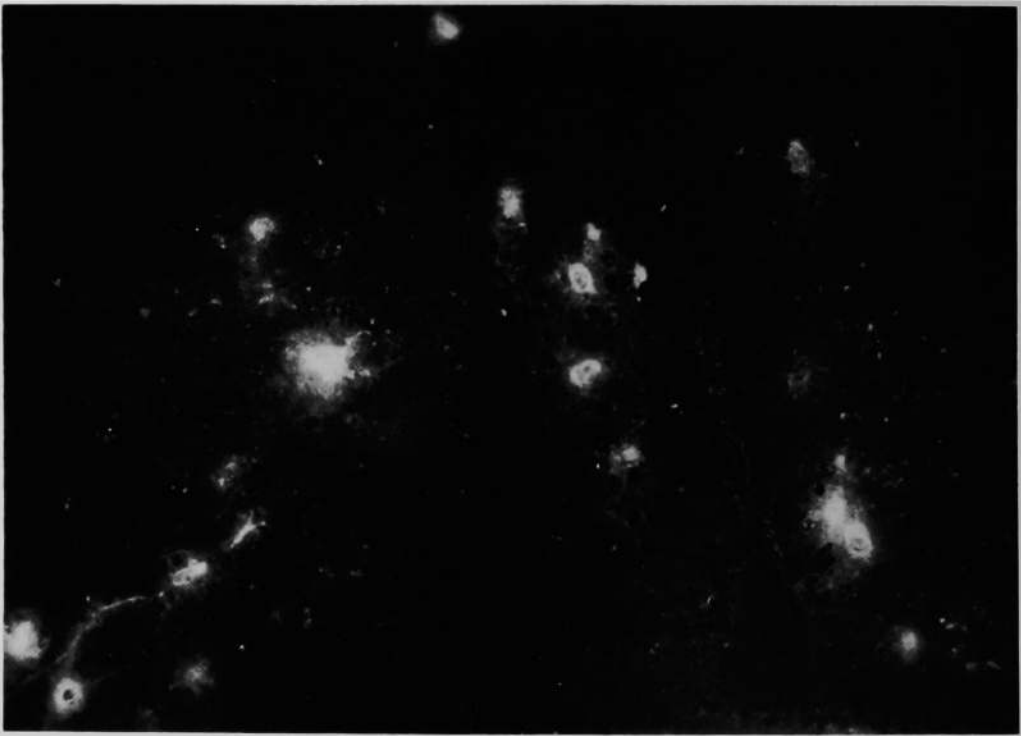


FIG. 8. Mount of the mesencephalon, which is the same section as in Fig. 7. TH-positive and DBH-negative dopaminergic neurons are observed in the arcuate nucleus. Some cells have long processes. $\times 225$

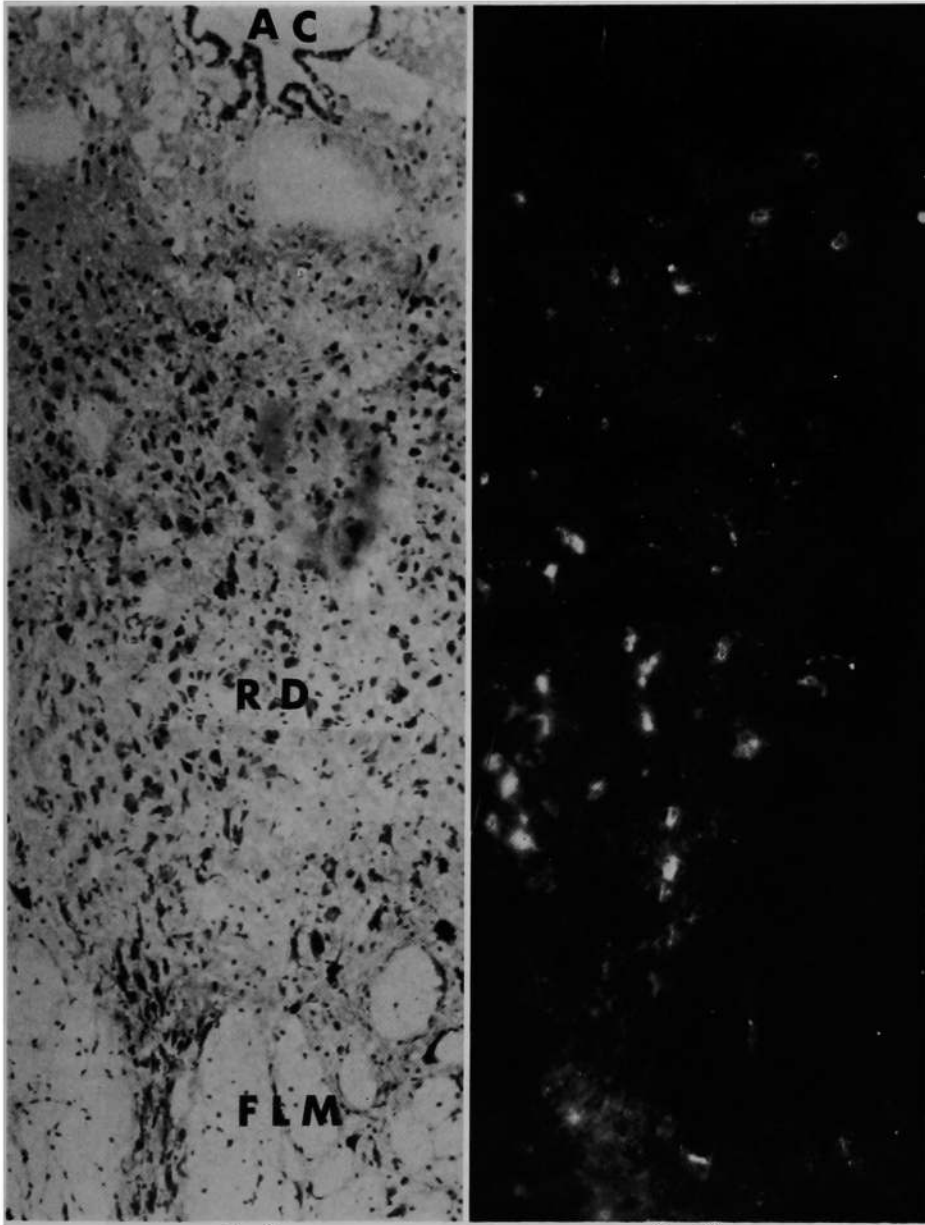


FIG. 9

FIG. 10

FIG. 9. Nucleus raphe dorsalis (a toluidine blue-stained frontal section). FLM, fasciculus longitudinalis medialis; AC, aqueductus cerebri; RD, nucleus raphe dorsalis. $\times 118$

FIG. 10. Nucleus raphe dorsalis. A TH-stained frontal section as indicated in Fig. 9. In the part just above and medial to the FLM, small to medium-sized immunofluorescent cells are scattered in the central gray ventral to the aqueduct of the mesencephalon. $\times 121$

same results were observed in the sagittal section (Fig. 12, TH-stained). On the other hand, serial sections stained with anti-DBH showed only very low levels of immunofluorescent terminals but no DBH-specific cell bodies in the nucleus raphe dorsalis (Fig. 13, a sagittal section).

Diencephalon

In diencephalon, we observed many dopaminergic neurons (TH-positive and DBH-negative) in the arcuate nucleus (Figs. 2, 3, 4, 7, 8) (A12 according to Dahlström and Fuxe (3)) and around the anterior hypothalamic nucleus (A14 according to Björklund and Nobin (1)), and also some cells of the anterior hypothalamic nucleus (Figs. 2, 3, 4, 5). In the lateral sagittal sections of the hypothalamus

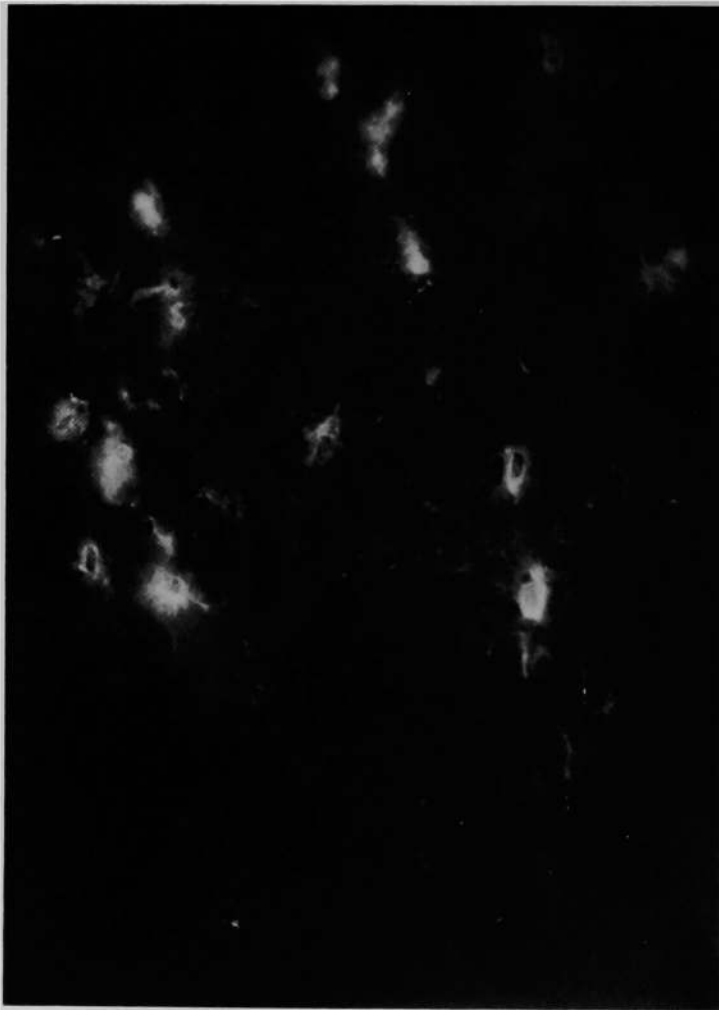


FIG. 11. Nucleus raphe dorsalis. An enlarged photograph of a part of Fig. 10. $\times 296$

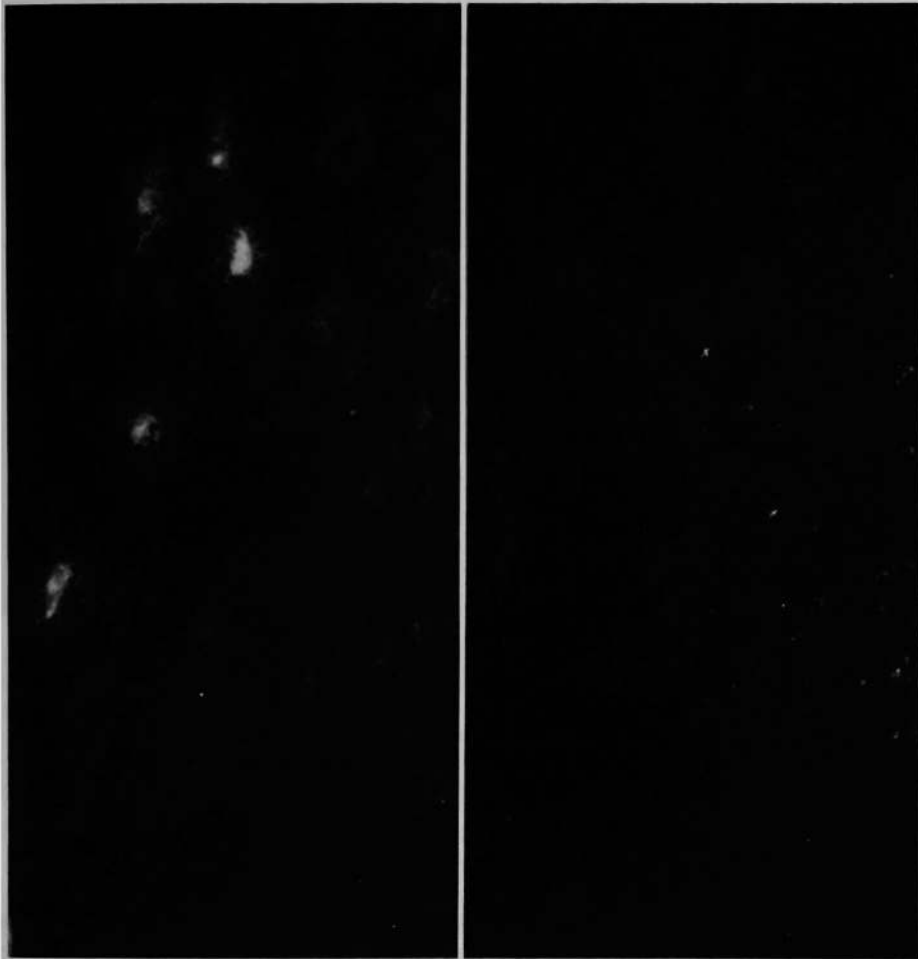


FIG. 12

FIG. 13

FIG. 12. Nucleus raphe dorsalis (a TH-stained sagittal section). In the central gray, green immunofluorescence specific for TH is found in cell bodies among many serotonergic neurons. $\times 296$

FIG. 13. Nucleus raphe dorsalis (a DBH-stained serial section in the sagittal plane). Only very low levels of immunofluorescent terminals but no DBH specific cell bodies are found. $\times 296$

(from middle, Figs. 4, 5, 6) and in one of the frontal sections of the diencephalon examined (Fig. 7), numerous dopaminergic neurons were observed in the zona incerta (Figs. 5, 6, 7) (A13 in the nomenclature of Fuxe *et al.* (8)), a few in the ventromedialis hypothalamic nucleus (Figs. 6, 7) and in the dorsomedialis nucleus (Figs. 2, 3, 5), and probably in the posterior hypothalamic nucleus (Figs. 2, 3).

The outer layer of the median eminence showed strong specific immunofluorescence for TH (Figs. 2, 14), but not for DBH (Fig. 15). The inner layer of the median eminence had many capillaries of the tuberoinfundibular sulcus and showed a very low level of autofluorescence, but distinguishably specific fluorescence for

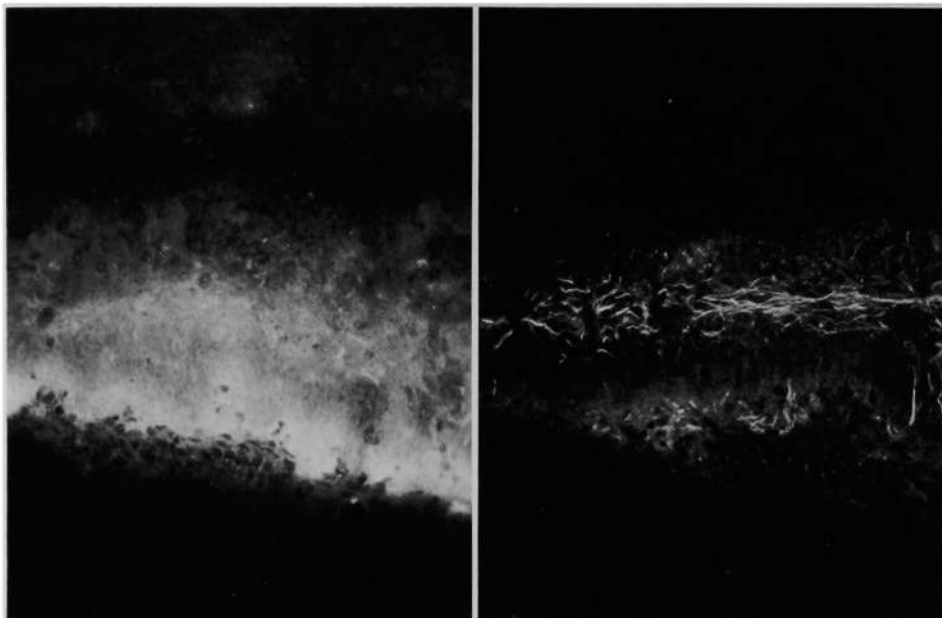


FIG. 14

FIG. 15

FIG. 14. Median eminence (a TH-stained sagittal section). In the outer layer TH-positive terminals are abundantly shown. $\times 185$

FIG. 15. Median eminence (a DBH-stained sagittal section). In the inner layer DBH-positive terminals are observed around capillaries of the tuberoinfundibular sulcus. In the most lateral parts of the external layer, thin strands of DBH terminals are found adjacent to the primary capillary plexus. $\times 185$

DBH (Fig. 15) and for TH (Fig. 14). In the most lateral parts of the external layer of the median eminence, thin strands of DBH terminals were found at certain levels adjacent to the primary capillary plexus (Fig. 15). The corpus mamillare showed no specific fluorescence for both TH and DBH (Fig. 2).

Telencephalon

One of the examined sagittal sections of the telencephalon is drawn in Fig. 16. In the olfactory bulb, some periglomerular cells were fluoresced specifically only for TH (Fig. 17), but not for DBH. These dopaminergic neurons were newly found and named A15 by Halasz *et al.* (10). Many TH terminals were found in the glomerular layer (Fig. 17).

TH terminals were abundantly observed in the nucleus caudate-putamen, nucleus accumbens and tuberculum olfactorium (Figs. 16, 18). DBH fibers were observed in the tractus olfactorius intermedius and fasciculus medialis prosencephali.

DISCUSSION

The present results of immunofluorescent work indicate that only when un-

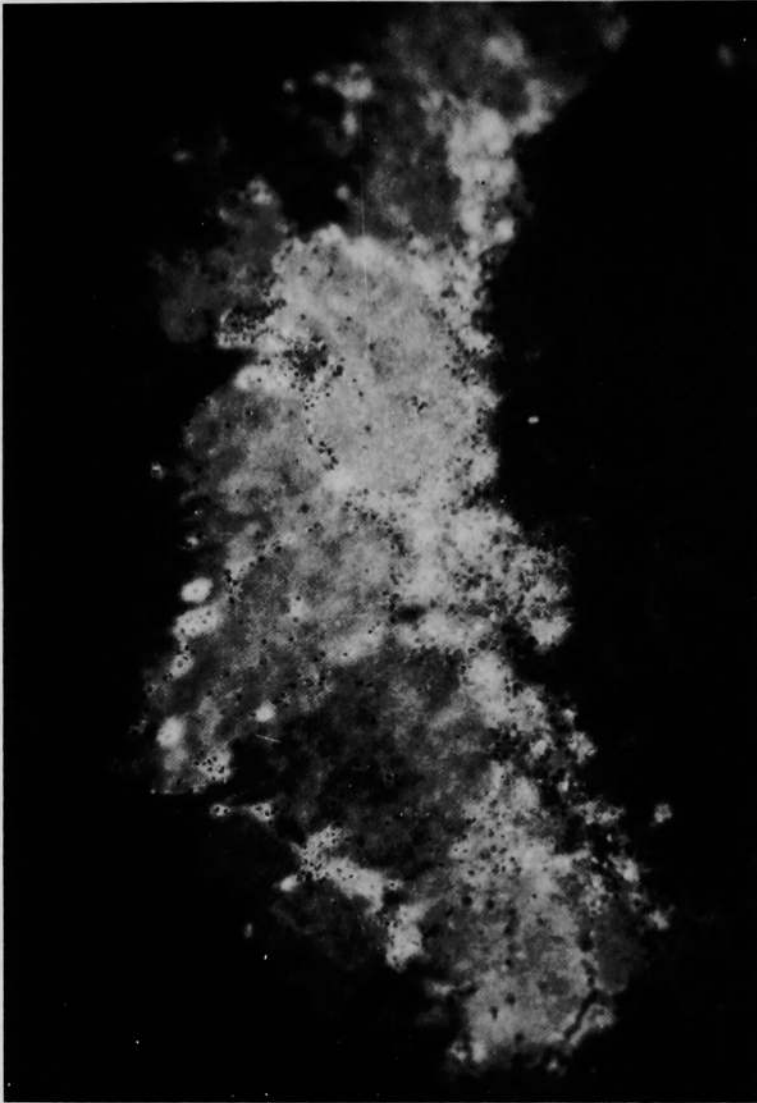


FIG. 17. Olfactory bulb. TH-positive periglomerular cells are shown around TH-positive fibers of the glomeruli. An olfactory nerve layer (the left side) and an external plexiform layer (the right side) show TH-negative. $\times 118$

specific immunofluorescence (Fig. 19, a frontal section) of extended processes was clearly observed in the substantia nigra, but the density of immunofluorescence in the cell body was much higher than that of the dendrites.

The occurrence of catecholamine-containing neurons in the nucleus raphe dorsalis and in other parts of the central grey has been mapped previously by Lindvall and Björklund (17). They also gave some data on the afferent and

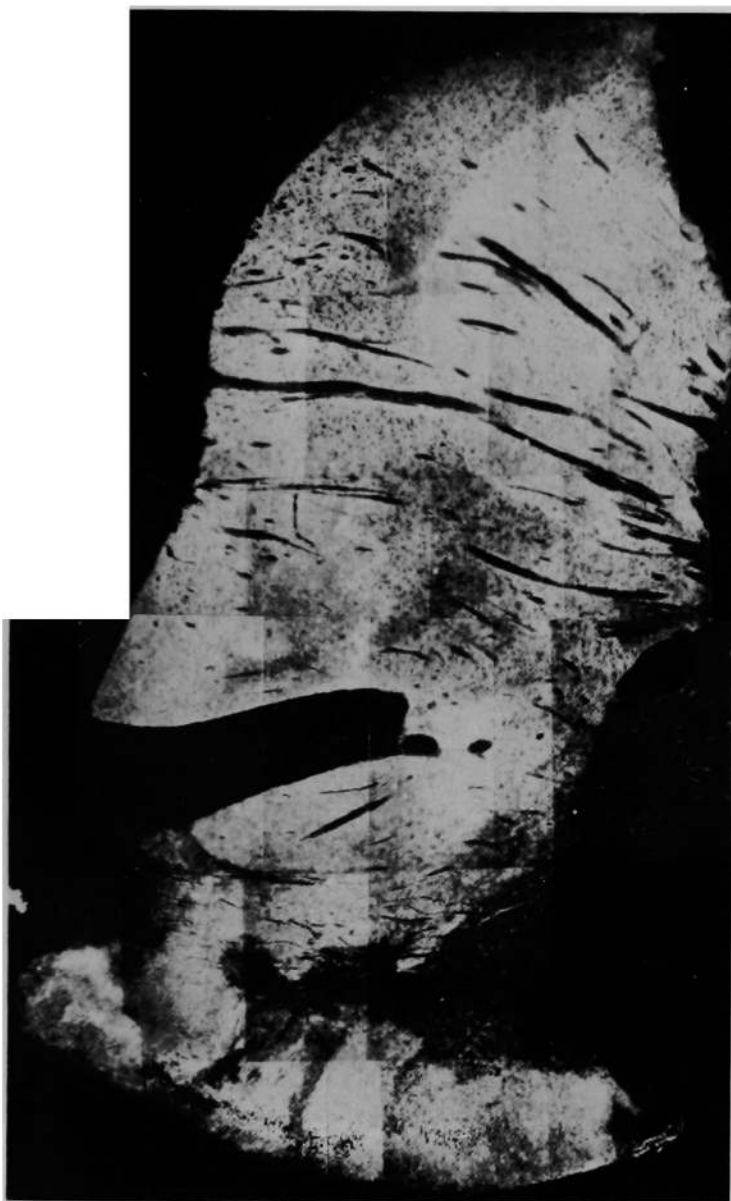


FIG. 18. Mount of the telencephalon, which is a part of the same section as in Fig. 16. $\times 28$

efferent catecholamine connections of this area as well as the catecholamine innervation pattern. The catecholamine neurons in the dorsal raphe belong to a periventricular paramedian cell system that is classified either as A10 or A11, depending on which the connections are. Subsequently Swanson and Hartman (24) reported the absence of DBH-positive neurons in this area, and Hökfelt *et al.*

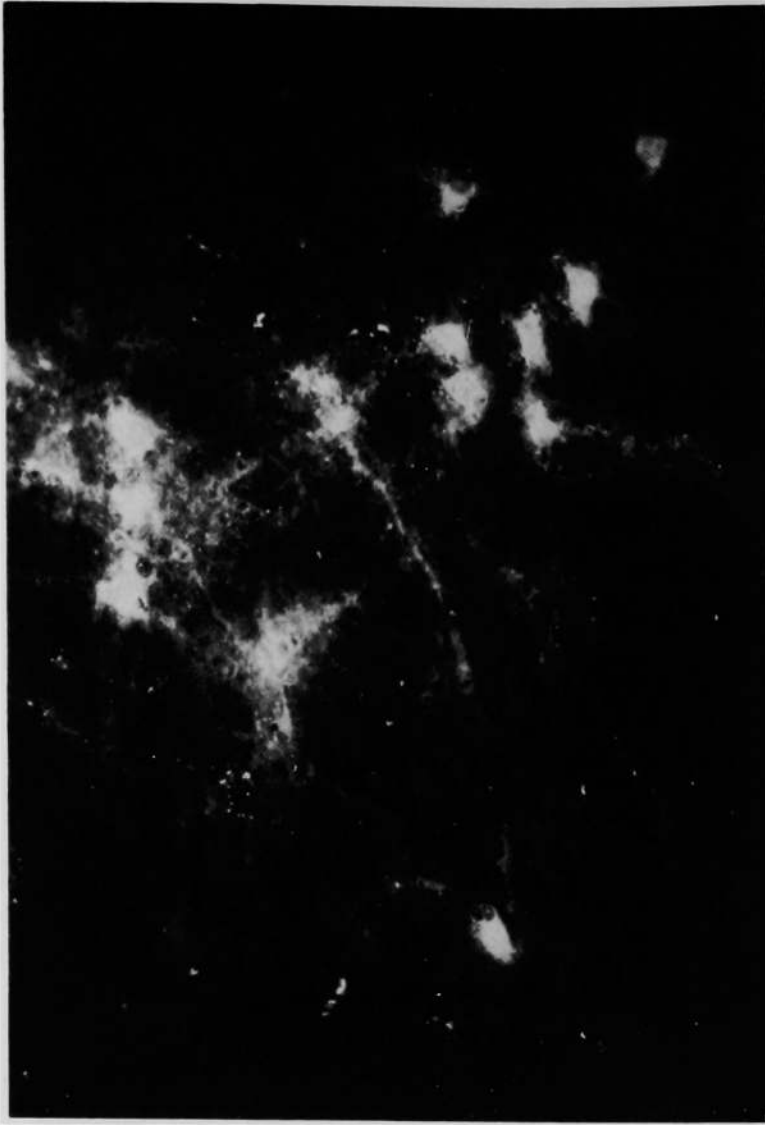


FIG. 19. Substantia nigra (a frontal section). TH-positive and DBH-negative dopaminergic neurons, processes and terminals are shown. Immunofluorescence specific for TH is very strong in the cell bodies showing halation. $\times 277$

(13) have shown TH-positive neurons in this area in their Fig. 11. Our results confirmed the above. Thus, dopaminergic neurons coexist in the nucleus raphe dorsalis with serotonin neurons (17, 27).

TH-positive and DBH-negative dopaminergic cell bodies were distributed numerously in the arcuate nucleus (A12) and their processes extended to the median eminence. These dopaminergic nerve terminals mostly located in the external

