Afferent Projections to the Ventral Tegmental Area of Tsai and Interfascicular Nucleus : A Horseradish Peroxidase Study in the Rat

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ABSTRACT Using the retrograde transport of horseradish peroxidase (HRP), a study has been made of projections to the ventral tegmental area of Tsai (VTA) and related dopaminergic cell groups (A 10). In order to minimise the possibility of damage to fibres of passage, a technique was evolved for the microiontophoresis of HRP such that minimal current strengths and durations were applied. In addition to a sham injection, control injections were also made to the medial lemniscus, red nucleus, deep tegmental decussations, mesencephalic reticular formation and brachium conjunctivum. Following HRP injections confined to the areas of the VTA containing the dopamine cell groups, labelled neurons appeared in prefrontal cortex, dorsal bank of rhinal sulcus, nucleus accumbens, bed nucleus of stria terminalis, amygdala, diagonal band of Broca, substantia innominata, magnocellular preoptic area, medial and lateral preoptic areas, anterior, lateral and postero-dorsal hypothalamus, lateral habenular, nucleus parafascicular nucleus of thalamus, superior colliculus, nucleus raphe dorsalis, nucleus raphe magnus and pontis, dorsal and ventral parabrachial nuclei, locus coeruleus and deep cerebellar nuclei. Regions containing catecholamine groups A 1, A 5, A 6, A 7, A 9, A 13 and the serotonin group B 7 corresponded to the topography of labeled cell groups. Injections of HRP to the interfascicular nucleus resulted in labeling predominantly confined to the medial habenular and median raphe nuclei. The results are discussed in relation to the known connections of these regions. Other regions of the brain labelled by VTA injections are assessed in relation to control injections and the limitations of the HRP technique.

A review of the organisation of some of these afferents in relation to the known cortical-subcortical-mesencephalic projection systems, suggests that the VTA is in a position to receive information from a massively convergent system derived ultimately from the entire archi-, paleo-, and neo-cerebral cortices. In addition A 10 dopaminergic neurons are known to project to restricted regions of both pre-frontal and entorhinal cortices, which themselves also receive massively convergent association cortico-cortical connections. It would appear reasonable to propose that these neurons perform a correspondingly important integrative function.

A considerable body of evidence exists to support the hypothesis that dopaminergic systems in the central nervous system provide a site of action for the antipsychotic actions of different chemical classes of neuroleptic drugs (Burt et al., '75; Seeman et al., '75; Iversen, '75). The dopaminergic neurons of the ventral tegmental area of Tsai (VTA) are known as the A 10 group (Dahlström and Fuxe, '64) and in the rat project to many "limbic" forebrain sites as well as to the frontal cortex. (Ungerstedt, '71; Berger et al., '74; Fuxe et al., '74; Lindvall et al., '74a; Lindvall, '75; Lindvall et al., '78). In addition, recent

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autoradiographic studies have demonstrated descending projections from VTA to paramedian midbrain structures (Domesick et al., '76). Findings obtained with pathway tracing methods have given some information about the nature of their afferent supply. Anterograde degeneration experiments have suggested that these pathways arise in septal area (Nauta, '56), lateral hypothalamic region (Guillery, '57; Wolf and Sutin, '66); lateral preoptic area (Nauta, '58), and lateral habenula (Agaki and Powell, '68). More recent autoradiographic evidence has confirmed these projections (Swanson, '76; Arbuthnott et al., '76; Herkenham and Nauta, '77a) and provided additional evidence of afferents to VTA from substantia innominata (Swanson, '76; Meibach and Siegel, '77), diagonal band, bed nucleus of stria terminalis, medial preoptic area, anterior hypothalamus (Swanson, '76; Conrad and Pfaff, '76a,b; Meibach and Siegel, '77) and dorsal raphe nucleus (Conrad et al., '74; Bobillier et al., '75; Taber-Pierce et al., '76).

More comprehensive information about the afferent supply to VTA neurons must be obtained, however, in order to understand how changes in activity in this predominantly dopaminergic group can arise. This in turn should help to clarify how the connections of neuronal systems, which are suspected as the targets for antipsychotic drug action, relate to the various clinical aspects of the psychotic syndrome.

The present study by the method of retrograde neuron labelling with horseradish peroxidase (HRP) (Kristesson and Olsson, '71; La Vail and La Vail, '72; La Vail et al., '73) was undertaken in order to obtain such information, and in addition to gain an accurate identification of the site of origin of the afferents. This is difficult to obtain with anterograde techniques because the cyto-architecture of many of the sources of afferents, such as the basal forebrain area, is heterogeneous and diffuse. In addition to confirming previously described afferents, the results of the present study suggest so far unknown systems projecting to the VTA from cerebral cortex, amygdala, hypothalamus, thalamus, tectum, pons and medulla.

A preliminary report of some of the present findings has been published previously (Phillipson, '78).

MATERIALS AND METHODS

Small unilateral and midline injections of HRP were placed in different regions of the ventral mesencephalon in 16 female adult Sprague-Dawley rats (145-180 g) by the technique of microelectrophoresis (Graybiel and Devor, '74). Glass micropipettes with bevelled tips (external diameters 20-40 μm) were prepared and filled with a 40% solution of HRP (Sigma, type VI) in 0.1 M NaOH. Rats were anaesthetised with chloral hydrate (400 mg/kg) and positioned in a Kopf stereotaxic frame. Electrodes were lowered with a retaining current to target sites determined from König and Klippel's ('63) stereotaxic atlas. Particular care was taken to avoid as far as possible the major neighbouring nuclei and fibre tracts during penetration. HRP was ejected with a constant positive current of 1 μA for 5 to 17 minutes (microiontophoresis programmer, model 160, W-P instruments, Inc. U.S.A.). In some cases intermittent current was applied, with one minute on and one minute off.

A retaining current was reapplied during withdrawal. About 24 hours later, rats were deeply anaesthetised and perfused through the heart with 100 ml saline followed by 500 ml 1% paraformaldehyde and 1.25% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2-7.4 with 5% sucrose. Brains were removed immediately and postfixed for one to two hours in the same fixative in 30% sucrose, and finally rinsed overnight in 0.05 M cacodylate buffer in 30% sucrose at 4°C. Frozen sections were cut at 40 μm in frontal plane and processed immediately according to the highly sensitive method of Hardy and Heimer ('77) using the non-carcinogenic substrate tetramethylbenzidine (TMB). Sections were counterstained in neutral red and examined for HRP positive neurones under bright field conditions. Control injections were made in addition into medial lemniscus, red nucleus, tegmental decussations, midbrain reticular formation, and superior cerebellar decussations and also by a sham injection in which the electrode was lowered into VTA but no HRP ejected.

In order to minimise damage at the injection site and to limit the spread of HRP around the needle tip preliminary experiments were carried out in seven animals to assess the minimum positive current strength and duration which would eject sufficient HRP for retrograde transport. Additional experiments were also carried out in order to assess the degree of local spread of HRP at the injection site at short survival times. A total of ten animals were thus sacrificed at 0.25,

2, 3, 6, 14 and 24 hours after HRP injection and the degree of spread of HRP around the pipette tip assessed in non counterstained sections.

Neurons labelled by retrograde transport in the main series of experiments were counted and categorised into cytoarchitectonic groups. Examples of sections bearing labelled neurons were then plotted for visual inspection at representative levels of the neuraxis.

RESULTS

Preliminary experiments

Different current parameters were applied to a 40% HRP solution to determine the minimum strengths and durations necessary to elicit measureable retrograde transport from VTA to basal forebrain areas. Since reproducible retrograde transport in this pathway was not obtained below 1,000 nA for any length of time of application, this was the current selected for routine use. Even when the total current × duration product was the same for very low current strengths (e.g., 100 nA for 100 minutes), as that used for higher currents (e.g., 1,000 nA for 10 minutes), and presumably the same total quantity of HRP ejected from the needle tip, adequate retrograde transport with low currents was not obtained. In such cases labelling was confined to local glial cells, vascular endothelium and some nerve fibre uptake. Thus demonstrable retrograde transport may only occur when HRP is ejected faster into the extracellular space than the capacity of local uptake processes in vascular endothelium, glial cells and axons to remove the HRP, and this rate is only achieved at current strenghts greater than 1 μA. Naturally, such low current strengths resulted in only low numbers of labelled neurons. This disadvantage, however, was balanced by a higher accuracy in interpretation, resulting from minimum tissue damage.

The spread of HRP into the surrounding tissues at a delivery current of 1 μA for seven minutes was then assessed after injection of the VTA. The main finding of these experiments was that there was rapid expansion and contraction of the injection spot soon after current application. The spread at short intervals after injection was much greater than that suspected from examining tissue fixed 24 hours after operation.

Exact determination of the diameters of spread was complicated because (1) the reaction product was rather granular and the margin not well defined; and (2) the staining was "squeezed" between the local tissue land marks e.g., the medial lemniscus, interpeduncular nucleus and ventral border of the brain. Nevertheless, the approximate diameter at 24 hours was about one-third of that seen at four hours; the point of maximal spread seen. This rapidly reduced by about six hours to a diameter only slightly greater than that measured at 24 hours. Neurons within the margin of the expanded HRP stain showed an even faint blue staining. Such neurones were observed in red nucleus, medial substantia nigra and a very few neurones in the lateral rim of the interpeduncular nucleus, at one-fourth and four hours after a VTA injection. Dark granular reaction product was seen within neurons in the same sites (but not the interpeduncular nucleus) at the 14 and 24 hours survival times, although in very much smaller numbers. At the one-fourth hour survival time, marked light evenly blue axonal labelling was seen in the fibres of the oculomotor nerve which ran through the injection site, while uptake to a lesser extent was also observed in medial lemniscus, cerebral peduncle, mammillary peduncle, ventral tegmental decussations and reticular formation around the needle track. It was noticed that although the oculomotor fibres were heavily labelled in these experiments, only occasionally did labelled neurons appear in the oculomotor nuclei. This indicated that although retrograde transport occurred from fibres of passage labelled by this procedure, it was uncommon relative to the number of fibres taking up the enzyme. This conclusion, however, could only be applied to undamaged axons of passage; later experiments showed clear evidence of uptake and retrograde transport from damaged fibres of the medial lemniscus. At 24 hours after injection, glial and vascular endothelial cells also accumulated HRP and this occurred mainly in the territory which had been occupied by the expanded HRP stain, at early survival times.

Injection sites

Figure 1 shows an example of the appearance of the injection site after 25 hours survival in case 15. The reaction product is largely confined to the VTA and its spread has been limited laterally by the medial lemniscus and also apparently ventro-medially since it did not extend (except at its lowest margins) into the interpeduncular nucleus. Outside the margin of the main HRP injection, accumulation of reaction product can be seen in

perivascular and glial cells and in the pia on the ventral surface of the brain.

Figure 2 shows diagrammatically the injection sites of all cases in which successful injections were made into dopaminergic cell groups (cases 16, 10, 11, 32, 15, 21, 12, 31, 29) and the control injection sites (cases 25, 17, 26, 22, 28, 14 — sham injection control is not shown).

Control injections. (Tables 1 and 2, right hand block)

(1) Injections into medial lemniscus

In case 25, a fairly large injection was made into medial substantia nigra compacta and lateral VTA. The needle track, however, instead of avoiding medial lemniscus as in other cases, was deliberately aimed through the medial edge of the lemniscus, (fig. 2). In this case, unlike injections to VTA alone, very large numbers of labelled neurons appeared in the principle nucleus of V. (264 neurones, compared to a total of 16 for *all* cases of HRP injection to VTA alone). In case 17, where a rather small HRP deposit was made directly into the medial edge of medial lemniscus, again a large number of labelled neurones appeared in the principal nucleus of V (101 neuro). Since dopamine neurons of the VTA extend into the medial edge of the lemniscus, concomitant labelling of other VTA afferents was to be expected (table 1).

(2) Injections to the red nucleus. (Case 26)

Large numbers of neurons appeared in dorsolateral cerebral cortex, but none in suprarhinal, cingulate or prefrontal cortex. Heavy labelling of zona incerta (several hundreds of neurons) and some labelling of the entopeduncular nucleus were observed. Many neurons were also labelled in the oculomotor nuclei, superior colliculus and pontine reticular formation, while many neurons were also seen in the principal nucleus of V. The dentate nucleus was heavily labelled (116 neurons). In case 11, where much of the HRP injection spread also to red nucleus, 67 neurones were observed in the dentate nucleus. In other cases where VTA alone was injected, only a few labelled neurons were observed in the dentate nucleus. A considerable number of labelled neurons appeared in the vestibular nuclei after injection of the red nucleus.

(3) Mesencephalic reticular formation. (Case 22)

The injection was centered on the reticular formation dorsal to medial lemniscus and lateral to the red nucleus. Labelled cells were

Abbreviations

aac, anterior commissure, anterior limb
ac, anterior commissure
ABL, basal amygdaloid nucleus, pars lateralis
AC, central amygdaloid nucleus
ALA, lateral amygdaloid nucleus, pars anterior
AM, medial amygdaloid nucleus
AVT, ventral tegmental area of Tsai
BST, bed nucleus of stria terminalis
cc, crus cerebri
DBB, diagonal band of Broca
EP, entopeduncular nucleus
f, fornix
fr, fasciculus retroflexus
GP, globus pallidus
IP, interpeduncular nucleus
LC, nucleus linearis raphe pars caudalis
LH, lateral hypothalamus
LHa, lateral habenula
LPO, lateral preoptic area
ml, medial lemniscus
MaPO, magnocellular preoptic area
MPO, medial preoptic area
MR, median raphe nucleus
mt, mammillo-thalamic tract
NA, nucleus accumbens
ntV, nucleus spinal tract of trigeminal nerve
och, optic chiasm
ON, olivary nuclei
ot, optic tract
OT, olfactory tubercle
OTpo, olfactory tubercle, polymorphic layer
OTpy, olfactory tubercle, pyramidal layer
p, corticospinal tract
poma, mammillary peduncle
pcs, superior cerebellar peduncle
PN, paraventricular nucleus of hypothalamus
R, red nucleus
SI, substantia innominata
SNC, substantia nigra pars compacta
SO, supraoptic nucleus
sm, stria medullaris
ZI, Zona incerta
III, third cranial nerve
VII, seventh cranial nerve

Fig. 1 Microphotographs (a) and (b) illustrate the centre of the injection site in the same section in case 15. (a) shows the full extent of spread of HRP reaction product (compare to diagram A1610, fig. 2). (b) shows the same section at a higher magnification and a different exposure to illustrate the extent of the densest central HRP deposit. The dotted line indicates the dorsal border of interpeduncular nucleus. Note how the HRP does not spread ventrally to the interpeduncular nucleus and is limited laterally in its spread by the medial lemniscus. Note also how perivascular cells outside the margin of the injection site accumulate HRP. In this case an ejection current of 1 μA was applied for seven minutes and the animal sacrificed at 25 hours after injection. Bar, 200 μm.

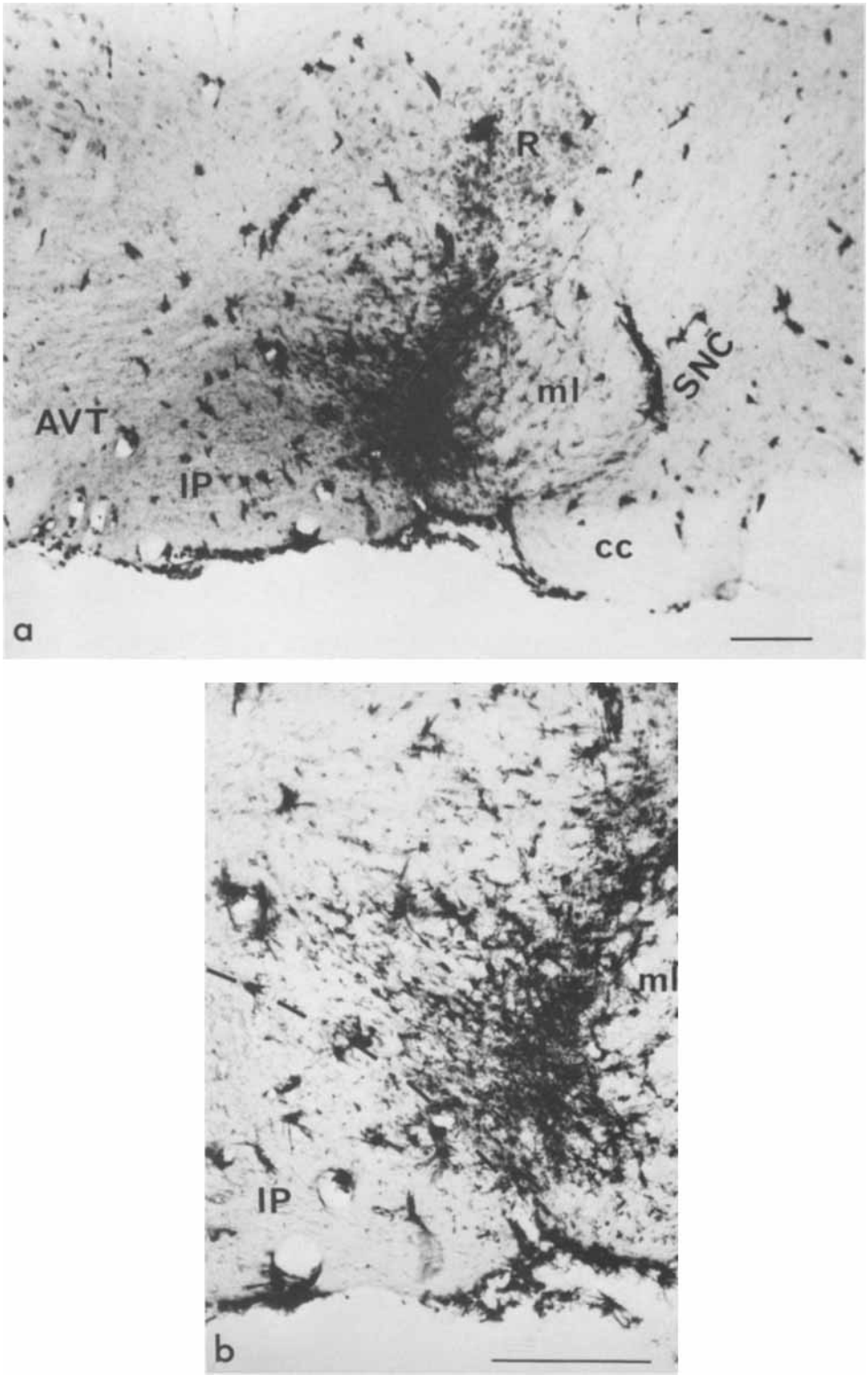

Figure 1

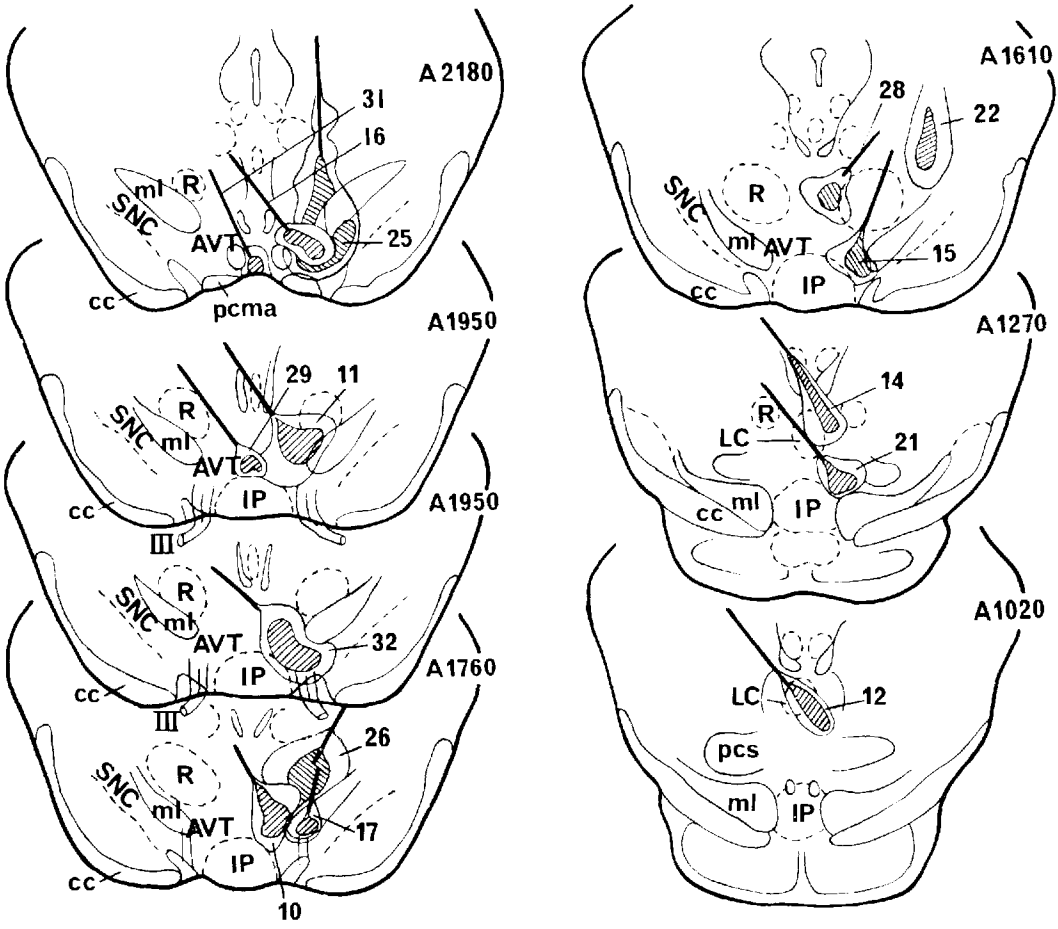

Fig. 2 Drawings of the central part of the injection site in different regions of the mesencephalon. The thick line represents pipette track. The area of densest extracellular HRP deposit is indicated by the hatched area and a more lightly stained zone by the clear surround. The present results suggest that only tissue very close to the point of ejection is taken up and transported in sufficient amounts to be detected by the present method (see technical discussion). No indication, thus, is given of the antero-posterior extent of the HRP deposit. Coordinates are according to König and Klippel ('63).

concentrated in the superior colliculus and substantia nigra pars reticulata, while only scattered labelling was seen elsewhere.

(4) Deep tegmental decussations. (Case 28)

Here the injection site centered on an area dorsal and medial to the red nucleus and dorsal to the main body of dopaminergic neurons of the VTA. Both dentate nucleus and red nucleus were heavily labelled in this case, while the parabrachial nuclei were also labelled, but to much lesser extent.

(5) Sham injection

In one case where the HRP containing pipette was lowered into the VTA but no HRP ejected by current, no labelled neurons could be observed in any part of the brain. This excludes the possibility in the brain that endogenous peroxidase activity, as recently shown for monkey brain, could account for labelled neurons (Wong-Riley, '76).

Sites of afferents detected after injections confined to VTA

(1) Afferents from cortical regions

Taking all cases of VTA injection together, labelled neurons appeared most frequently in the prefrontal cortex (deep layers) and to a lesser extent in the dorsal bank of rhinal

TABLE 1

Distribution of reactive neurons in forebrain and rostral brain stem

Area	Injections to dopaminergic cell groups									Control injections					
	16	10	11	32	15	21	12	31	29	25	17	26	22	28	14
I. *Telencephalon*															
Pre-frontal cortex	+ +	0	+	0	+	+	+	0	0	+	0	0	0	0	+
Suprarhinal cortex	+	0	+	0	0	0	+	0	0	0	+	0	+	0	0
Cingulate cortex	0	0	+	0	0	0	0	0	0	+	0	0	0	0	0
Parietal cortex	0	0	+	0	0	0	0	0	0	0	0	+ + +	0	0	0
Olfactory tubercle	+	0	+ +	+	0	0	0	0	0	0	+	0	0	0	0
Nucleus accumbens	+ + +	0	+	0	0	0	+	+	0	0	0	0	0	0	0
Bed nucleus stria terminalis	+ + +	0	+	0	+	+	+	0	0	+	+	+	0	+	+
Amygdala	+	0	+	0	0	0	0	0	0	+	+	0	0	0	0
Diagonal band Broca	+ + +	+	+	0	+	+	+	+	+	0	+	0	0	+	+
Substantia Innominata	+ + +	+	+ +	0	+	0	+	0	+	+	0	0	0	+	0
Lateral preoptic area	+ + +	+	+	+	+	+	+	0	0	+	+	0	0	0	0
Medial preoptic area	+	0	0	0	+	0	+	0	0	+	0	0	0	0	0
Magnocellular preoptic area	+ +	0	+ +	0	+	0	0	+	0	+	+ +	0	0	+	0
II. *Diencephalon*															
Anterior hypothalamus	+	0	+	0	0	0	+	0	0	0	0	0	0	0	0
Lateral hypothalamus	+ + +	+	+ +	+	+	+	+ +	+	+	+ +	+ +	+	+	+	+
Posterior/dorsal hypothalamus	+	0	+	0	0	+	0	0	+	+	+	0	+	0	+
Parafascicular nucleus	+	0	+	+	+	+	+	0	0	+	+	0	0	0	0
Zona incerta	+	+	+	+	0	0	+	+	0	+ + +	+	+ + +	+	0	0
H_1 and H_2 Fields of Forel	+	0	+	+	0	+	0	+	0	+ + +	+	0	+	0	0
Habenula — lateral nucleus	+	+ +	+	+	+	+ + +	+	+	0	+	+	0	0	0	+
Habenula — medial nucleus	0	0	0	0	0	0	0	+ + +	+ + +	0	0	0	0	0	0

On left: Injections confined mainly to ventral tegmental area (16, 10, 11, 32, 15, 21), nucleus linearis raphe caudalis (12), interfascicular nucleus (31, 29).

On right: Control injections confined mainly to medial lemniscus, medial substantia nigra and red nucleus (25), medial lemniscus (17), red nucleus (26), tegmental decussations (22), mesencephalic reticular formation (28), and cerebellar decussations (14).

In every case the actual total number of neurons in each region was counted and the results summarised according to the following code: numbers of neurons labelled − + +, more than 20; + +, 10 to 20; +, 1 to 10; 0, nil.

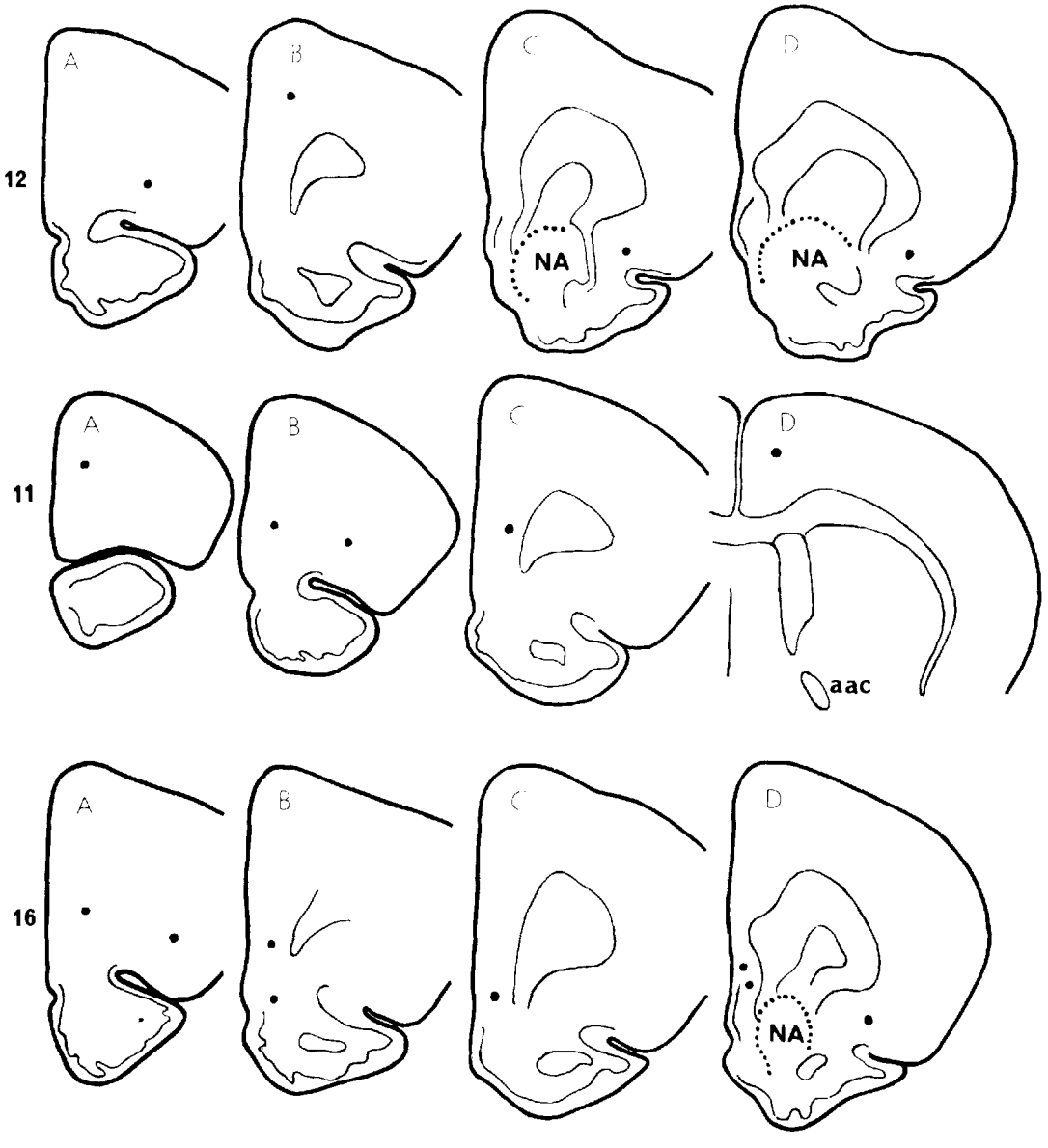

Fig. 3 Positions of labelled neurones in the frontal and cingulate cortex in three cases. Each dot represents the position of a single neuron in a representative section. Number in the left hand margin indicates the experimental animal.

sulcus. Prefrontal cortex described here corresponds to two cytoarchitecturally distinct regions (1) the infralimbic area of Rose and Woolsey ('48) (equivalent to area 25 of Brodmann, '09) and (2) the prelimbic area of Krettek and Price ('77) (equivalent to area 32 of Brodmann). Dorsal bank of rhinal sulcus contained labelled neurons in the ventral agranular insular area and less commonly in ventrolateral orbital area (Kerttek and Price, '77). Their distribution corresponded closely with the sites of dopaminergic terminal innervation in those regions (Lindvall et al., '78). Occasionally, labelled cells also appeared in the dorsal division of the anterior cingulate area (part of area 24 of Brodmann), (table 1,

fig. 3). Neurons only appeared in dorsolateral parts of cortex when the injection site encroached on the red nucleus, while injections confined entirely to the red nucleus resulted in a label only in dorsolateral cortex and none in pre-frontal cortex or dorsal bank of rhinal sulcus (case 11 and 26, table 1). No cortical neurons were labelled after injections of the interfascicular group in the mid-line.

(2) Afferents from basal forebrain regions. (Fig. 4)

The terminology used in the description of this section is based on that of Swanson ('76). Labelled neurons were found in the medial segment of nucleus accumbens anteriorly (fig. 8), the central segment of accumbens merging with the subcommissural portion of the bed nucleus of stria terminalis more posteriorly, and the posterior segment of the bed nucleus caudal to the anterior commissure. Neurons of the accumbens and rostral bed nucleus were small and round and in the caudal bed nucleus medium sized and stellate or spindle shaped. Although neurons in the bed nucleus were labelled after injections of red nucleus (table 1), no excess labelling was observed over that seen in many cases of VTA injections, and labelling was less than that seen in some VTA injections. Many small to medium sized neurons were found to be labelled in lateral preoptic area (fig. 4), and in smaller numbers also in the medial preoptic area (fig. 9). Labelled neurons were observed anteriorly in the vertical limb of the diagonal band, and further posteriorly in larger numbers in the horizontal limb where they merged with the large and medium sized cells of the substantia innominata. Labelled neurons were observed in substantia innominata particularly at the level of the anterior commissure, and in the polymorphic layer of olfactory tubercle. These smaller cells of olfactory tubercle were much less numerous than the large cells of the overlying substantia innominata diagonal band and magnocellular preoptic area. The most dorsal component of substantia innominata identified by Heimer and Wilson ('75) as "ventral pallidum" also contained some labelled neurons (fig. 9). More posteriorly and laterally the cells of the substantia innominata appear to aggregate into the magnocellular preoptic area (Swanson, '76). This nucleus has also been called the nucleus of the horizontal limb of the diagonal band (Price and Powell, '70) and may correspond to nucleus basalis of primates (Nauta and Haymaker, '69). This nucleus also contained prominently labelled large neurons in most cases (fig. 9). When taken together, labelled neurons of both limbs of diagonal band, substantia innominata and magnocellular preoptic area, make up a prominent component of the afferent systems to VTA as a whole. Neurons labelled in these structures were usually large but medium sized neurons also occurred. In primates (Jones et al., '76) such neurons may comprise a single complex stretching from vertical limb of diagonal band anteriorly to nucleus basalis posteriorly, and in rats a similar chain of "magnocellular nuclei of the basal forebrain" has been described (Divac, '75).

(3) Afferents from hypothalamic areas. (Fig. 5)

The lateral hypothalamus was labelled in all cases, and appeared to provide one of the principal sites of afferents to the VTA. The labelled neurons were usually spindle shaped and oriented in a dorsoventral axis slanted medially. Neurons were surrounded by many labelled axons travelling towards forebrain sites in the medial forebrain bundle. In the minority of cases, neurons were labelled in the anterior hypothalamus and posterior and dorsal regions of the hypothalamus ventral and medial to the mammillo-thalamic bundle (fig. 5, case 16). More posteriorly labelled cells were observed amongst the assembling fibres of the mammillothalamic tract. Neurons were labelled in the mammillary body itself only when the injection site encroached upon the mammillo-tegmental fibres, which runs immediately ventral to the VTA.

(4) Afferents from habenular nuclei. (Fig. 5)

An important source of fibres to VTA was from the lateral habenula. This nucleus was labelled in every case. Neurons were only labelled in the medial habenula following injections confined to the mid-line interfascicular group (table 1, cases 31, 29). In no case of injection of VTA alone was label observed in the medial habenula indicating that although demonstrable HRP activity was almost certainly present in the interpeduncular nucleus shortly after injection *(Preliminary experiments),* that this was insufficient to elicit detectable retrograde transport.

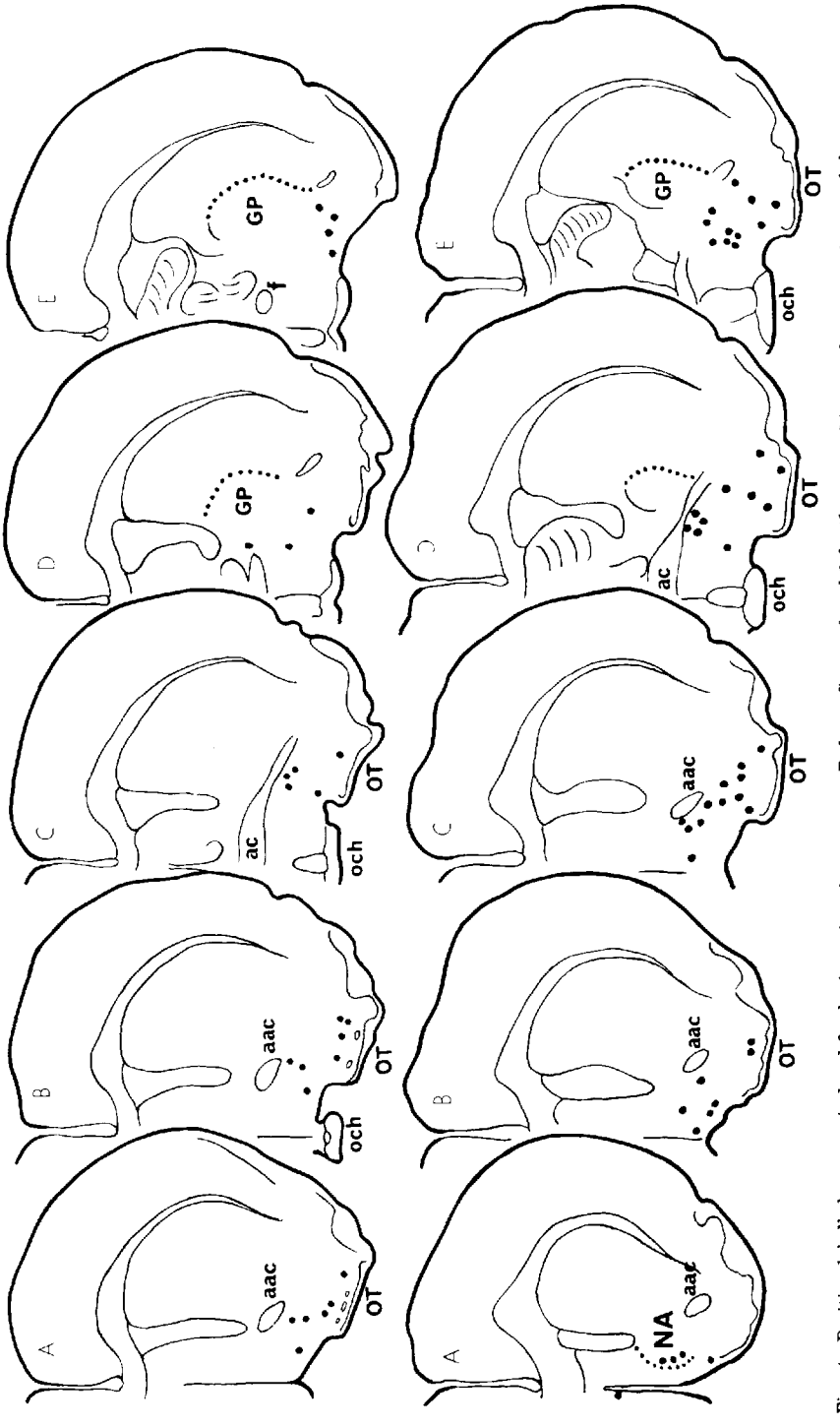

Fig. 4 Position labelled neurons in basal forebrain regions in two cases. Refer to figures 8 and 9 for the cytoarchitectural position of some of these neurons.

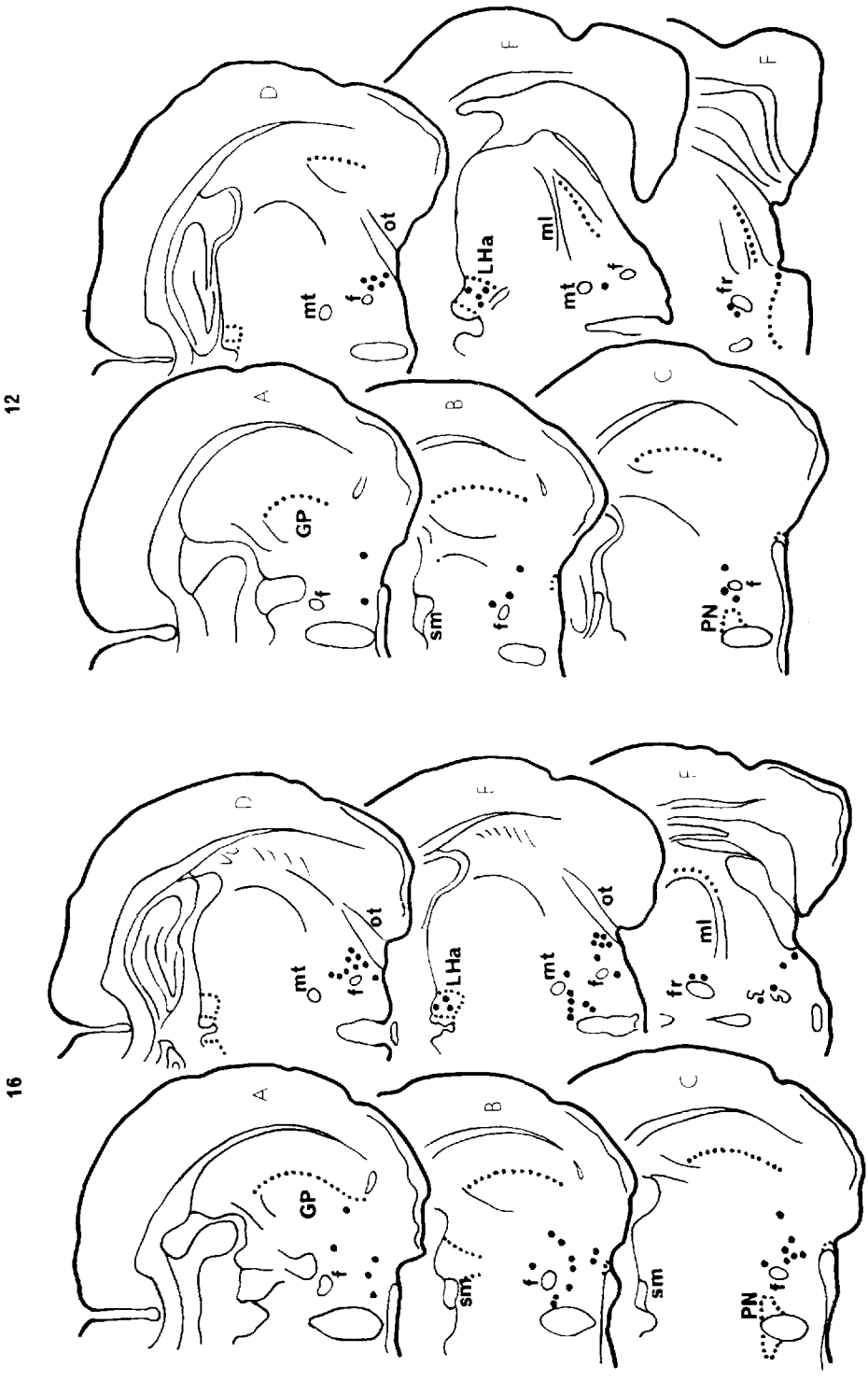

Fig. 5 Position of labelled neurons of diencephalon and epithalamus in two cases.

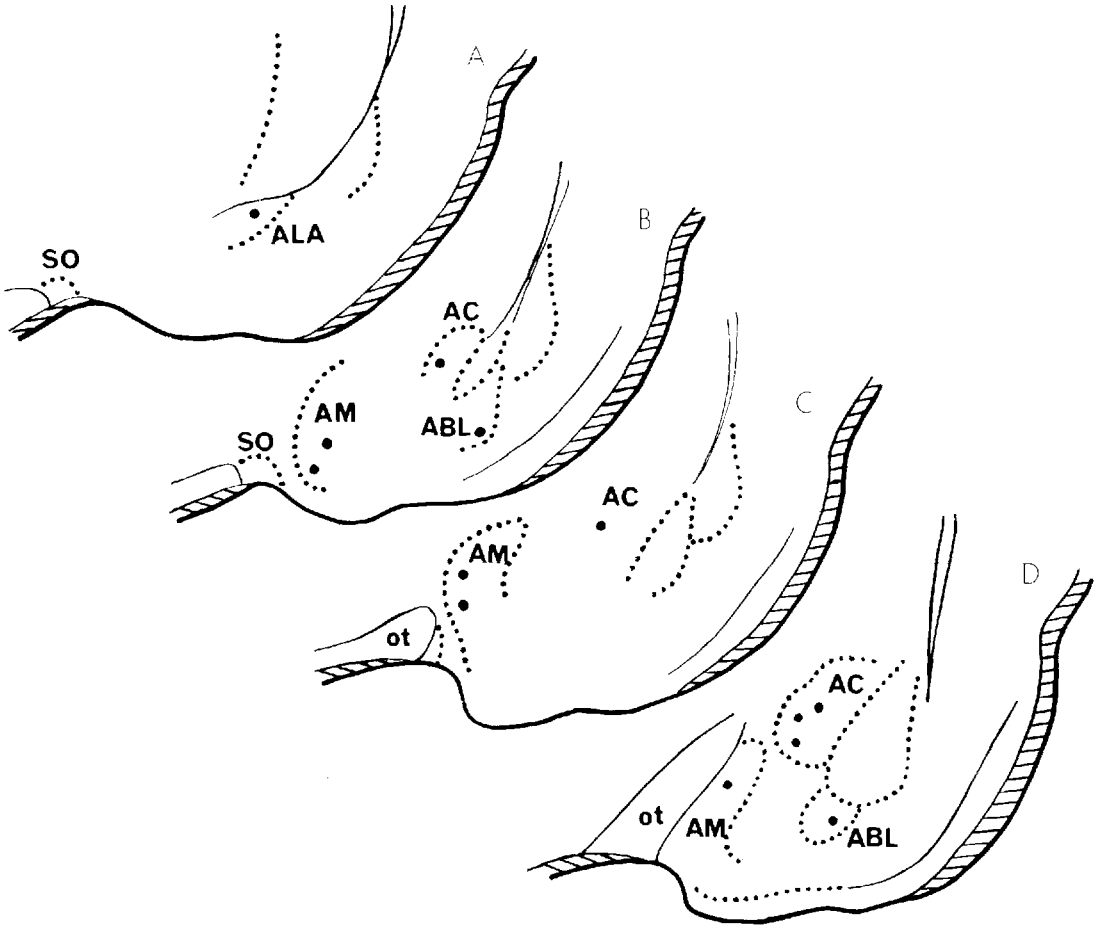

Fig. 6 Cytoarchitectonic position of labelled neurons in the amygdala.

(5) Afferents from parafascicular nucleus

A few labelled neurones were observed in this area in every case of VTA injection except one. Earlier experiments not reported here showed that after the ejection of large quantities of HRP to the VTA, this nucleus was fairly heavily labelled.

(6) Afferents from amygdala. (Fig. 6)

Labelled neurones were found in the amygdala in a minority of cases. In the case resulting in most labelled neurones, the injection site was in the anterior portion of VTA. Neurons were observed in anterolateral, basolateral, medial and central divisions. The number of labelled neurons was small.

(7) Afferents from zona incerta and Fields of Forel

Most cases contained labelled neurones in zona incerta and both regions of Forel's fields. Since however there was a marked increase in labelled neurones in both regions when the needle tract passed through medial lemniscus or the injeciton was in the lemniscus itself these results must be treated with caution (table 1). Injections of red nucleus, also led to large number of neurons appearing in zona incerta. Thus it seems likely that after VTA injections, the labelled neurons in these regions resulted from labelling of damaged fibres of passage in the medial lemniscus or of labelling of synaptic terminals in the red nucleus.

(8) Afferents from midbrain regions

The superior colliculus was labelled in all cases of VTA injections. The neurons labelled always appeared in the deeper layers (album profundum, album mediale griseum profun-

TABLE 2

Distribution of reactive neurons in mid- and hind-brain

Area	Injections to dopaminergic cell groups									Control injections					
	16	10	11	32	15	21	12	31	29	25	17	26	22	28	14
I. *Mesencephalon*															
Superior colliculus	++	++	++	+	+	+	+	0	+	++	+	+++	++	+	+
Central grey	+	0	+	0	+	0	+	+	+	+++	+	0	+	0	+
Oculomotor complex	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+
Red nucleus	0	+	++	+	+	0	0	+	++	0	+	+	+	+++	+++
Substantia nigra	++	+	++	0	+	0	+	0	0	++	0	0	++ [1]	0	0
II. *Pons medulla*															
Nucleus linearis rostralis	+	+	0	0	0	+	0	+	0	0	0	0	0	0	+
Nucleus raphe dorsalis	+++	++	+++	+	++	+	+++	+	+	++	++	0	0	++	+
Nucleus raphe medianus	+	0	+++	0	0	+	+	+	+++	0	0	0	0	0	0
Nucleus raphe magnus	+	+	+	+	0	0	0	0	0	+	+	++	0	0	0
Nucleus raphe pontis	+	+	+	+	0	0	0	0	0	+	0	+	0	0	0
Principal nucleus nV	0	0	+	++	0	0	0	0	0	+++	+++	+++	0	0	0
Nucleus cuneatus	++	++	+	0	+	0	+	0	0	+	0	+	0	0	0
Nucleus parabrachialis	++	+	+++	+	+	0	++	0	0	+++	++	+	+	+++	0
Nucleus reticularis pontis	+	0	+	+	0	0	+	0	0	++	+	+++	+	+	0
Locus coeruleus	+	+	+	+	0	0	0	0	0	+	+	+	0	+	0
Vestibular nuclei	0	0	+	0	0	0	0	0	0	+	0	+++	+	0	0
Nucleus reticularis lateralis	+	0	+	+	0	0	0	0	0	+	0	0	0	0	0
III. *Cerebellum*															
Dentate nucleus	+	++	+++	+	+	+	+	0	0	+++	+	+++	0	+++	0

See footnote to table 1 for abbreviations.

[1] Pars reticulata.

Fig. 7 Position of labelled neurons in mid- and hind-brain in two cases.

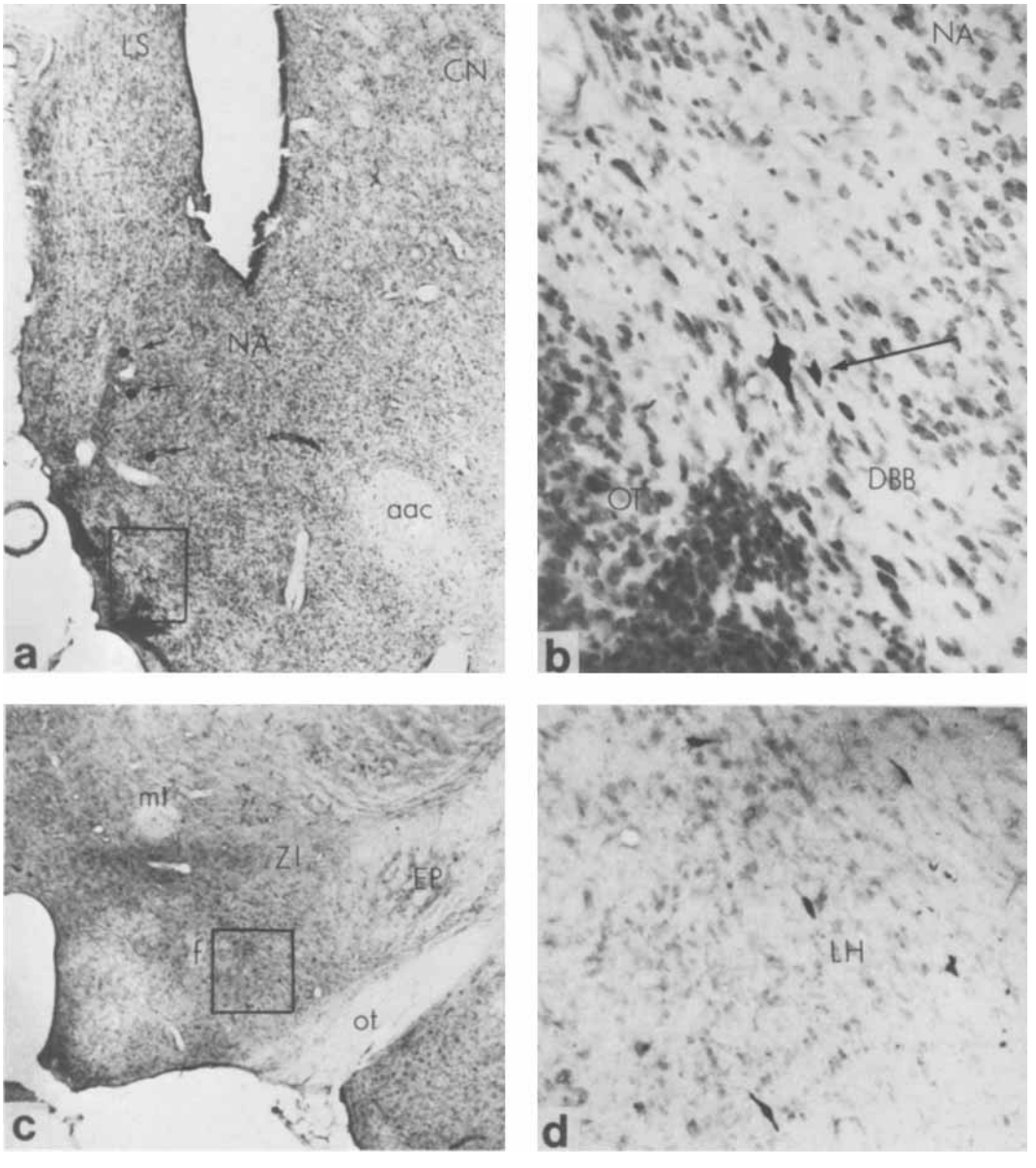

Fig. 8 Cytoarchitecture of regions containing labelled neurons in case 16. a. Anterior septal reigon and nucleus accumbens. Arrows indicate position of labelled neurons in the accumbens; b. magnification of area in a (box) to show large and small (arrow) labelled neuron ventral to nucleus accumbens and dorsal to the olfactory tubercle; c. lateral hypothalamic area; d. magnification of box in c.

dum, according to König and Klippel, '63). Injection of the red nucleus and other control areas also gave rise to considerable labelling of the superior colliculus (table 2). Since also the needle track inevitably traversed many tectotegmental fibres the specificity of projection to VTA is open to question. The few labelled neurons appearing in the oculomotor nuclei were probably due to HRP entering damaged or undamaged oculomotor rootlets as they course through the ventral tegmental region, (see discussion in technical section).

Similar caution relates to neurons labelled in the red nucleus. In cases of injection to the anterior VTA, heavily labelled neurons were observed in substantia nigra compacta. Since efferent axons of compacta neurons assemble in the medial edge of substantia nigra compacta (Lindvall and Björklund, '74) some of this label could represent uptake and retrograde transport of HRP in damaged axons.

(9) Afferents from pons and medulla

Neurons in the dorsal raphe nucleus were prominently labelled in all cases. Of all the nuclei throughout the brain, this nucleus was the most heavily labelled. More caudally, labelled neurons occasionally appeared in nucleus raphe magnus and pontis. Large injections of the VTA which involved the midline interfascicular catecholamine group gave rise to labelled neurons in the median raphe nucleus particularly its dorsal segment (fig. 7, case 11). Smaller laterally placed VTA injections did not label this nucleus (fig. 7, case 16), whereas injections confined to the interfascicular nucleus gave rise to labelling of many median raphe neurons (table 2, cases 31, 29).

In more lateral positions neurons were labelled in caudal portions of nucleus cuneatus amongst fibres of the cerebellar peduncle in an area containing the A 8 catecholamine group (Palkovits and Jacobowitz, '74). Further posteriorly, many neurons were also labelled in both dorsal and ventral divisions of the nucleus parabrachialis. In most cases the ventral division contained more labelled neurons than the dorsal. Many of these ventrally located parabrachial neurons were located within an area containing the A 7 catecholamine group (nucleus subcoeruleus). As the ventral parabrachial group was followed posteriorly and medially, labelled neurones appeared in positions immediately ventral to the locus coeruleus and also, though only in small numbers, in the locus coeruleus itself (A 6) in the periaqueductal grey.

The reticular nuclei of the pons contained occasionally labelled neurones, but much larger numbers were observed after red nucleus injections (table 2). In some cases labelled neurons were also observed around the roots of the VII nerve towards the ventral surface of the brain in the region where neurons of the A 5 catecholamine group have been described (Dahlström and Fuxe, '64; Palkovits and Jacobowitz, '74). Further caudal, occasionally labelled neurons were seen in or near the lateral segment of nucleus reticularis lateralis. Their position was equivalent to the region containing the A 1 catecholamine group (Dahlström and Fuxe, '64; Palkovits and Jacobowitz, '74). Furthermore their cell size and dendritic morphology were distinct from nearby neurons which were occasionally labelled in the principal nucleus of fifth nerve laterally and dorsally. This nucleus was heavily labelled after injections of red nucleus or medial lemniscus (table 2) indicating that light labelling after VTA injections was probably not significant. *(Control injections).*

(10) Other afferents

Neurons in the deep cerebellar nuclei were frequently labelled by injections confined to the VTA. Heavy labelling of the dentate nucleus was obtained after injections of red nucleus and light labelling obtained after injections confined to the anterior VTA (table 2, case 16). Most prominent dentate labelling in VTA injections was obtained when the injection area overlapped the borders of the red nucleus e.g., case 10. In every case of VTA injection the pipette track inevitably traversed the dense cerebellar-rubral projections either at the tegmental decussations or the cerebellar peduncles more posteriorly. Vestibular neurons were occasionally labelled but much less frequently than control injections to red nucleus or medial lemniscus.

(11) Contralateral projections

In case 12 where HRP was deposited in the most caudal and midline dopamine group, the nucleus linearis raphe caudalis, afferents were distributed about equally to both sides of the brain. In cases 29 and 31, where HRP was deposited in the midline interfascicular nucleus, again the main afferent source, from the medial habenular nucleus, was represented bilaterally.

In all other cases of unilateral VTA injections, only occasionally labelled neurones were observed contralateral to the injection site, with the exception of the deep cerebellar nuclei and oculomotor nuclei which were

Fig. 9 a. Cytoarchitecture of basal forebrain at level of anterior commissure. Dots represent position of neurons labelled and their relative density in this and other similar sections. b. Illustration of a large labelled neuron in horizontal limb of diagonal band and smaller neurons in lateral preoptic area; c. Large labelled neuron in magnocellular preoptic area close to polymorphic layer of the olfactory tubercle.

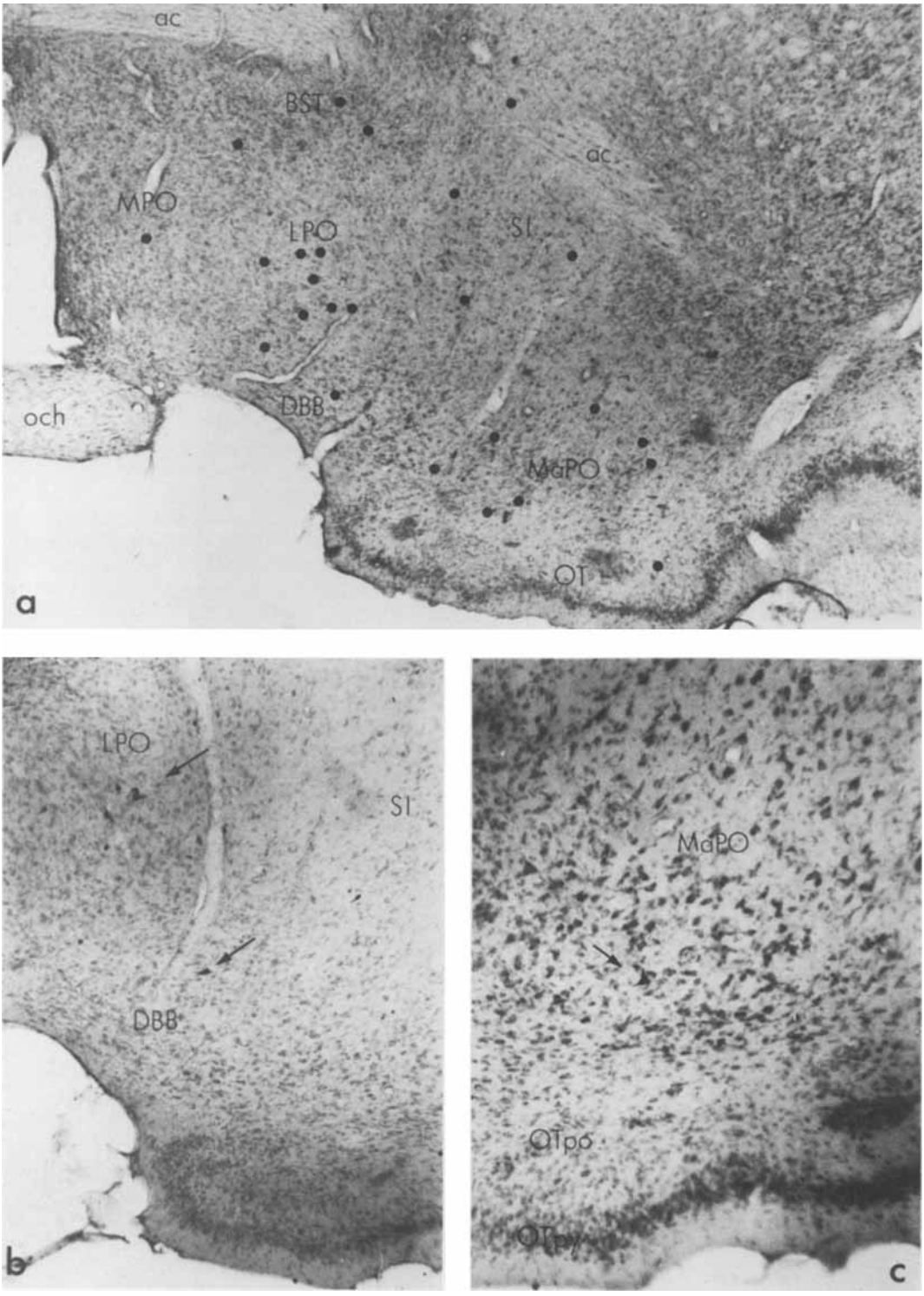
ac
BST
ac
MPO
LPO
SI
och
DBB
MaPO
OT
a
LPO
SI
DBB
b
MaPO
OTpo
OTpl
c

Figure 9

usually labelled contralateral to the injection, and superior colliculus which was frequently labelled contralateral to the side of injection.

DISCUSSION

(1) *Technical factors*

It has recently been clearly demonstrated that the technique of microiontophoresis of HRP in the central nervous system will not only label neuron cell bodies as a result of uptake and retrograde transport of enzyme from axon terminals, but will also label neurons whose axons pass through the site of injection and are damaged by the injection procedure (Herkenham and Nauta, '77b). Thus although there are advantages in using the microiontophoretic technique, in that extremely small deposits of HRP can be made, there is still a significant risk of labelling neurons by "injecting" damaged fibres of passage, as had been earlier noticed in the case of peripheral nerves whose axons had been deliberately transected or damaged (Kristensson and Olsson, '74; Halperin and La Vail, '75; Furstman et al., '75; Kuypers and Maisky, '75; Kristensson and Olsson, '76; Oldfield and McLachlan, '77). It is possible that the type of reaction product seen within the neuron can give an idea of whether or not the cells are labelled from injured axons; evenly filled nongranular reaction product indicating filling of a damaged fibre and granular reaction product indicating axon terminal uptake (Jones and Leavitt, '74; Turner and Harris, '74; Halperin and La Vail, '75; Colman et al., '76; Parent, '76). Unfortunately, however, neurons labelled from cut or damaged nerves do not always show even staining (Furstman et al., '75); while with the highly sensitive TMB method used in this study the increased amount of reaction product in neurons well labelled by retrograde transport may give a rather solidly stained profile (Phillipson, '78). The appearances of the reaction product is thus of no value in distinguishing those neurons labelled from damaged fibres of passage.

A related technical problem is the degree to which surrounding undamaged fibres will take up HRP ejected from the pipette and subsequently transport it to the neuronal soma. The present results from VTA injections show that although fibres of passage in the oculomotor nerve are prominently labelled soon after injection, there is only a very small degree of resultant labelling of neuronal cell bodies at 24 hours survival. Similarly, after injections of the VTA which avoid direct damage to the medial lemniscus, few or no labelled neurons were seen after 24 hours survival in the principal nucleus of the fifth nerve, whose efferent fibres are well known to travel in the medial edge of the medial lemniscus (Walker, '39). However, after direct damage to the lemniscus, large numbers of labelled neurons appeared in this nucleus. This effect is very similar to the result obtained in the peripheral nervous system by Oldfield and McLachlan ('77) using the diamino-benzidine substrate. Thus axons will take up HRP even if not damaged. However, retrograde transport will not occur, except to a minor extent, unless axons are cut or damaged directly.

In summary, differentiation between neurons labelled by terminal uptake and those from damaged axons of passage is not possible with the present technique alone. Only ultrastructural or physiological evidence can definitely confirm the presence of a pathway. Since the VTA is an area densely traversed by many fibre tracts of different origin, this factor must strongly influence the interpretation of the present results.

Finally, it is not certain to what extent HRP which spreads from the pipette tip to synaptic regions outside VTA will be taken up and transported to cell bodies. This factor might be of importance because the expanded HRP injection spot observed at short intervals after injection invaded many neighbouring structures, even at such low ejection currents as 1 μA. Our preliminary experiments showed early staining of red nucleus, medial substantia nigra, and slightly, of lateral interpeduncular nucleus after test VTA injections. However, in animals killed 24 hours later no labelled neurons were seen in regions projecting to those nuclei e.g., dorsolateral cerebral cortex; caudate nucleus or medial habenula respectively. Neurons labelled in the deep cerebellar nuclei (a site of afferents to red nucleus) are discussed below (DISCUSSION: *Control injections*). Thus synaptic sites invaded by the periphery of the HRP spread, did not result in detectable retrograde labelling. The area of effective uptake appears to be restricted to tissues very close to the pipette tip. This result agrees with that obtained with the diaminobenzidine substrate by Vanegas et al. ('78) who found similar rapid expansion and contraction of the HRP spot following hydraulic

injections to the visual cortex and also that uptake and transport occurred only from sites close to the needle tip.

Neurons may also become labelled by a direct route by means of vesicular uptake of extracellular HRP by dendrites or neuronal perikarya (Turner and Harris, '74). This may account for occasionally labelled neurons seen in red nucleus, substantia nigra and lateral margin of interpeduncular nucleus in the region of early expansion on the HRP spot. Labelled neurons observed close to the injection site should thus be treated with caution although real short projections cannot be discounted by these methods.

(2) *Control injections*

It is clear from the results obtained in cases 25 and 17, that the principal nucleus of V did not provide a projection system to the VTA, and that any labelled neurons seen in this nucleus were due to labelling of fibres travelling to the thalamus in the medial edge of the medial lemniscus. These results are in agreement with the arrangement of projection pathways of the V nerve nucleus found by Walker ('39) in the monkey. A similar conclusion can be made about neurons labelled in the vestibular nuclei, fields of Forel, zona incerta, oculomotor complex, and pontine reticular formation. The superior colliculus is discussed below. In the case of the red nucleus, it would seem simplest to assume that labelled neurons appearing in the deep cerebellar nuclei resulted from labelling of damaged fibres of passage in the cerebello-rubral pathway running in the tegmental decussations, or labelling due to spread of HRP into the synaptic terminal area of this pathway in the red nucleus itself. However, recent investigation of the cerebellar-mesencephalic projections with the Fink-Heimer technique has suggested the presence of a pathway from deep cerebellar nuclei to ventral mesencephalic dopaminergic neurons (Snider et al., '76). The present VTA HRP injections did not label the well documented cortico-rubral tracts, and were mostly made anterior to the cerebello-rubral fibres crossing in the main in the brachium conjunctivum. Therefore, labelling of deep cerebellar nuclei may indicate, in agreement with Snider et al., a cerebello-tegmental pathway innervating VTA neurons. Clearly further details of such a pathway at electron microscopic levels, or physiological investigations, are necessary.

(3) *Afferents from cortical regions*

The present results showing labelling of prefrontal cortex after HRP injection to the VTA are compatible with earlier degeneration studies in which lesions of large areas of frontal cortex in the monkey gave rise to degeneration in or near the VTA (De Vito and Smith, '64; Nauta, '64). More restricted lesions confined to medial frontal cortex in the monkey, however, gave rise to preterminal degeneration only in substantia nigra, (Leichnetz and Astruc, '76). Comparisons of results obtained from rat and monkey, however, are hazardous because of the difficulty of accurately defining homologous cortical regions. Moreover, negative results obtained with silver techniques used by these authors does not exclude the presence of a pathway.

Efferent dopaminergic fibres from VTA innervate exactly those deep cortical regions shown here to contain HRP labelled neurons (Lindvall et al., '78). Thus it seems probable that in the rat there is a reciprocal loop between VTA and prefrontal cortex, and to a lesser extent also the dorsal bank of rhinal sulcus and the cingulate cortex. There is evidence that this prefrontal VTA loop may have an important role to play in motor behaviour (Tassin et al., '78).

(4) *Afferents from subcortical dopamine rich areas*

Afferents to VTA from nucleus accumbens described here confirm with a retrograde technique previous reports in the rat in which anterograde autoradiographic methods have been used (Swanson and Cowan, '75; Conrad and Pfaff, '76c; Nauta et al., '78). The results of these workers, however, show that the accumbens-VTA projection is a minor one compared to the accumbens-substantia nigra (pars compacta) projection. This may account for the failure of other studies to identify the VTA input from accumbens (Powell and Leman, '76; Williams et al., '77). The present results, in agreement with the autoradiographic findings of Nauta et al. ('78), and Troiano and Siegel ('78) show that the main input to VTA is from the medial segment of nucleus accumbens, as well as from the more ventral and posterior accumbens as it merges with bed nucleus of stria terminalis. It is interesting, also, that the most caudal and ventral neurons labelled in frontal cortex occurred very close to

the labelled neurons in the anterior and medial border of accumbens and in some sections the borders between these two structures were hard to define.

Judged by the numbers of neurons labelled in all cases, however, the accumbens is not a major source of afferents to VTA. This is in sharp contrast to the striato-nigral system where an extremely heavy projection exists (Bunney and Aghajanian, '76) accompanied by marked development of the pars reticulata. It is interesting that there is no well developed analog to pars reticulata in the VTA.

Olfactory tubercle also provides some afferent fibres and the neurons of origin are usually situated in the deep polymorphic layers. This pathway has not so far been described.

Bed nucleus of the stria terminalis, both dorsal and ventral divisions provided afferent fibres, a finding in agreement with the autoradiographic data of Swanson ('76), Conrad and Pfaff ('76a) and Meibach and Seigel ('77).

(5) *Afferents from other basal forebrain regions*

Fibres from the vertical and horizontal limbs of the nucleus of the diagonal band have been described with anterograde methods by Meibach and Seigel ('77) and Conrad and Pfaff ('76a) to project the VTA. The present experiments, confirm those results with a retrograde method and show that such fibres originate from, large neurons near the apex of the vertical limb, and large and medium sized neurons in the middle and horizontal limbs of diagonal band. Large labelled neurons were also seen in the horizontal limb to merge laterally and posteriorly with large labelled neurons of substantia innominata and magnocellular preoptic area. These results are in agreement with autoradiographic data (Swanson, '76). The distribution of large labelled neurons in medial septum (vertical limb of diagonal band), horizontal limb of diagonal band, magno-cellular preoptic area and substantia innominata agrees well with the distribution of magnocellular basal forebrain neurons described by Divac ('75). Neurons were also labelled in the "ventral pallidum" i.e., that region ventral to the region of the anterior commissure which receives afferents from the dopamine rich olfactory tubercle and nucleus accumbens (Heimer and Wilson, '75; Nauta et al., '78). Other neurons which are part of the magnocellular basal forebrain "system" but which were not labelled by injections to the VTA occur in globus pallidus. These neurons may project to pars compacta of substantia nigra (Hattori et al., '75; Carter and Fibiger, '78).

(6) *Afferents from preoptic regions*

The present results showing afferent projections to VTA from both medial and lateral preoptic areas agree well with autoradiographic data of Swanson ('76) and Conrad and Pfaff ('76a). Furthermore, like Swanson, the presents results suggest the projection from the lateral preoptic area is more prominent than that of medial preoptic area. These VTA projections seem to arise from neurons which are cytologically and cytoarchitecturally distinct from those of the magnocellular basal forebrain system.

(7) *Afferents from hypothalamic regions*

The lateral hypothalamic region was the most consistently and heavily labelled hypothalamic group. These results are in agreement with the results of fibre degeneration (Guillery, '75; Nauta, '58; Wolf and Sutin, '66) and autoradiographis data (Arbuthnott et al., '76; Nauta et al., '78) in the rat which show prominent fibre bundles projecting to VTA as well as to substantia nigra.

The anterior hypothalamus also contained labelled neurons, though their numbers were small. Autoradiographic and degeneration evidence has been obtained for such a pathway in rat (Conrad and Pfaff, '76b).

The posterior and dorsal regions of hypothalamus were labelled to a minor extent and extend the suggestion from fibre degeneration studies (Guillery, '57) of such a projection. The connections of this region are poorly understood. Sakai et al. ('77a) noticed after HRP injections of the locus coeruleus in the cat that labelled neurons appeared in the same region, while similar results were obtained after HRP injection of nucleus raphe dorsalis in the cat. (Sakai et al., '77b). Thus it may be that neurons of this region send information to several midbrain and pontine nuclei in addition to the VTA. It is interesting to note also that this region corresponds closely with the position of the A 13 catecholamine group (Lindvall et al., '74).

(8) *Afferents from habenula*

The lateral habenular nucleus was a prominent source of fibres to the VTA. These results

confirm the results of degeneration (Akagi and Powell, '68) and autoradiographic studies (Herkenham and Nauta, '77a). Neurons in both medial and lateral divisions of the lateral nucleus were labelled. The lateral habenula appears to provide a site of convergence for afferents from basal forebrain structures; nucleus of diagonal band, substantia innominata, lateral preoptic area and magno-cellular preoptic area; as well as the more caudal lateral hypothalamic and entopeduncular nuclei. It is thus in a position to integrate information from both "limbic" and "caudatoputamen" systems (GENERAL DISCUSSION).

The medial habenula was only labelled after HRP injections confined to the interfascicular nucleus. This suggests that the interfascicular nucleus is related functionally to the interpeduncular nucleus, since the bulk of the efferent projections of the medial habenula are confined almost entirely to the interpeduncular nucleus (Herkenham and Nauta, '77a). It should be emphasized that no evidence of a projection from medial habenula to VTA was obtained, and this agrees with anterograde labelling studies (Akagi and Powell, '68; Herkenham and Nauta, '77a).

(9) *Afferents from parafascicular area*

The number of neurons labelled in this region was small. Nevertheless the labelling was seen in most cases. It is interesting that the ascending projections of this nucleus to the pre-frontal cortex, and caudate nucleus-areas containing dopamine terminals—(Divac et al., '76; Powell and Cowan, '54) complement the descending projection shown here to VTA. Some labelled neurons of the parafascicular area also occurred near the region containing the A 11 dopamine group of the posterior thalamus (Jacobowitz and Palkovits, '74).

(10) *Afferents from amygdala*

Neurons were unexpectedly labelled in amygdala. Anatomical studies usually state that efferent fibres of the amygdala cannot be traced further caudally than posterior hypothalamus (Nauta, '61; Cowan et al., '65; Hall, '63; Leonard and Scott, '71). Recently, however, some evidence of amygdalotegmental, (Hopkins, '75) and amygdalo-nigral pathways (Bunney and Aghajanian, '76) has been obtained with the horseradish peroxidase technique. In addition there is electrophysiological evidence of an amygdalo-mesencephalic projection (Gloor, '55) although these results suggest multisynaptic rather than direct influence on the midbrain. Autoradiographic tracing of amygdalo-tegmental connections has recently confirmed that amygdala projects to VTA in the cat (Krettek and Price, '78). However, this was only noted after injections to the anterior and basomedial nuclei. In other cases of injection to central nucleus in rat, label was transported to lateral substantia nigra. Thus it is possible that label in central amygdala seen in the present experiments was due to damage and transport of HRP in fibres of passage to substantia nigra. Further work is necessary to resolve this question, and the status of pathways from anterolateral basolateral and medial nuclei. Only a minority of cases showed such label and the number of neurons labelled was small. In any case, it seems that the amygdala does project to the mesencephalic area, and the present results, in conjunction with those of others, suggest that both A 9 and A 10 dopaminergic systems may be one such input area.

(11) *Afferents from midbrain areas*

It is very likely that neurons labelled in oculomotor complex, red nucleus and perhaps substantia nigra compacta represent labelling of damaged and/or undamaged axons of passage. Some label in red nucleus and substantia nigra may also have resulted from direct perikarya uptake from the HRP injections which had spread from the nearby pipette tip. Such spread to red nucleus was deduced in some cases because of concomitant uptake and transport of HRP to the dentate nuclei contralateral to the injection site.

More interesting, however, was labelling more distant from the electrode tip, in the deeper layers of the superior colliculus. Of course it is possible that damaged tectospinal or tectotegmental fibres may be responsible for this labelling. Furthermore, both degeneration and autoradiographic studies of efferents from the superior colliculi fail to demonstrate colliculo-VTA fibres (Kawamura et al., '74; Graham, '77). Nevertheless, recent anatomical and electrophysiological demonstrations of nigro-tectal pathways arising from pars reticulata of substantia nigra and projecting to deep layers of the superior colliculus (Deniau et al., '77; Graybiel, '78) suggest that a return collicular-VTA connection should not be ruled out on negative grounds alone. Fur-

ther evidence for this pathway is obviously necessary.

(12) *Afferents from pons and medulla*

The efferent projections of the dorsal raphe nucleus have been repeatedly studied with anterograde methods and all are in agreement that the VTA receives fibres from this nucleus (Conrad et al., '74; Bobillier et al., '75; Taber-Pierce, '76; Ruda, '76; Graybiel, '77). The present results confirm this pathway with a retrograde method. Biochemical evidence suggests that the transmitter used by this pathway may be 5HT (Saavedra et al., '74a; Brownstein et al., '75), a suggestion consistent with the earlier histochemical evidence (Fuxe, '65) showing 5HT containing cell bodies in dorsal raphe nucleus and 5HT varicosities in VTA. Definite proof that synaptic terminals to the VTA are derived from this pathway, however, is still lacking and this will require ultrastructural and electrophysiological evidence.

Neurons in median raphe nucleus were occasionally labelled by VTA injections. This occurred only when the injection involved the midline of the VTA. Whether such labelling results from uptake from fibres of passage from the habenula to median raphe (Pasquier et al., '76) remains to be seen. However, after labelling restricted to the midline anterior VTA group, the interfascicular nucleus, many neurones were labelled in this nucleus, particularly its dorsal segment. Since the interfascicular nucleus also receives afferents from the medial habenula (see above) it seems that afferent relations of the interfascicular group differ fundamentally from the rest of the VTA. Thus it receives afferents from medial habenula and median raphe, whereas the rest of VTA receives afferents from lateral habenula and dorsal raphe.

Neurons of the raphe magnus and pontis were sometimes labelled in these experiments. However, since labelling was also obtained after HRP injections of the red nucleus, these results should be treated with caution.

The most consistently and heavily labelled group in caudal pons were neurons lying amongst the decussated fibres of the brachium conjuntivum in the ventral parabrachial nucleus, but also to a lesser extend in the dorsal nucleus. This region also includes the A 7 catecholamine group (Palkovits and Jacobowitz, '74), Labelled neurons lay in an area which appears to correspond to the nucleus tegmenti pedunculopontinus described by other workers in the monkey brain (Nauta and Mehler, '66; Kim et al., '76). This area appears to receive the most caudal fibres from the medial segment of globus pallidus in monkey (Nauta and Mehler, '66) and possibly also from the analogous structure, the entopeduncular nucleus, in the rat (Carter and Fibiger, '78). Furthermore, since the pedunculopontine region also receives fibres from the lateral hypothalamus, (Arbuthnott et al., '76) and substantia innominata (Swanson, '76) it may represent, like the lateral habenula, another important point of convergence of efferents from caudatoputamen and "limbic" systems. (GENERAL DISCUSSION).

As the ventral parabrachial neurons were followed posteriorly they merged with neurons labelled immediately ventral to the locus coeruleus and some of these at least may well have been catecholamine neurons (Palkovits and Jacobowitz, '74). The number of neurons labelled within the locus coeruleus itself was small and in animals where the posterior A 10 group were injected no neurons were found in this nucleus at all. Degeneration silver methods have failed to demonstrate a pathway from locus coeruleus to VTA (Shimizu et al., '74). Swanson and Hartman ('75), however, showed fibres containing adrenaline or noradrenaline in the ventral tegmental area, with the more sensitive immunohistochemical method. Scattered labelling was found in some animals in regions containing the A 5 and A 1 catecholamine groups. The former group seems to be noradrenergic in nature (Swanson and Hartman, '75) while the latter probably uses adrenaline as its neurotransmitter (Hökfelt et al., '74; Koslow and Schlumpf, '74; Saavedra et al., '74b; Moore and Phillipson, '75). Of course, in none of these cases where labelled neurons were observed in regions containing catecholamine cell bodies, is it possible, except in the case probably of principal locus coeruleus and substantia nigra compacta, to say whether the afferents observed are aminergic or not. Double labelling procedures will be necessary to prove this point. Nevertheless, the present results suggest that there may be an extensive interconnective network between the different aminergic systems of the brainstem such that A 10 neurons receive fibres from A 7, A 6, A 5, A 2 and A 1 catecholamine neurons (possibly also A 9 and A 8) as well as the B 7 serotoninergic group in the dorsal raphe nucleus. In the case of A 6, A 7, B 7 groups this network may involve reciprocal

connections with other amine systems (Sakai et al., '77a,b; Cedarbaum and Aghajanian, '78).

(13) *Negative findings*

No retrogradely labelled neurons were observed in any case in entorhinal cortex or lateral septum, in spite of careful search of these regions. These are the only forebrain areas which receive dopamine terminals from VTA neurons but which do not contain labelled cells. Of course negative results obtained with the HRP method do not exclude the presence of a pathway, and it may well be that larger HRP injections would reveal such projections. Further evidence is clearly required about these exceptions.

GENERAL DISCUSSION

An attempt to place the findings of the present study within the context of a more general neuroanatomical framework must start with a brief summary of cortico-subcortical pathways and their relation to dopaminergic systems. These have been reviewed recently by Heimer and Wilson ('75). The major conclusion can be drawn that, in parallel with the well-known neocortico-strio-pallidal pathway in which the A 9 dopamine system innervates the striatal stage (caudate nucleus); there appears to be an analogous allocortico-strio-pallidal pathway in which the striatal stage is innervated by the A 10 dopamine system. In the second system, according to Heimer and Wilson, the cortical component is represented by hippocampus and pyriform cortex, and the striatal component by nucleus accumbens and olfactory tubercle respectively ("ventral striatum"). The main feature of the system for the present discussion is that the pallidal component of the second pathway is represented by a region which, although quite discrete as determined in their lesion studies, is poorly defined in currently available rat brain atlases. According to the cytoarchitectural study of Swanson ('76) it corresponds to the dorsal area of substantia innominata at the level of the anterior commissure. The cytology and ultrastructure of this region support the evidence on connectional grounds, that this area should be considered "pallidal." An important gap in the scheme which remained at that stage was the question of the efferent relationship of this "ventral pallidum." The present study clearly indicates that neurons of the "ventral pallidum" project to the VTA i.e., the midbrain region providing the dopaminergic innervation of the "ventral striatum." In this respect the analogy to the neocortico-strio-pallidal system is complete for in that case some neurons in the globus pallidus ("dorsal pallidum") are known to project to pars compacta of substantia nigra (Nauta and Mehler, '66; Hattori et al., '75; Carter and Fibiger, '78). Thus, for both neocortical and allocortical systems the "striatal," as well as "pallidal" stages project back to the midbrain dopamine nuclei. As might be expected from the size of the caudate nucleus, the volume of afferents to substantia nigra is much greater than the volume of fibres from the smaller accumbens and olfactory tubercle to VTA and this may explain the lack of a well developed analog in VTA to pars reticulata of substantia nigra — the recipient of striatal fibres.

The "ventral pallidal" system so defined here, only occupies a portion of substantia innominata. The definition of substantia innominata is variable (for discussion see Heimer and Wilson, '75; Divac, '75; Jones et al., '76; Swanson, '76). However, it is interesting that the distribution of neurons included in the term "magnocellular nuclei of the basal forebrain" (MNBF) (Divac, '75) corresponds in general very well with the sites of labelled neurons seen in the present study. These occur in vertical and horizontal limbs of diagonal band, substantia innominata (as defined by Swanson, '76, in the rat), magnocellular preoptic area and appear to project to cortex. These MNBF neurons may correspond to the chain of "aggregated and unaggregated large cells that lie in the substantia innominata" described by Jones et al. ('76) in the monkey. Since the present results show that they appear also to project back to mesencephalic dopaminergic neurons, this provides additional evidence that neurons in these regions may have some functional equivalence. There is some electrophysiological evidence of connection between neurons of the vertical limb of the diagonal band and VTA. Thus, macroelectrode recordings from the VTA will register rhythmic potential fluctuations (Trembly and Sutin, '62; Le Moal and Cardo, '75) which appear to be related to hippocampal theta and are driven from the medial septal area (Le Moal and Cardo, '75).

In addition to these important afferent systems from neurons of diagonal band, substantia innominata and magnocellular preoptic

area to VTA, there appears to be a series of afferents from a different type of neuron in the lateral preoptic/lateral hypothalamic continuum or "path neurons" of the medial forebrain bundle (Millhouse, '69). The anatomical relationship between these two apparently distinct systems and their relation in turn to the cortico-strio-pallidal systems of Heimer and Wilson should provide important clues to the function of the VTA as a target for afferents from both MNBF and "path" neurons. Behavioural experiments indicate that the responses of some neurons in the lateral hypothalamus and substantia innominata are modulated by learning during discrimination and extinction tests (Mora et al., '76). This suggests that neurons in the VTA, by virtue of their connection with these regions, may play a role in learning mechanisms.

The lateral habenula has been shown to be a site where information from both "limbic" and "caudatoputamen" systems can collaborate (Herkenham and Nauta, '77b). Since the present results repeatedly demonstrate afferent fibres to VTA from this nucleus, the VTA is in a position to sample information integrated not only from both the limbic "ventral" strio-pallidal system via substantia innominata but also from the extrapyramidal "dorsal" neocortico-strio-pallidal pathway via the entopeduncular nucleus. It is interesting to note that the medial segment of lateral habenula is supplied with dopaminergic fibres (Lindvall et al., '74) which appear to be derived from the VTA (Herkenham and Nauta, '77b). Thus the VTA, in addition to receiving fibres from lateral habenula, is in a position to exercise direct control over the lateral habenula. The VTA also receives fibres from neurons embedded in the decussated parts of the brachium conjunctivum. This area may correspond to the nucleus tegmenti pedunculopontinus of the monkey. As pointed out above this may be another integrative area where, like the lateral habenula, fibres from substantia innominata and entopeduncular neruons converge, and where in addition information from the "path" neurons via the lateral hypothalamus can be integrated.

The VTA neurons are thus in a position to detect the resultant integration, via lateral habenula and pedunculopontine region, of a vast array of neuronal activity. They are the only ones known which also provide a restricted innervation to the deep layers of the pre-frontal cortex (Lindvall et al., '78) and entorhinal cortex (Fallon et al., '78). Since these cortical regions are themselves at the highest levels of convergence of association fibres from many sensory cortical areas (Jacobson and Trojanowski, '77; Van Hoesen et al., '72), taken together the evidence indicates that both axon terminals and dendrites of VTA dopaminergic neurons lie in regions of the nervous system characterised by truly massive convergence. It would appear to be reasonable to propose a correspondingly important integrative or coordinating function for these neurons.

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LITERATURE CITED

Akagi, K., and E. W. Powell 1968 Differential projections of habenular nuclei. J. Comp. Neur., *132:* 263-274.

Arbuthnott, G. W., M. J. Mitchell, I. F. Tulloch and A. K. Wright 1976 Efferent pathways from lateral hypothalamic neurones. J. Phsyiol., 33P.

Berger, B., J. P. Tassin, G. Blanc, M. A. Moyne and A. M. Thierry 1974 Histochemical confirmation for dopaminergic innervation of the rat cerebral cortex after destruction of the ascending noradrenergic pathways. Brain Res., *81:* 332-337.

Bobillier, P., F. Pettitjean, D. Salvert, M. Ligier and S. Seguin 1975 Differential projection of the nucleus raphe dorsalis and nucleus raphe centralis as revealed by autoradiography. Brain Res., *85:* 205-210.

Brodmann, K. 1909 Vergleichende Lokalisationslehre derr Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. J. Barth, Leipzig, 324 pp.

Brownstein, M. J., M. Palkovits, J. M. Saavedra and J. S. Kizer 1975 Tryptophan hydroxylase in the rat brain. Brain Res., *97:* 163-166.

Bunney, B. S., and G. K. Aghajanian 1976 The precise localisation of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res., *117:* 423-435.

Burt, D. R., S. J. Enna, I. Creese and S. H. Snyder 1975 Dopamine receptor binding in the corpus striatum of mammalian brain. Proc. Nat. Acad. Sci. (U.S.A.), *72:* 4655-4659.

Carter, D. A., and H. C. Fibiger 1978 The projections of the entopeduncular nucleus and globus pallidus in rat as demonstrated by autoradiography and horseradish peroxidase histochemistry. J. Comp. Neur., *177:* 113-124.

Cedarbaum, J. M., and G. K. Aghajanian 1978 Afferent

projections to the rat locus coeruleus as determined by a retrograde tracing technique. J. Comp. Neur., *178:* 1-16.

Colman, D. R. F., F. Scalia and E. Cabrales 1976 Light and electron-microscopic observations on the anterograde transport of horseradish peroxidase in the optic pathway in the mouse and rat. Brain. Res., *102:* 156-163.

Conrad, L. C. A., C. M. Leonard and D. W. Pfaff 1974 Connections of the median and dorsal raphe nuclei in the rat: An autoradiographic and degeneration study. J. Comp. Neur., *156:* 179-206.

Conrad, L. C. A., and D. W. Pfaff 1976a Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J. Comp. Neur., *169:* 185-220.

——— 1976b Efferents from medial basal forebrain and hypothalamus in the rat. II. An autoradiographic study of the anterior hypothalamus. J. Comp. Neur., *169:* 221-262.

——— 1976c Autoradiographic tracing of nucleus accumbens efferents in the rat. Brain Res., *113:* 589-596.

Cowan, W. M., G. Raisman and T. P. S. Powell 1965 The connections of the amygdala. J. Neurol. Neurosurg. Psychiat., *28:* 137-151.

Dahlström, A., and K. Fuxe 1964 Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. *62:* Suppl. 232.

Deniau, J. M., C. H. -Le Guyader, J. Feger and J. S. McKenzie 1977 Bilateral projections of nigrocollicular neurons: an electrophysiological analysis in the rat. Neurosci. Letters, *5:* 45-50.

De Vito, J. L., and O. A. Smith 1964 Subcortical projections of the prefrontal lobe of the monkey. J. Comp. Neur., *123:* 413-424.

Divac, I. 1975 Magnocellular nuclei of the basal forebrain project to neocortex brain stem, and olfactory bulb. A review of some functional correlates. Brain Res., *93:* 385-398.

Divac, I., A. Kosmal, O. Lindvall and A. Björklund 1976 Subcortical projections to the prefrontal cortex in the rat. Neurosci. Abst., Vol. 2 part 1, no. 189.

Domesick, V. B., R. M. Beckstead and W. J. H. Nauta 1976 Some ascending and descending projections of the substantia nigra and ventral tegmental area in the rat. Neurosci. Abstr., Vol. 2 part 1, no. 82.

Fallon, J. H., D. A. Koziell, and R. Y. Moore 1978 Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J. Comp. Neur., *180:* 509-532.

Furstman, L., S. Saporta and L. Kruger 1975 Retrograde axonal transport of horseradish peroxidase in sensory nerves and ganglion cells of the rat. Brain Res., *84:* 320-324.

Fuxe, K. 1965 Evidence for the existence of monoamine neurones in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. *64* (Suppl. 247): 37-85.

Fuxe, K., T. Hökfelt, O. Johansson, G. Jonsson, P. Lindbrink and A. Ljungdahl 1974 The origin of the dopamine nerve terminals in limbic and frontal cortex. Evidence for mesocortical dopamine neurons. Brain Res., *82:* 349-355.

Gloor, P. 1955 Electrophysiological studies of the connections of the amygdaloid nucleus in the cat. Part I: the neuronal organisation of the amygdaloid projection system. Electroenceph. Clin. neurophysiol., *7:* 223-242.

Graham, J. 1977 An autoradiographic study of the efferent connections of the superior colliculus in the cat. J. Comp. Neur., *173:* 629-654.

Graybiel, A. M. 1977 Direct and indirect preoculomotor pathways of the brainstem: an autoradiographic study of the pontine reticular formation in the cat. J. Comp. Neur., *175:* 37-78.

——— 1978 Organisation of the nigrotectal connection: an experimental tracer study in the cat. Brain Res., *143:* 339-348.

Graybiel, A. M., and M. Devor 1974 A microelectrophoretic delivery technique for use with horseradish peroxidase. Brain Res., *68:* 167-173.

Guillery, R. W. 1957 Degeneration in the hypothalamic connexions of the albino rat. J. Anat. (London), *91:* 91-115.

Hall, E. A. 1963 Efferent connections of the basal and lateral nuclei of the amygdala in the cat. Am. J. Anat., *113:* 139-151.

Halperin, J. J., and J. H. LaVail 1975 A study of the dynamics of retrograde transport and accumulation of horseradish peroxidase in injured neurones, Brain Res., *100:* 253-269.

Hardy, H., and L. Heimer 1977 A safer and more sensitive substitute for diaminobenzidine in the light microscopic demonstration of retrograde and anterograde axonal transport of HRP. Neurosci. Letters, *5:* 235-240.

Hattori, T., H. C. Fibiger and P. L. McGeer 1975 Demonstration of a pallido-nigral projection innervating dopaminergic neurones. J. Comp. Neur., *162:* 487-504.

Heimer, L., and R. D. Wilson 1975 The subcortical projections of the allocortex: Similarities in the neural associations of the hippocampus, the piriform cortex and the neocortex. In: Perspectives in Neurobiology. M. Santini, ed. Raven Press, N.Y., pp. 177-193.

Herkenham, M., and W. J. H. Nauta 1977a Projections of the habenular nuclei in the rat. Anat. Rec., *187:* 603.

——— 1977b Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study with a note on the fibre of passage problem. J. Comp. Neur., *173:* 123-146.

Hökfelt, T., K. Fuxe, M. Goldstein and O. Johansson 1974 Immunohistochemical evidence for the existence of adrenaline neurons in the rat brain. Brain Res., *66:* 235-251.

Hopkins, D. A. 1975 Amygdalotegmental projections in the rat, cat and rhesus monkey. Neurosci. Letters, *1:* 263-270.

Iversen, L. L. 1975 Dopamine receptors in the brain. Science, *188:* 1084-1089.

Iwahori, N. 1977 A Golgi study on the habenular nucleus of the cat. J. Comp. Neur., *171:* 310-344.

Jacobowitz, D. M., and M. Palkovits 1974 Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). J. Comp. Neur., *157:* 13-28.

Jacobson, S., and J. Q. Trojanowski 1977 Prefrontal granular cortex of the rhesus monkey. I. Intrahemispheric cortical afferents. Brain Res., *132:* 209-233.

Jones, E. G., H. Burton, C. B. Sapor and L. W. Swanson 1976 Midbrain, diencephalic and cortical relationships of the basal nucleus of Meynert and associated structures in primates. J. Comp. Neur., *167:* 385-420.

Jones, E. G., and R. Y. Leavitt 1974 Retrograde axonal transport and the demonstratin of non-specific projections to the cerebral cortex and striatum from thalamic intralaminar nuclei in the rat, cat and monkey. J. Comp. Neur., *154:* 349-378.

Kawamura, K., A. Brodal and G. Hoddevik 1974 The projection of the superior colliculus onto the reticular formation of the brain stem. An experimental anatomical study in the cat. Exp. Brain Res., *19:* 1-19.

Kim, R., K. Nakano, A. Jayaraman and M. B. Carpenter 1976 Projections of the globus pallidus and adjacent

structures: An autoradiographic study in the monkey. J. Comp. Neur., *169:* 263-290.

König, J. F. R., and R. A. Klippel 1963 The Rat Brain. Krieger, Huntington, New York.

Koslow, S. H., and M. Schlumpf 1974 Quantitation of adrenaline in rat brain nuclei and areas by mass fragmentography. Nature, *251:* 530-531.

Krettek, J. E., and J. L. Price 1977 The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neur., *171:* 157-192.

——— 1978 Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J. Comp. Neur., *178:* 225-254.

Kristensson, K., and Y. Olsson 1971 Retrograde axonal transport of protein. Brain Res., *29:* 363-365.

——— 1974 Retrograde transport of HRP in transected axons. I. Time relationships between transport and induction of chromatolysis. Brain Res., *79:* 101-109.

——— 1976 Retrograde transport of horseradish peroxidase in transected axons. III. Entry into injured axons and subsequent localisation in perikaryon. Brain Res., *115:* 201-213.

Kuypers, H. G. J. M., and V. A. Maisky 1975 Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. Neurosci. Letters, *1:* 9-14.

LaVail, J. H., and M. M. LaVail 1972 Retrograde axonal transport in the central nervous system. Science, *176:* 1416-1417.

LaVail, J. H., K. R. Winston and A. Tish 1973 A method based on retrograde intraaxonal transport of protein for identification of cell bodies or origin of axons terminating within the CNS. Brain Res., *58:* 470-477.

Leichnetz, G. R., and J. Astruc 1976 The efferent projections of the medial prefrontal cortex in squirrel monkey *(Saimiri sciureus).* Brain Res., *109:* 455-472.

Le Moal, M., and B. Cardo 1975 Rhythmic slow wave activity recorded in the ventral mesencephalic tegmentum in the rat. Electroenceph. and Clin. Neurophysiol., *38:* 139-147.

Leonard, C. M., and J. W. Scott 1971 Origin and distribution of the amygdalofugal pathways in the rat: an experimental neuroanatomical study. J. Comp. Neur., *141:* 313-330.

Lindvall, O. 1975 Mesencephalic dopaminergic afferents to the lateral septal nucleus of the rat. Brain Res., *87:* 89-95.

Lindvall, O., A. Björklund and I. Divac 1978 Organisation of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res., *142:* 1-24.

Lindvall, O., A. Björklund, R. Y. Moore and U. Stenevi 1974a Mesencephalic dopamine neurons projecting to neocortex. Brain Res., *81:* 325-333.

Lindvall, O., A. Björklund, A. Nobin and U. Stenevi 1974b The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. J. Comp. Neur., *154:* 317-348.

Meibach, R. C., and A. Siegel 1977 Efferent connections of the septal area in the rat: an analysis utilizing retrograde and anterograde transport methods. Brain Res., *119:* 1-20.

Millhouse, O. E. 1969 A Golgi study of the descending medial forebrain bundle. Brain Res., *15:* 341-363.

Moore, K. E., and O. T. Phillipson 1975 Effects of dexamethasone on phenylethanolamine N-methyltransferase and adrenaline in the brains and superior cervical ganglia of adult and neonatal rats. J. Neurochem., *25:* 289-294.

Mora, F., E. T. Rolls and M. J. Burton 1976 Modulation during learning of the responses of neurons in the lateral hypothalamus to the sight of food. Experim. Neurol., *53:* 508-519.

Nauta, W. J. H. 1956 An experimental study of the fornix system in the rat. J. Comp. Neur., *104:* 247-271.

——— 1968 Hippocampal projections and related neural pathways to the midbrain in the cat. Brain, *81:* 319-340.

——— 1961 Fiber degeneration following lesions of the amygdaloid complex in the monkey. J. Anat. (London), *95:* 515-531.

——— 1964 Some efferent connections of the prefrontal cortex in the monkey. In: The Frontal Granular Cortex and Behaviour. J. M. Warren and K. Akert, eds. McGraw-Hill, New York., pp. 397-409.

Nauta, W. J. H., and W. Haymaker 1969 Hypothalamic nuclei and fiber connections. In: The Hypothalamus. W. Haymaker, E. Anderson and W. J. H. Nauta, eds. Thomas, Springfield, Illinois, pp. 136-209.

Nauta, W. J. H., and W. R. Mehler 1966 Projections of the lentiform nucleus in the monkey. Brain Res., *1:* 3-42.

Nauta, W. J. H., G. P. Smith, R. L. M. Faull and V. B. Domesick 1978 Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience, *3:* 385-401.

Oldfield, B. J., and E. M. McLachlan 1977 Uptake and retrograde transport of HRP by axons of intact and damaged peripheral nerve trunks. Neurosci. Letters, *6:* 135-141.

Palkovits, M., and D. M. Jacobowitz 1974 Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). J. Comp. Neur., *157:* 29-42.

Parent, A. 1976 Striatal afferent connections in the turtle (chrysemys picta) as revealed by retrograde axonal transport of horseradish peroxidase. Brain Res., *108:* 25-36.

Pasquier, D. A., C. Anderson, W. B. Forbes and P. J. Morgane 1976 Horseradish peroxidase tracing of the lateral habenalar-midbrain raphe nuclei connections in the rat. Brain Res. Bull., *1:* 443-451.

Phillipson, O. T. 1978 Afferent projections to A10 dopaminergic neurons in the rat as shown by the retrograde transport of horseradish peroxidase. Neurosci. Letters, *9:* 353-359.

Powell, T. P. S., and W. M. Cowan 1954 The connexions of the midline and intralaminar nuclei of the thalamus of the rat. J. Anat. (London), *88:* 307-319.

Powell, E. W., and R. B. Leman 1976 Connections of the nucleus accumbens. Brain Res., *105:* 389-403.

Price, J. L., and T. P. S. Powell 1970 An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb in the rat. J. Anat. (London), *107:* 215-237.

Rose, J. E., and Woolsey 1948 Structure and relations of limbic cortex and anterior thalamic nuclei in rabbit and cat. J. Comp. Neur., *89:* 279-347.

Ruda, M. 1976 Autoradiographic Study of the Efferent Projections of the Midbrain Central Grey in the Cat. Ph.D. Thesis in University of Pennsylvania.

Saavedra, J. M., M. Brownstein and M. Palkovits 1974a Serotonin distribution in the limbic system of the rat. Brain Res., *79:* 437-441.

Saavedra, J. M., M. Palkovits, M. J. Brownstein and J. Axelrod 1974b Localisation of phenylethanolamine N-methyl-transferase in the rat brain nuclei. Nature, *248:* 695-696.

Sakai, K., M. Touret, D. Salvert, L. Leger and M. Jouvet 1977a Afferent projections to the cat locus coeruleus as visualized by the horseradish peroxidase technique. Brain Res., *119:* 21-41.

Sakai, K., D. Salvert, M. Touret and M. Jouvet 1977b Afferent connections of the nucleus raphe dorsalis in the cat as

visualised by the horseradish peroxidase technique. Brain Res., *137:* 11-35.

Scheibel, M. E., and A. B. Scheibel 1958 Structural substrates for integrative patterns in the brainstem reticular core. In: The Reticular Formation of the Brain. H. H. Jasper, L. D. Procton, R. S. Knighton, W. C. T. Noshay and R. Costello, eds. Little Brown & Co., Boston. Ch. 2, pp. 31-55.

Seeman, P., M. Chan-Wong, J. Tedesco and K. Wong 1975 Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc. Nat. Acad. Sci. (U.S.A.), *72:* 4376-4380.

Shimizu, N., S. Ohnishi, M. Tohyama and T. Maeda 1974 Demonstration by degeneration silver method of the ascending projection from the locus coeruleus. Exp. Brain Res., *21:* 181-192.

Snider, R. S., A. Maiti and S. R. Snider 1976 Cerebellar pathways to ventral midbrain and nigra. Exper. Neurol., *53:* 714-728.

Swanson, L. W. 1976 An autoradiographic study of the efferent connections of the preoptic region in the rat. J. Comp. Neur., *167:* 227-256.

Swanson, L. W., and W. M. Cowan 1975 A note on the connections and development of the nucleus accumbens. Brain Res., *92:* 324-330.

Swanson, L. W., and B. K. Hartman 1975 The central adrenergic system: An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. J. Comp. Neur., *163:* 467-506.

Taber-Pierce, E., W. E. Foote and J. A. Jobson 1976 The efferent connections of the nucleus raphe dorsalis. Brain Res., *107:* 137-144.

Tassin, J.-P., L. Stinus, H. Simon, G. Blanc, A. M. Thierry, M. Le Moal, B. Cardo and J. Glowinski 1978 Relationship between locomotor hyperactivity induced by A10 lesions and the destruction of the frontocortical dopaminergic innervation in the rat. Brain Res., *141:* 267-281.

Trembly, B., and J. Sutin 1962 Slow wave activity in the ventral tegmental area of Tsai related to barbiturate anaesthesia. Exper. Neurol., *5:* 120-130.

Troiano, R., and A. Siegel 1978 Efferent connections of the basal forebrain in the cat: the nucleus accumbens. Experim. Neurol., *61:* 185-197.

Turner, P. T., and A. B. Harris 1974 Ultrastructure of exogenous peroxidase in cerebral cortex. Brain Res., *74:* 305-326.

Ungerstedt, U. 1971 Stereotaxic mapping of the monamine pathways in the rat brain. Acta Physiol. Scand. (Suppl.), *367:* 1-48.

Vanegas, H., H. Hollander and H. Distel 1978 Early stages of uptake and transport of horseradish peroxidase by cortical structures and its use for the study of local neurons and their processes. J. Comp. Neur., *177:* 193-212.

Van Hoesen, G. W., D. N. Pandya and N. Butters 1972 Cortical afferents to the entorhinal cortex of the rhesus monkey. Science, *175:* 1471-1473.

Walker, A. E. 1939 The origin, course and terminations of the secondary pathways of the trigeminal nerve in primates. J. Comp. Neur., *71:* 59-89.

Williams, D. J., A. R. Crossman and P. Slater 1977 The efferent projections of the nucleus accumbens in the rat. Brain Res., *130:* 217-227.

Wolf, G., and J. Sutin 1966 Fiber degeneration after lateral hypothalamic lesions in the rat. J. Comp. Neur., *127:* 137-156.

Wong-Riley, M. T. T. 1976 Endogenous peroxidatic activity in brainstem neurons as demonstrated by their staining with diaminobenzidine in normal squirrel monkey. Brain Res., *108:* 257-277.