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EFFERENTS AND AFFERENTS OF THE VENTRAL TEGMENTAL-A10 REGION STUDIED AFTER LOCAL INJECTION OF [^{3}H]LEUCINE AND HORSERADISH PEROXIDASE

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SUMMARY

The efferents and the afferents of the VMT-A10 region were studied by using anterograde ([^{3}H]leucine) and retrograde (HRP) tracing techniques. In order to produce very small injections in various parts of the VMT-A10 region, a slow diffusion technique for [^{3}H]leucine labelling and a microiontophoretic injection for horseradish peroxidase labelling were developed. According to the histochemical and biochemical data, the [^{3}H]leucine anterograde results were separated into three main types of projections.

(1) *Projections to regions rich in DA terminals.* These projections certainly correspond to the efferents of the dopaminergic A10 neurones. According to various injection sites, we have been able to identify mesolimbic projections originating from the VMT-A10, pars medialis and mesostriatal-mesolimbic projections originating from the VMT-A10, pars lateralis.

The mesolimbic projections include the prefrontal cortex, the medial part of the lateral septum, the interstitial nucleus of the stria terminalis, the accumbens nucleus and the olfactory tubercle. The mesostriatal-mesolimbic projections include the anteromedial part of the caudate nucleus, the cingular cortex, the entorhinal cortex, the amygdaloid complex, the accumbens nucleus, the olfactory tubercle and the piriform cortex to a lesser extent.

(2) *Projections to regions suspected of containing DA terminals.* These ascending and descending projections which could represent the dopaminergic efferents of the VMT-A10 neurones have been demonstrated. Ascending projections originating either from the VMT-A10 pars medialis or pars lateralis region were found in the claustrum, the nucleus of the tractus diagonalis, the olfactory nuclei, the lateral habenula, the

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medial hypothalamus and the median eminence. The projections observed in the medial hypothalamus included the periventricular region, the arcuate nucleus, the ventral part of the ventromedial nucleus and the dorsomedial nucleus. The labelling of the anteromedial part of the dorsal hippocampus appeared to originate from the VMT-A10, pars posterior. The projections to the medial hypothalamus, median eminence and hippocampus may have a great functional significance, but further proof of their dopaminergic nature is needed. Descending projections were found ipsilaterally to the dorsal raphe and to the cerebellum, and bilaterally to the locus coeruleus. The projections to the cerebellum are distributed to the nuclei interpositus and dentatus and to the Purkinje cell layer and granular layer of the cortex. These results raise the problem of descending dopaminergic projections from the A10 neurones.

(3) *Projections to regions not known to contain DA terminals.* Anterior projections were found ipsilaterally to the supraoptic nucleus and bilaterally to the anterodorsal thalamic nucleus. Posterior projections were traced ipsilaterally to the limbic midbrain area, including the median raphe, the ventral and dorsal tegmental nucleus and the central gray.

The horseradish peroxidase experiment supplied some clues as to the posterior afferents of the VMT-A10 region. Some labelled cells were found ipsilaterally in the substantia nigra, the median raphe and the ventral tegmental nucleus. Numerous cells were labelled ipsilaterally in the dorsal raphe nucleus, and nuclei interpositus and dentatus of the cerebellum, and contralaterally in the locus coeruleus. These structures are likely to play an important role in the modulation of the activity of VMT-A10 neurones.

The results of [^{3}H]leucine and HRP experiments permitted us to demonstrate reciprocal connections between VMT-A10 region and anterior raphe nuclei, locus coeruleus and cerebellum.

INTRODUCTION

During the last few years, we have tried to show the functional importance of the ventral mesencephalic tegmentum (VMT) region[31,41,42,43,73] containing the dopaminergic (DA) cell group A10 (ref. 21, 82). It is, however, obvious that this region is not the only one to control the observed forms of behaviour. On the one hand, it modulates the activity of the nervous structures on which it projects, and on the other hand its own activity is regulated by the afferents it receives. Thus, it seemed that a thorough knowledge of the efferents and afferents of the VMT-A10 is necessary to specify its exact function.

Since the work of Ungerstedt[82], the efferents of the DA-A10 neurones have been the subject of an extensive study mainly employing histochemical[6,7,30,44–50,54,55] and biochemical methods[25,38,80,81].

Thanks to the use of silver impregnating techniques combined with 6-hydroxydopamine (6-OHDA) lesions, we have suggested for the first time the existence of a topographical organization of the A10 neurones[65], and recent results, principally

obtained by the use of the horseradish peroxidase method (HRP)[4,15,28,48], have come to support this notion.

The aim of the present experiments is to complete our previous study on the anatomical organization of the VMT-A10 system by using anterograde and retrograde tracing techniques. In order to investigate the efferents and afferents of this region we have in separate experiments injected [^{3}H]leucine and HRP in the VMT-A10 area.

While preparing this manuscript, Fallon and Moore[26–28] published an important study concerning the efferents of the A10 neurones using the autoradiographic method. To the results of these authors, with whom we agree completely on the essential points, we here add certain important supplementary data concerning anterior projections, and some entirely new data about posterior projections of the VMT-A10. Part of our results have been the subject of preliminary publications[66–68].

MATERIALS AND METHODS

Male rats (250–300 g body weight) of the Sprague–Dawley strain were used. All surgical procedures were carried out under ketamine chlorhydrate anaesthesia.

[^{3}*H*]*Leucine experiment*

Ten rats were used for autoradiographic analysis. The animals' brains were implanted stereotaxically with the aid of micropipettes (40 μm o.d. at the tip) filled with [^{3}H]leucine (specific activity 43 Ci/mmol from Saclay, France). During the animals' survival time, [^{3}H]leucine is allowed to diffuse slowly from the tip of the micropipette. This method provides a more intensive labelling of fibres and terminals than the conventional pressure injection method for two main reasons: (1) it is less traumatic and minimizes cellular damage; (2) it gives the equivalent of several survival times, since the [^{3}H]leucine diffuses continuously.

The amount of radioactivity delivered by this method varied from 10 μCi to 20 μCi in volumes of saline ranging from 0.1 μl to 0.2 μl. The implanted micropipettes were centered in three parts of the VMT-A10 region: VMT-A10, pars medialis (2 rats), VMT-A10, pars lateralis (5 rats) and VMT-A10, pars posterior (3 rats). After survival times ranging from 24 h to 20 days, the animals were killed by intracardiac perfusions with a 10% formaldehyde solution. Their brains were removed, stored in the same fixative, and then dehydrated, embedded in paraffin and finally sectioned in the frontal or sagittal plane at 10 μm. These sections were then prepared for autoradiography by coating with Ilford K5 nuclear emulsion and, after exposure at 20 °C during 15 days, were developed in Kodak D 19 b. They were then stained with cresyl violet and examined with a light microscope under bright- or dark-field illuminations.

HRP experiment

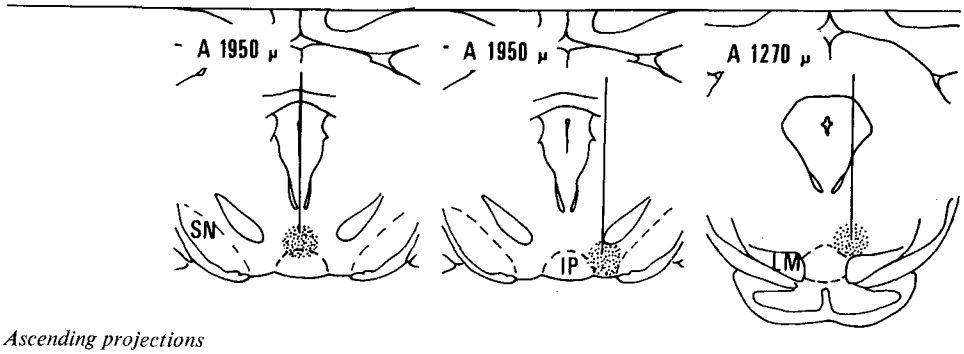
The HRP experiment was performed on 5 rats. HRP (Sigma type VI or Boehringer type I) was dissolved in sterile water (50% w/v) and electrophoretically delivered into the VMT-A10 region through a glass cannula (40 μm o.d. at the tip). Thus, the labelling was more intense and restricted to a very limited region, and the

tissue damage was kept at a minimum. After a survival period of 24 h, the animals received a perfusion of saline, containing polyvinylpyrrolidone (PVP) concentrated at 3%, followed by a mixture of paraformaldehyde–glutaraldehyde and PVP buffered with 0.1 M phosphate buffer at pH 7.4. The brains were then removed, postfixed for a period of 2–5 h in the same solution, and soaked overnight in a 4 °C phosphate buffer with 5% sucrose. The following day, the brains were cut in a cryostat at 40 μm in either the frontal or sagittal planes. The sections were incubated according to the procedure of Graham and Karnovsky, mounted on slides, and finally studied microscopically with bright- or dark-field illuminations.

TABLE I

Labelled terminals found in anterior structures after [^{3}H]leucine injection in various parts of the VMT-A10 region

According to these different injection placements, the density of labelled terminals is indicated in different ways: +++, very abundant; ++, moderate; +, sparse; 0, never observed.



Ascending projections of vmt-A10	*P. medialis*	*P. lateralis*	*P. posterior*
Frontal cortex	++	+	+
Cingular cortex	0	+	+
Suprarhinal cortex	0	0	0
Piriform cortex	0	+	+
Entorhinal cortex	0	+	++
Claustrum	0	+	++
Amygdaloid complex	0	+	++
Stria terminalis	++	++	++
Lateral septum	++	+	+
Hippocampus	0	0	+
Tractus diagonalis	++	++	++
Medial hypothalamus	+	+	++
Caudate nucleus	+	++	+
Accumbens nucleus	+++	+++	+++
Olfactory tubercle	+++	+++	+++
Olfactory nuclei	+	+*	+*
Lateral habenula	+	++	++
Anterodorsal nucleus	0	+*	+*

* Bilateral projections.

RESULTS

Anterograde transport studies

Injection sites

Table I shows the 3 types of unilateral injections made. In the VMT-A10 region pars medialis, the injection is centered on a frontal plane which corresponds approximately to the plane A 1950 μm of König and Klippel[39]. The labelled area corresponds to the medial part of the supra-interpeduncular region and the dorsal part of the interpeduncular nucleus. Its extent in an anteroposterior plane was about 100–200 μm. In the VMT-A10 region, pars lateralis, the injection is centered on the same frontal plane as the median injection, i.e. plane A 1950 μm of König and Klippel[39] but laterally with regard to the median line. The labelled zone corresponds to the ventral tegmental area of Tsai, the lateral part of the interpeduncular nucleus, a very small internal part of the substantia nigra and of the medial lemniscus. Its extent was about 100–200 μm anteriorly and posteriorly to the injection site.

In the VMT-A10, pars posterior, the injection centered on a frontal plane

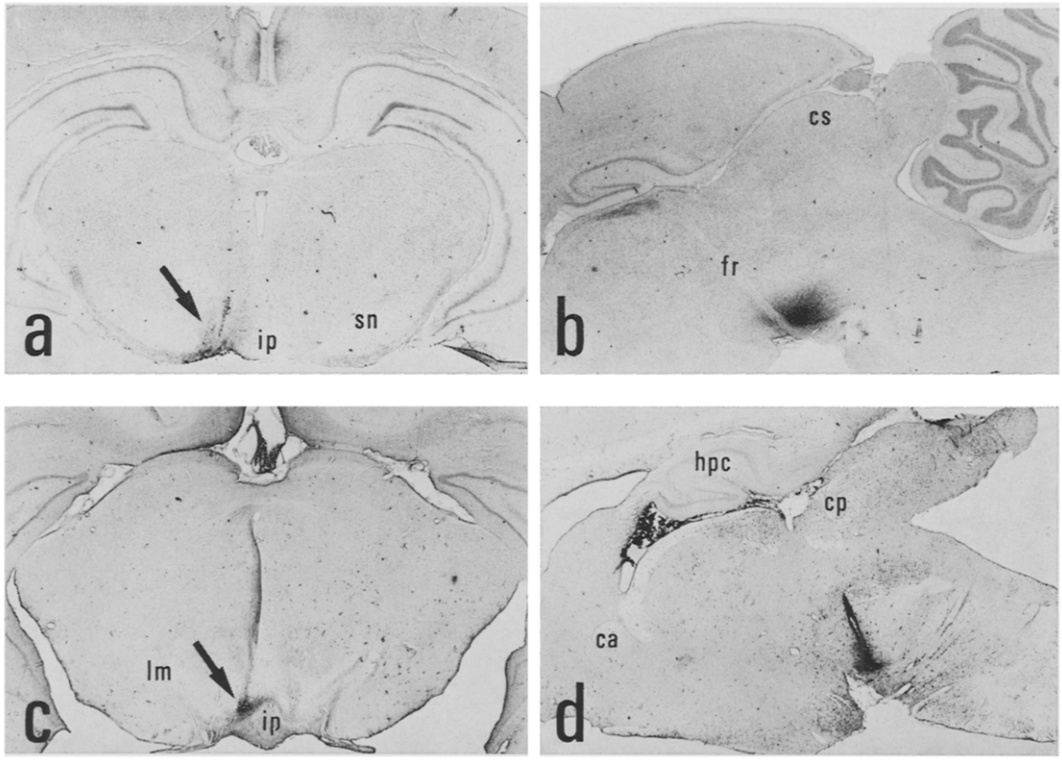
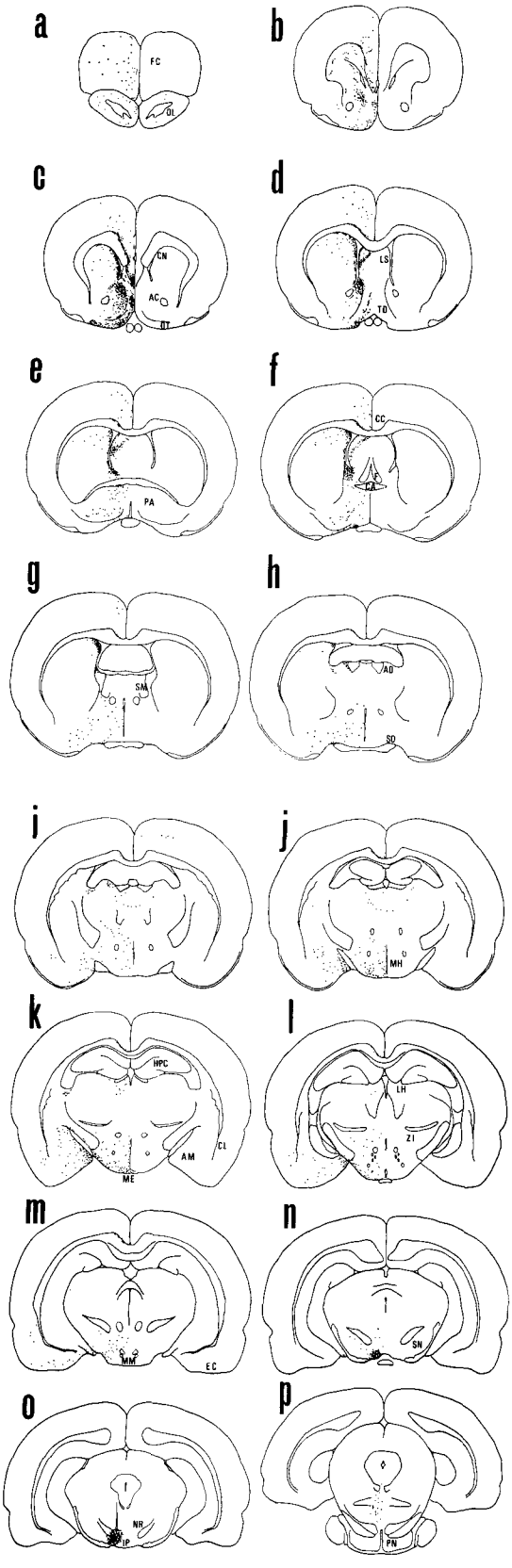


Fig. 1. Photomicrographs of representative coronal (a,c) and sagittal (b,d) brain sections showing the location and the size of [^{3}H]leucine (a,b) and HRP (c,d) injections in the VMT-A10, pars lateralis. A small injection of [^{3}H]leucine is presented in (a), while the most extensive injection is shown in b. For the identification of nervous structure, see list of abbreviations.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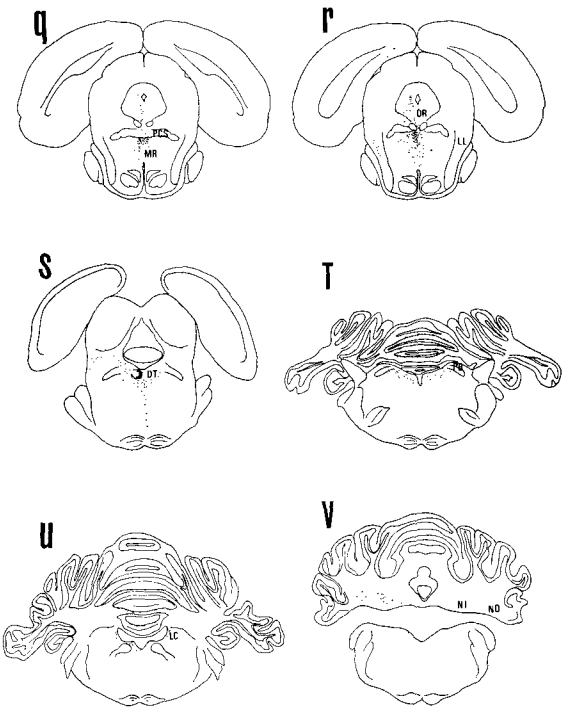

Fig. 2. Schematic representations of various frontal planes (in part from König and Klippel[39]) showing labelled fibres and terminals after [^{3}H]leucine injection in the VMT-A10, pars lateralis.

corresponding approximately to the plane A 1270 μm of König and Klippel[39]. The labelled zone is lateral (0.5 mm with regard to the median line) and covers an area of about 100–200 μm anteriorly and posteriorly to the injection site. It is important to note that, in 2 rats, the size of the labelled zone, visible only with microscopic examination, was considerably smaller.

Nevertheless, we have to admit that it is extremely difficult to appreciate the volume labelled by the injection. Indeed, the picture obtained of the labelled zone at the level of the injection is determined by different factors[32]. For example, the apparent size of the labelled zone increases with the exposure time of the sections. This suggests to us that, in the conditions of our experiment (exposure during 15 days at room temperature), the labelled zone appears larger than it actually is.

The labelled structures shown after an injection of [^{3}H]leucine in the VMT-A10 region, pars lateralis (Fig. 1a, b) are presented schematically in Figs. 2 and 3, in the frontal and sagittal planes. In connection with these data, some different results obtained by median (VMT-A10, pars medialis) or posterior injections (VMT-A10, pars posterior) will eventually be described.

Ascending projections

From the VMT-A10 region, the labelled fibres follow the course of the medial

forebrain bundle in the ventral position. A first group of fibres, orientated laterally and through the ansa lenticularis (Fig. 3f), is massively distributed in the amygdaloid complex (Fig. 2i, j, k, l), less densely in the entorhinal cortex (Fig. 2m) and claustrum (Fig. 2k, l), and very sparsely in the posterior piriform cortex (Fig. 2l).

Some more posterior injections (VMT-A10, pars posterior) labelled more intensively the amygdaloid complex (Table I) including the medial, cortical, basal and central amygdaloid nuclei. It also seems that some fibres follow the course of the stria terminalis (Fig. 2h, i, k) to the amygdaloid nucleus.

A second group of fibres innervate almost all of the medial hypothalamus, especially the ventral part (Fig. 2i, j, k, l). The ventral part of the ventromedial nucleus, the dorsomedial nucleus, the arcuate nucleus and the median eminence (Fig. 2k) show considerable labelling, whereas the paraventricular nucleus is more sparsely labelled.

In the lateral hypothalamus, where most of the fibres transit, very few terminals seem labelled. Anteriorly many terminals appear in the medial and lateral preoptic region (Fig. 2e, f) as well as in the supraoptic nucleus (Fig. 2g, k). The suprachiasmatic nucleus is labelled in cases of injection in the VMT-A10 region, pars posterior (Table I). More dorsally, scattered terminals mark the zona incerta (Fig. 2j, k).

In the preoptic region, a group of fibres run in a ventral and lateral position corresponding to the pathway of the lateral corticohypothalamic tract, ending in the olfactory tubercle (Fig. 2b, c, d). In a mediodorsal position, corresponding to the pathway of the striohypothalamic tract, a group of fibres innervate the accumbens nucleus (Fig. 2b, c, d) and the interstitial nucleus of the stria terminalis, principally in the dorsal part at the level of the rostral edge of the anterior commissure (Fig. 2e, f). Some fibres running in a direction parallel to the lateral corticohabenular tract also seem to give off terminals in the nucleus of the stria terminalis. These fibres continue to ascend dorsally, thus leaving terminals in the nucleus of the diagonal band (Fig. 2d) and in the anteromedial part of the caudate nucleus (Fig. 2c, d, e, f, g). In cases of more laterally located injection, the labelling of the caudate nucleus is more massive and more lateral. The medial part of the lateral septal nucleus (Fig. 2d, e) seems to be innervated by some fibres following a pathway corresponding to the septohypothalamic tract. From injections placed in the VMT-A10, pars posterior, we can observe some labelled terminals in the gyrus dentatus region and at the internal edge of the hippocampus. These terminals seem to originate from fibres passing through the septum. More anteriorly, the labelled fibres were divided in two groups. One ventral group seems to follow the pathway of the medial olfactory tract (Fig. 3a) and is distributed to the medial, lateral and dorsal olfactory nuclei. This projection is bilateral and most massively involves the medial olfactory nucleus (Fig. 2a). The other group of labelled fibres ascends anteriorly following the pathway of two bundles: the septocortical tract and the olfactocortical tract. One part of these fibres are distributed in the superficial layers of the prefrontal cortex (Fig. 2a and Fig. 3b) and the other part in the deep layers of the cingular cortex (Fig. 2b, c). In the suprarhinal cortex no labelling was observed. The most abundant labelling obtained from the injections located in the VMT-A10, pars medialis, can be found in the lateral nucleus of the septum and in the prefrontal cortex, whereas the cingular cortex was most abundantly

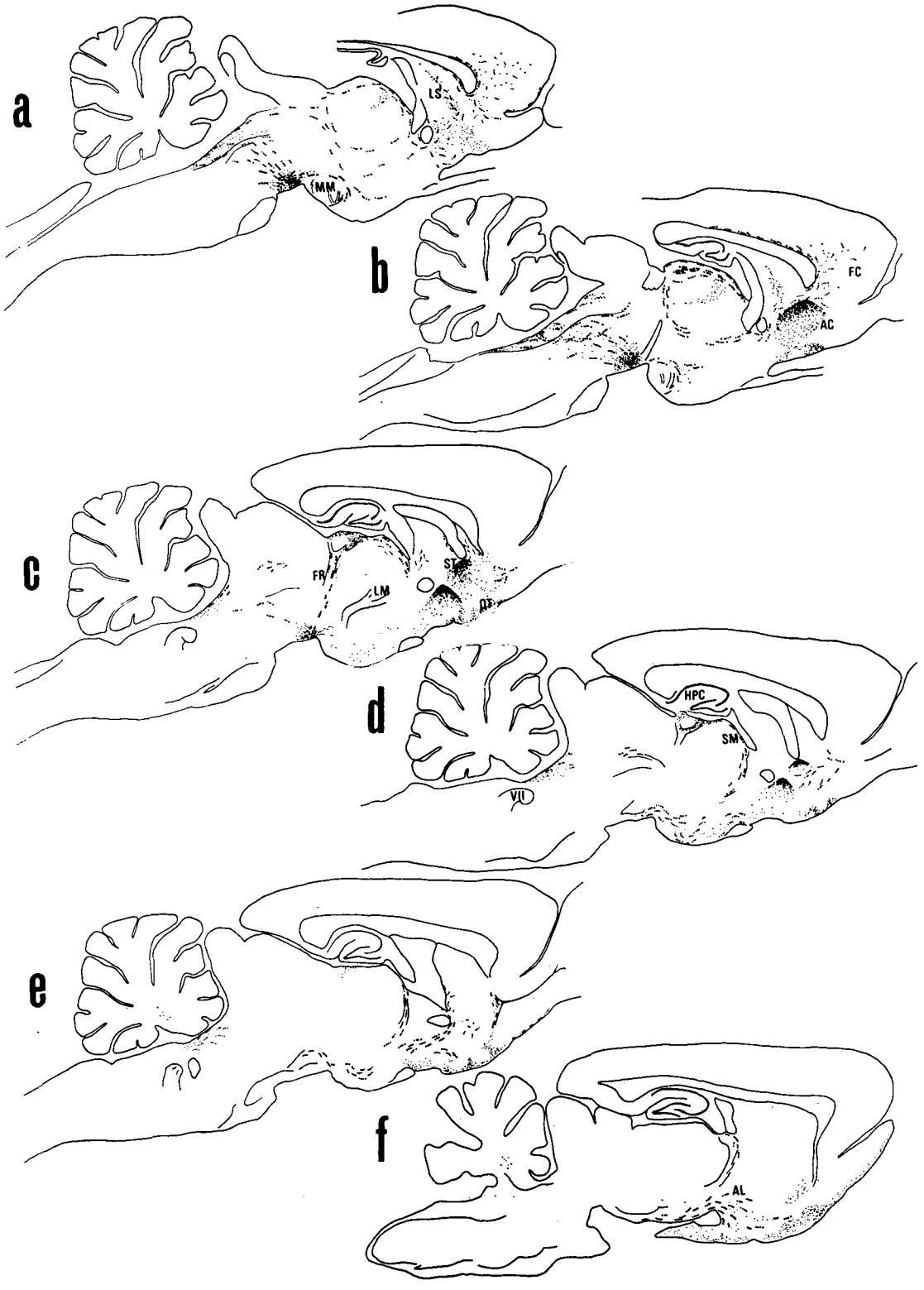

Fig. 3. Schematic representations of various sagittal planes (from König and Klippel[39]) showing labelled fibres and terminals after [^{3}H]leucine injection in the VMT-A10, pars lateralis.

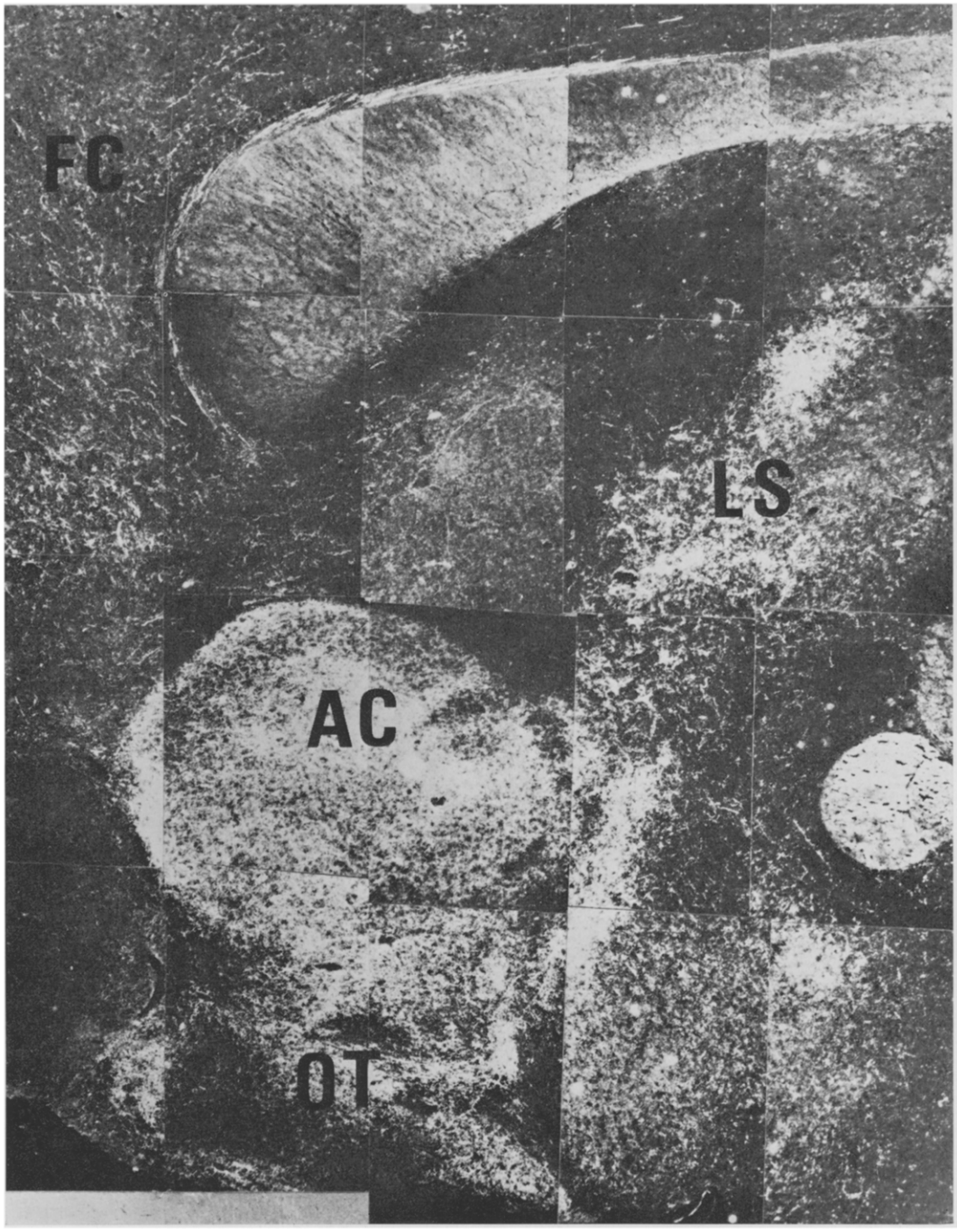

Fig. 4. Dark-field photomicrograph (montage) showing the anterior labelled structures from a brain cut in the sagittal plane. Note the abundant density of labelled terminals in the accumbens nucleus, olfactory tubercle and lateral nucleus of the septum. Sparsely distributed fibres and terminals can be observed in the prefrontal cortex, the cingular cortex and the cingulate bundle (cingulum).

labelled when the injections were placed in the VMT-A10 pars lateralis region (Table I). The labelling at anterior forebrain levels is represented in Fig. 4 by a photomontage obtained in dark-field illumination. Following injections in the VMT-A10 pars lateralis region, we can observe some labelled terminals bilaterally in the anterodorsal thalamic nucleus (Fig. 2h, i, j) and ipsilaterally in the lateral habenular nucleus (Fig. 2k, i). The lateral nucleus of the habenula seems to be innervated by two different pathways: the fasciculus retroflexus (Fig. 3c) and the stria medullaris (Fig. 3c, d). Table I summarizes the different intensities of the labelling obtained according to the various injection sites.

Descending projections

In contrast to the anterior projection results, we have not found important topographical differences in relation to the three types of injections performed. It seems that the majority of the descending projections follow the pathway of the mamillotegmental tract. However, some fibres take a lateral orientation and can be observed in the lateral lemniscus (Fig. 2r, s). The fibres following the pathway of the mamillotegmental tract are distributed essentially in and near the median plane. Numerous labelled terminals can be observed in the ventral tegmental nucleus, and in the dorsal and the median nuclei of the raphe (Fig. 2q, r). The dorsal part of the median nucleus of the raphe is the most intensively labelled. More posteriorly, massive labelling marks the dorsal tegmental nucleus and surrounding regions of the central grey substance on both sides (Fig. 2s). Ipsilaterally and also contralaterally, the locus coeruleus is very clearly labelled (Fig. 2u). Only ipsilaterally a moderately dense labelling appears in the median and lateral parabrachial nuclei (Fig. 2t). Finally, in the ipsilateral half of the cerebellum, widespread labelling marks not only the nuclei interpositus and dentatus (Fig. 2v) but also the granular and Purkinje cell layers of the cortex (Fig. 2u, v).

Retrograde transport studies

The peroxidase injection is centered in the VMT-A10, pars lateralis region (Fig. 1c, d) and its diameter does not exceed 100 μm. The posterior afferents only have been studied, apart from the lateral nucleus of the habenula which presents numerous labelled cells (Fig. 5a).

In the injection plane, we can note many labelled cells in the substantia nigra, located for the most part in the pars compacta (Fig. 5b). Posteriorly, we can find some HRP-positive cells in the dorsal and median nucleus of the raphe and in the ventral tegmental nucleus (Fig. 5d). Some labelled cells can also be observed in the pontine nucleus (Fig. 5c). More posteriorly labelled cells are scattered ipsilaterally and contralaterally in the central gray (Fig. 5d, e) and contralaterally in the locus coeruleus (Figs. 5f, g and 6a). Finally, numerous labelled cells have been shown to be present in the nucleus raphe magnus (Fig. 5g) and in the nuclei interpositus and dentatus of the cerebellum (Fig. 5h and 6b).

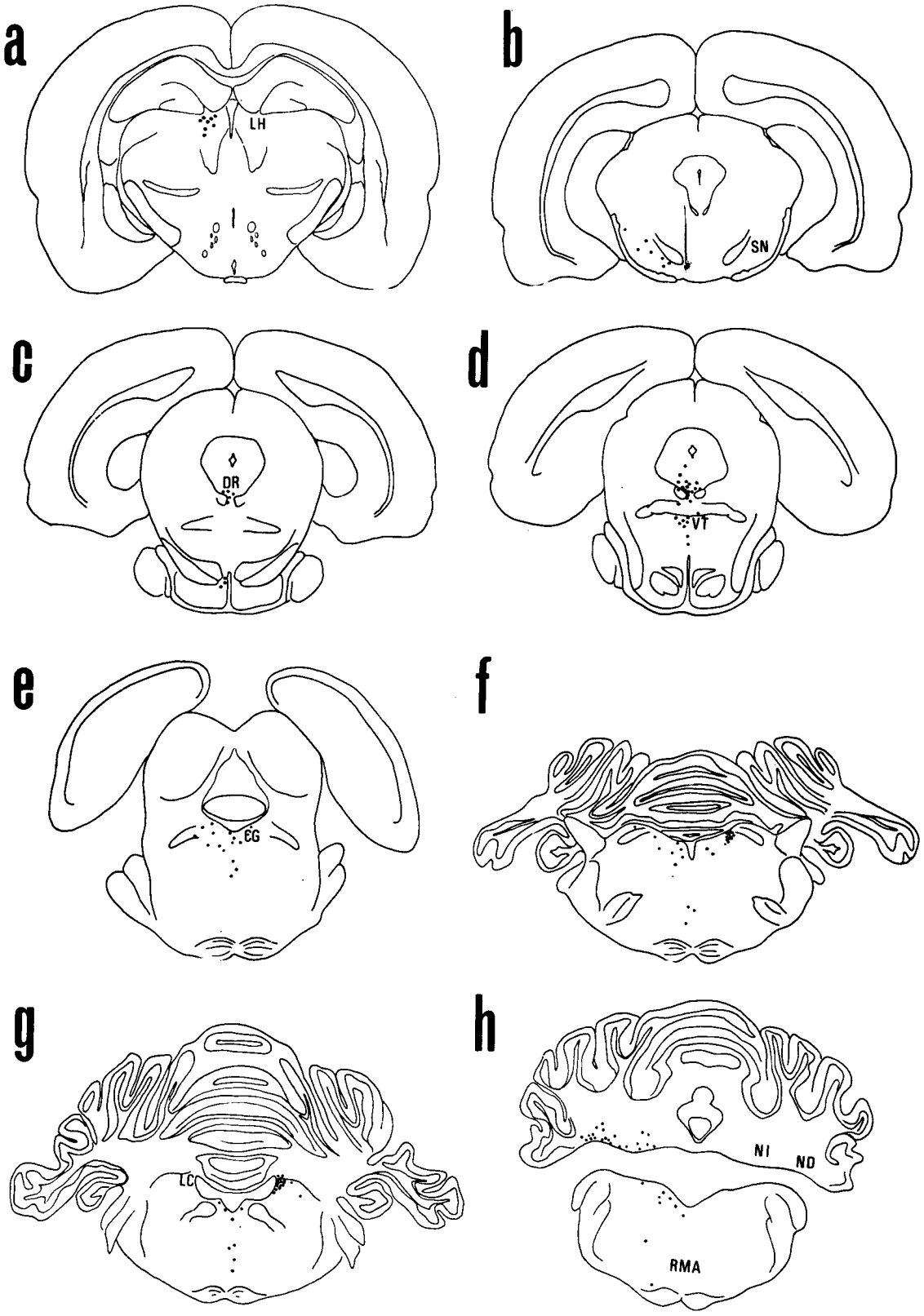

Fig. 5. Schematic representation of retrogradely labelled cells following HRP injection in the VMT-A10 region.

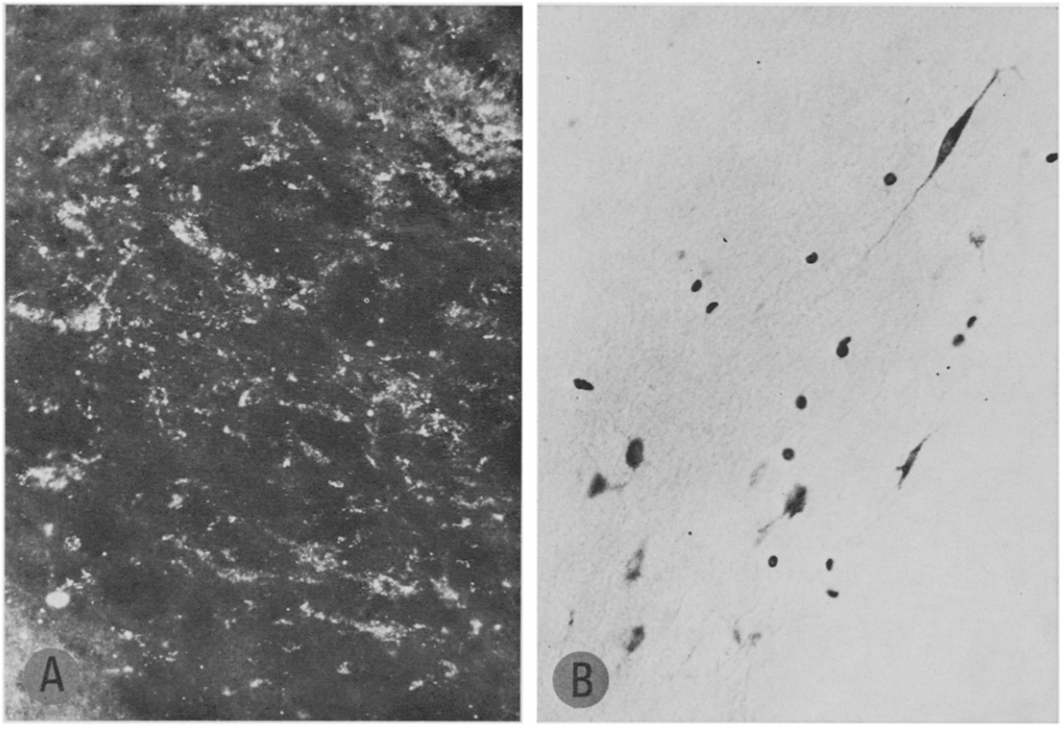

Fig. 6. Microphotographs of retrogradely labelled cells after HRP injection in the VMT-A10. A: locus coeruleus; B: nucleus dentatus of the cerebellum.

DISCUSSION

Anterograde transport studies

By using very localized injections of [^{3}H]leucine, we have been able to show the ascending and descending efferents of the VMT-A10 region, thus avoiding the fibre of passage problem occurring with the use of lesion experiments in combination with silver impregnating methods[65], histochemical methods[30,44,49,50,82], and biochemical methods[25,38,63,80]. Even though the [^{3}H]leucine does not reveal the biochemical nature of the demonstrated anatomical systems, it is important to be able to dissociate the dopaminergic efferents originating from the A10 cell bodies to the non-dopaminergic efferents originating from other cell bodies localized in the VMT. This is why the visualized projections will be discussed according to their presumed biochemical nature. All the anterior and posterior projections have been divided into three groups according to the data collected from the literature (Fig. 7). First, a group of projections are found in regions known to be rich in dopaminergic terminals. Even though the existence of some non-dopaminergic projections cannot be excluded in these regions, it would be reasonable to assume that these projections mainly correspond to the dopaminergic efferents of the A10 cell group. Second, a group of projections are found in the regions where some dopaminergic terminals may exist.

These projections represent the possible dopaminergic efferents of the A10 cell group. Finally a third group of projections are observed in regions in which dopaminergic terminals thus far have not been demonstrated. We will consider that it consists of projections corresponding to the non-dopaminergic neurones situated in the VMT region.

Projections to regions rich in DA terminals (Fig. 7a)

All these projections are ascendant. In agreement with a previous study by the silver impregnating method after 6-OHDA lesion of the VMT region[65], we found in this work projections to the accumbens nucleus, olfactory tubercle, the bed nucleus of the stria terminalis, the caudate nucleus, the prefrontal cortex, the cingular cortex and the entorhinal cortex. In addition, we found some projections to the central, medial, cortical, basal and lateral nuclei of the amygdala, the lateral nucleus of the septum, and the piriform cortex. In the present study we could confirm the existence of a

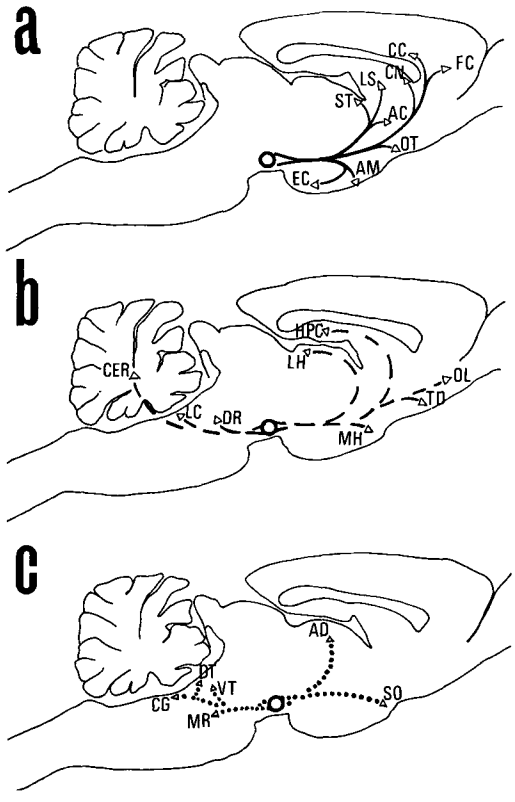

Fig. 7. Diagram summarizing in a sagittal plane the efferents of the VMT-A10 region, as demonstrated by [^{3}H]leucine experiment. These projections have been divided into three groups according to their presumed biochemical nature. a: projections to regions rich in DA terminals; b: projections to regions suspected of containing DA terminal regions; c: projections to regions not known to contain DA terminal regions. Note that bilateral distribution of some projections has not been represented in this diagram.

topographical mediolateral and anteroposterior organization of the dopaminergic A10 cell bodies in the VMT region (Table I) already suggested in 1976 (ref. 65) and noted recently also by other authors[28,46–49].

The projections to the accumbens nucleus, the olfactory tubercle and the bed nucleus of the stria terminalis correspond to the classical efferents of the A10 cell bodies (Table I) shown by histofluorescence methods[2,45–47]. A topographical arrangement of these neurones does not seem to exist as the labelling is similar regardless of the localization of the injection site in the VMT-A10 (medialis, lateralis or posterior). Additionally, our results showed that the caudate nucleus, only in its anteromedial part, receives efferents from the VMT-A10, pars lateralis region. This result, already suggested in 1976 (ref. 65), is in agreement with recent results of biochemical studies[14,78], and anatomical studies by the methods of retrograde[15,59] and anterograde tracer transport[28]. These projections give an anatomical basis for some recent notions concerning the physiology of the caudate nucleus[18,23,51]. The origin of the projections observed in the pre-frontal cortex and in the cingular cortex is in good agreement with our previous results as well as with those of Lindvall and Björklund[45–49]. The cell bodies of the VMT-A10, pars medialis project to the prefrontal cortex and to the lateral septal nucleus while the cingular cortex is innervated by the VMT-A10 cell bodies, pars lateralis and also probably by the A9 cell bodies[80], contrary to some other authors[4,15,30].

In agreement with several previous reports[5,15,28,30], it seems that the VMT-A10 pars lateralis projects to the entorhinal cortex and the amygdaloid complex in an anteroposterior organization (Table I). The piriform cortex also seems innervated by VMT-A10 cells, but this projection seems sparse, and restricted to the posterior part of the piriform cortex[28]. However, in the suprarhinal cortex, which is described as receiving the lateral dopaminergic extension of the fibres projecting to the prefrontal cortex from the A10 cells[6,7,46,47,49], we have not found any labelling. In our work, the lack of labelling, which is in agreement with the results of Beckstead[4], can be explained either by a minor innervation of the suprarhinal cortex, or by an innervation arising from the cell bodies situated outside the zone labelled by our [^{3}H]leucine injections.

The study of all these projections shows, on the one hand, that the injections centered on the VMT-A10 region, pars medialis, produced a labelling exclusively in the limbic structures and, on the other hand, that the injections centered on the VMT-A10 region, pars lateralis, labelled fibres to both limbic and striatal structures. As demonstrated by these two types of injection, our results suggest the existence of a mesolimbic pathway, originating from the VMT-A10, pars medialis and of a mesostriatal-mesolimbic pathway originating from the VMT-A10, pars lateralis.

Projection to regions suspected of containing DA terminals (Fig. 7b)

Ascending projections. In agreement with the results of Fallon and Moore[28], we have observed VMT-A10 projections to the medial, lateral and dorsal olfactory nuclei. However, contrary to these authors and for the first time in the rat, we have been able to show, in the olfactory nuclei, bilateral projections from the VMT-A10 region. Bilateral projections to the frontal cortex have been described by Avendaño et al.[3] in

the cat, but these crossed projections have not been found in the rat either by us or by other authors[28,47]. The nucleus of the tractus diagonalis, which receives numerous dopaminergic fibres[28,48–50] is abundantly innervated by the VMT-A10 region and therefore may constitute a supplementary structure of projection for the A10 neurones.

The VMT-A10 also projects to the lateral nucleus of the habenula. This result is confirmed by the VMT-A10 cell labelling after peroxidase injections in the habenula[34]. Furthermore, dopamine which is abundant in the habenula[36,47] falls 75% after lesion of the VMT-A10 region[38]. Contrary to Fallon and Moore[28], we observed an important quantity of labelled terminals in the medial hypothalamus and in the median eminence after injection in the VMT-A10 region. The medial hypothalamus is known to contain not only dopaminergic cell bodies but also dopaminergic terminals[9–11]. One important argument in favour of a dopaminergic A10 pathway to the medial hypothalamus and to the median eminence is given by Kizer et al.[38], who found a DA reduction of 40% after electrolytic lesion of VMT-A10. However, this effect may be due to an interruption of the DA fibres arising from more posteriorly situated cell bodies. Moreover Lindvall and Björklund[45,47] have suggested that some of the dopamine fibres in the medial hypothalamus could arise from the posterior extension of the A10 group. Even though this possibility cannot be excluded, it is clear from our work that the pathway originates from localized cell bodies surrounding the interpeduncular nucleus. It is also interesting to note that the distribution of the labelled terminals in the medial hypothalamus corresponds very closely to the distribution of the peptidergic neurones[12,35]. Very recently, Saavedra et al.[63] were unable to obtain a modification of the DA of the median eminence after an unilateral injection of 6-OHDA centered in the substantia nigra but also reaching the A10 neurones. This negative result can be explained by the fact that the biochemical analysis of the DA affects both sides of the median eminence, whereas the injection destroyed the A10 neurones only on one side; a decrease of the DA of the half of the median eminence ipsilateral to the injection site could have been masked by a compensating hyperactivity of the remaining half of the system. Furthermore, since these authors did not obtain any ipsilateral decrease of DA in the bed nucleus of the stria terminalis it could be suspected that the A10 neurones were not completely destroyed. In view of the important role of the medial hypothalamus and median eminence in the hormonal regulation mechanisms (for review see ref. 62), the existence of an afferent pathway from the VMT-A10 region suggested by our results deserves careful analysis. Finally, injections situated in the VMT-A10, pars posterior, allow us to observe a moderately intense labelling at the level of the internal edge and in the hilus region of the dentate gyrus of the hippocampus. The existence of dopamine as a transmitter in the hippocampus, and a fortiori the existence of an A10-hippocampus pathway, is far from being definitively demonstrated. However, anatomical, pharmacological, biochemical and behavioural data are in favour of such an hypothesis. Swanson and Hartman[76], based on an apparent lack of dopamine-β-hydroxylase in some regions of the hippocampus, suggested that many catecholamine containing fibres in the hippocampal formation, previously thought to be NA fibres, are actually DA fibres. According to

Swanson and Hartman[76] and Storm-Mathisen[75], the catecholamine fluorescent fibres in the fimbria, as well as those entering via the ventral route could be DA fibres. Bischoff et al.[8] reported that the amount of DOPAC is increased after systemic injections of haloperidol, and is decreased after injections of apomorphine. These authors also observe that 6-OHDA lesion at the level of the superior cerebellar peduncle of the locus coeruleus efferents innervating the hippocampus provokes a dissociated decrease in NA and DA concentration, respectively. These results suggest that the DA found in the hippocampus plays a role not only as a NA precursor. In another respect, Smialowski[69] observes that intrahippocampal injection of DA and of apomorphine, but not of NA, has a stimulating action on the electroencephalogram. This author suggests the existence of DA-sensitive receptors in the hippocampus. In agreement with this idea, Dolphin and Bockaert[24] have shown in the hippocampus an adenylate cyclase sensitive to DA and blocked by fluphenazine. The stimulation occurs in the presence of alprendol and is therefore not due to a stimulation of β-adrenergic receptors. The affinity of the receptors coupled at the DA sensitive cyclase is similar in the hippocampus to the affinity obtained in the cortex and in the caudate nucleus. Matthies[52] has shown that dopamine and apomorphine, but not intrahippocampal NA, influence positively the consolidation of the memory trace after a learning task in the rat.

DA and apomorphine are capable in vitro of provoking the same macromolecular changes which are observed in vivo in the hippocampus, for the same type of learning during the consolidation period of the memory trace. Therefore, these previously described experiments suggested the existence of DA terminals in the hippocampus but did not exclude the possibility that DA is linked to intrahippocampal interneurones. However, the destruction of the A10 neurones, and not of the A9 neurones, by 6-OHDA provokes a decrease of DOPAC in the hippocampus (Scatton, personal communication). Taking into account our results, the hypothesis of a dopaminergic A10 projection to the hippocampus is to be seriously considered.

Descending projections. We have found numerous labelled terminals coming from the cell bodies of the VMT-A10 region. According to Versteeg et al.[83], the dorsal nucleus of the raphe, known to contain serotoninergic cell bodies[21,82], contains an amount of DA as high as that of the substantia nigra. However, the technique used by these authors does not allow a differentiation of DA contained in cell bodies and DA localized within afferent fibres. Although the dorsal nucleus of the raphe seems to contain some DA cell bodies[45,47,58], it is possible that it also contains some DA terminals, as was already noted by Fuxe et al.[29]. In the present study, VMT-A10 efferents were found distributed bilaterally to the locus coeruleus. These results confirm and specify the data obtained by the silver impregnating technique combined with lesion of the VMT-A10 region[64]. The locus coeruleus contains a large amount of DA[83], probably represented by terminals. The existence of a dopaminergic A10 projection to the locus coeruleus could explain on the one hand the biochemical data paradoxically obtained after self-stimulation within the VMT-A10 region[53,74], and on the other hand, the role of dopamine in the self-stimulation obtained from the locus coeruleus region[19]. Finally, the present results suggest a significant VMT-A10

projection to the cerebellum, involving both the deep nuclei (interpositus and dentatus nuclei) and the cerebellar cortex (granular layer and Purkinje cell layer). These results confirm earlier, unpublished observations in a fibre-degeneration study (Simon and Le Moal). Dolphin and Bockaert[24] have shown that a DA-sensitive adenylate cyclase, as in the hippocampus, exists in the cerebellum. In addition, Kizer et al.[38] have obtained a 50% decrease of the DA concentration in the cerebellum by destroying the VMT-A10 region and the substantia nigra. Although these authors consider the entirety of the cerebellum, it is possible to admit the existence of a DA pathway from the A10 group to the deep nuclei and to the cerebellar cortex.

Projections to structure not known to contain DA terminals (Fig. 7c)

Rostral to the VMT-A10 region, we have observed bilateral projection to the anterodorsal thalamic nucleus. This nucleus, which is known to be innervated by the lateral mammillary nucleus[72], thus seems to be innervated also by the VMT region. Some ipsilateral projections were found in the supraoptic nucleus. This pathway is interesting because of the role of the supraoptic nucleus in the antidiuretic hormonal secretion[61].

Caudal to the VMTregion, we have observed some projections in the dorsal and ventral tegmental nucleus of Gudden, the central grey and the median nucleus of the raphe. These structures correspond to the limbic midbrain area of Nauta[57]. Thus, it is interesting to note that the VMT-A10 region is related to the limbic system, not only by its ascending, but also by its descending afferents.

Retrograde transport studies

After peroxidase injections in the VMT-A10 region, some labelled cells are observed in the substantia nigra, the median nucleus of the raphe and the ventral tegmental nucleus of Gudden. The structures containing the greatest number of labelled cells are the lateral nucleus of the habenula, the dorsal nucleus of the raphe, the locus coeruleus and the nuclei dentatus and interpositus of the cerebellum (Fig. 8). The lateral nucleus of the habenula projects through the fasciculus retroflexus to the interpeduncular nucleus and to some structures situated more posteriorly[1,20,34,59,60]. The present findings suggest the possibility that fibres originating from the habenula also terminate in the VMT-A10 region. The evidence of afferents arising from the dorsal nucleus of the raphe is compatible with the results obtained by anterograde transport[13,17,56,77] methods. The dorsal raphe being a structure rich in serotoninergic cell bodies, it is possible that the pathway terminating in the VMT-A10 region is of serotoninergic nature. This hypothesis is consistent with some preliminary findings showing a modification of the turnover of the A10 neurones after lesion of the dorsal nucleus of the raphe (Tassin and Simon, unpublished observations). A crossed pathway afferent to the VMT-A10 region is shown from the locus coeruleus[66]. The present evidence of this pathway is in agreement with the results of a recent anterograde tracing study[37]. In addition, Tassin et al.[79] have reported evidence of a regulatory effect of the locus coeruleus on A10 neurones. These results suggest the

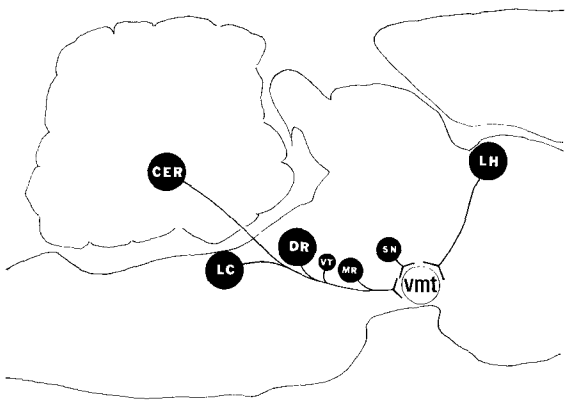

Fig. 8. Diagram summarizing, in a sagittal plane, the posterior afferents of the VMT-A10 region, as demonstrated by HRP experiments. The relative importance of these afferents, as indicated by the number of labelled cells, is schematized by the different sizes of the represented structures.

existence of a probably noradrenergic pathway from the locus coeruleus neurones to the dopaminergic cells of the A10 group. Finally, we have shown the existence of ipsilateral afferents arising from the deep nuclei of the cerebellum. In agreement with these results, degenerated terminals have been demonstrated in the VMT by the silver technique[16,70] following lesion of the deep nuclei of the cerebellum. In addition, Snider and Snider[71] have been able to show that this pathway of cerebellar origin modulates the activity of the dopaminergic neurones in the brain.

In interpreting the results of these retrograde labelling experiments one must take into account the limitations of the methods used. Indeed, in some cases, HRP may be taken up and transported not only by axons terminating at the injection site, but also by fibres passing through the injected area[22,33,34,40]. In addition, the peroxidase method cannot reveal the biochemical nature of either the afferent cell bodies shown, or the efferent cell bodies situated at the injection site. However, the utilization of the peroxidase has been largely developed these last few years, since it is an invaluable method for showing or suggesting the afferents of a given structure. All the labelled cells demonstrated in this work after injections in the VMT-A10 region provide a stimulating basis for future research concerning the regulation of A10 neurones. A first step in the study of these regulations is in progress. It consists in testing the effects of lesions of the major afferent structures shown in this work on the activity of the A10 neurones.

In proposing a complete anatomical study of the efferents and the afferents pathways of the VMT-A10 region, this work can help towards a better understanding concerning the physiology of these neurones.

ABBREVIATIONS

AC, Nucleus accumbens
AD, Anterodorsal thalamic nucleus
AL, Ansa lenticularis
AM, Amygdaloid complex
CA, Anterior commissure
CC, Cingular cortex
CG, Central grey
CL, Claustrum
CN, Caudate nucleus
CP, Posterior commissure
CS, Superior colliculus

DT, Dorsal tegmental nucleus
DR, Dorsal raphe nucleus
EC, Entorhinal cortex
F, Fornix
FC, Prefrontal cortex,
FR, Fasciculus retroflexus
HPC, Hippocampus
IP, Interpeduncular nucleus
LC, Locus coeruleus
LH, Lateral nucleus of the habenula
LL, Lateral lemniscus
LM, Medial lemniscus
LS, Lateral nucleus of the septum
ME, Median eminence
MH, Medial hypothalamus
MM, Mammillary nuclei
MR, Median raphe nucleus
ND, Nucleus dentatus
NI, Nucleus interpositus
NR, Red nucleus
OL, Olfactory nuclei
OT, Olfactory tubercle
PA, Preoptic area
PB, Parabrachial nucleus
PCS, Superior cerebellar peduncle
PN, Pontine nuclei
RMA, Nucleus raphe magnus
SM, Stria medullaris
SN, Substantia nigra
SO, Supraoptic nucleus
ST, Interstitial nucleus of the stria terminalis
TD, Nucleus of the tractus diagonalis
VMT, Ventral mesencephalic tegmentum
VT, Ventral tegmental nucleus
VII, Facial nerve
ZI, Zona incerta

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REFERENCES

1 Aghajanian, G. K. and Wang, R. Y., Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique, *Brain Research*, 122 (1977) 229–242.
2 Anden, N. E., Dahlström, A., Fuxe, K., Larsson, K., Olson, L. and Ungerstedt, U., Ascending monoamine neurons to the telencephalon and diencephalon, *Acta physiol. scand.*, 67 (1966) 313–326.
3 Avendaño, C., Reinoso-Suarez, F. and LLamas, A., Projections to gyrus sigmoïdeus from the substantia nigra in the cat, as revealed by the horseradish peroxidase retrograde transport technique, *Neurosci. Lett.*, 2 (1976) 61–65.
4 Beckstead, R. M., Convergent thalamic and mesencephalic projections to the anterior media cortex in the rat, *J. comp. Neurol.*, 166 (1976) 403–416.
5 Beckstead, R. M., Afferent connections of the entorhinal area in the rat as demonstrated by retrograde cell-labelling with horseradish peroxidase, *Brain Research*, 152 (1978) 249–264.
6 Berger, B., Tassin, J. P., Blanc, G., Moyne, M. A. and Thierry, A. M., Histochemical confirmation of dopaminergic innervation of the rat cerebral cortex after destruction of the noradrenergic ascending pathways, *Brain Research*, 81 (1974) 332–337.
7 Berger, B., Thierry, A. M., Tassin, J. P. and Moyne, M. A., Dopaminergic innervation of the rat prefrontal cortex: a fluorescence histochemical study, *Brain Research*, 106 (1976) 133–145.
8 Bischoff, S., Scatton, B. and Korf, J., Biochemical evidence for a transmitter role of dopamine in rat hippocampus, *Brain Research*, 165 (1979) 161–165.
9 Björklund, A., Lindvall, O. and Nobin, A., Evidence of an incerto-hypothalamic dopamine neurone system in the rat, *Brain Research*, 89 (1975) 29–42.
10 Björklund, A., Moore, R. Y., Nobin, A. and Stenevi, U., The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain, *Brain Research*, 51 (1973) 171–191.
11 Björklund, A. and Nobin, A., Fluorescence histochemical and microspectrofluorometric mapping of dopamine and noradrenaline cell groups in the rat diencephalon, *Brain Research*, 51 (1973) 193–205.

12 Bloom, F., Battenberg, E., Rossier, J., Ling, N. and Guillemin, R., Neurons containing β-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies, *Proc. nat. Acad. Sci. (Wash.)*, 75 (1978) 1591–1595.
13 Bobillier, P., Seguin, S., Petitjean, F., Salvert, D., Touret, M. and Jouvet, M., The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography, *Brain Research*, 113 (1976) 449–486.
14 Bockaert, J., Premont, J., Glowinski, J., Thierry, A. M. and Tassin, J. P., Topographical distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. II. Distribution and characteristics of dopamine adenylate cyclase. Interaction of D-LSD with dopaminergic receptors, *Brain Research*, 107 (1976) 303–315.
15 Carter, D. A. and Fibiger, H. C., Ascending projections of presumed dopamine containing neurons in the ventral tegmentum of the rat as demonstrated by horseradish peroxidase, *Neuroscience*, 2 (1977) 569–576.
16 Castro, A. J., Projections of the superior cerebellar peduncle in rats and the development of new connections in response to neonatal hemicerebellectomy, *J. comp. Neurol.*, 178 (1978) 611–628.
17 Conrad, L. C. A., Leonard, C. M. and Pfaff, D. W., Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study, *J. comp. Neurol.*, 156 (1974) 179–206.
18 Cools, A. R., Two functionally and pharmacologically distinct dopamine receptors in the rat brain. In E. Costa and G. L. Gessa (Eds.), *Nonstriatal Dopaminergic Neurons, Advanc. Biochem. Psychopharmacol., Vol. 16*, Raven Press, New York, 1977, pp. 215–235.
19 Cooper, B. R., Ervin, G. and Breese, G. R., A role for dopamine in the stimulant effects of amphetamine on locus coeruleus self-stimulation, *Pharmacologist*, 16 (1974) 666.
20 Cuello, A. C., Emson, P. C., Paxinos, G. and Jessell, T., Substance P containing and cholinergic projections from the habenula, *Brain Research*, 149 (1978) 413–429.
21 Dahlström, A. and Fuxe, K., 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.*, Suppl. 232 (1964) 1–55.
22 De Vito, J. L., Clausing, K. W. and Smith, O. A., Uptake and transport of horseradish peroxidase by cut end of the vagus nerve, *Brain Research*, 82 (1974) 269–271.
23 Divac, I., Markowistch, H. J. and Pritzel, M., Behavioral and anatomical consequences of small intrastriatal injections of kainic acid in the rat, *Brain Research*, 151 (1978) 523–532.
24 Dolphin, A. and Bockaert, J., β-Adrenergic receptors coupled to adenylate cyclase in cat brain: regional distribution, pharmacological characteristics and adaptative responsiveness. In E. Szabadi et al., (Eds.), *Recent Advances in the Pharmacology of Adrenoceptors*, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, in press.
25 Emson, P. C. and Koob, G., The origin and distribution of dopamine-containing afferents to the frontal cortex, *Brain Research*, 142 (1978) 249–267.
26 Fallon, J. H., Koziell, D. A. and Moore, R. Y., Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex, *J. comp. Neurol.*, 180 (1978) 509–532.
27 Fallon, J. H. and Moore, R. Y., Catecholamine innervation of the basal forebrain III. Olfactory bulb, anterior olfactory nuclei, olfactory tubercle and piriform cortex, *J. comp. Neurol.*, 180 (1978) 533–544.
28 Fallon, J. H. and Moore, R. Y., Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum, *J. comp. Neurol.*, 180 (1978) 545–580.
29 Fuxe, K., Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system, *Act physiol. scand.*, 64, Suppl. 247 (1965) 39–85.
30 Fuxe, K., Hökfelt, T., Johansson, O., Jonsson, G., Lidbrink, P. and Ljungdahl, A., The origin of the dopamine nerve terminals in limbic and frontal cortex: evidence for meso-cortico dopamine neurons, *Brain Research*, 82 (1974) 349–355.
31 Galey, D., Simon, H. and Le Moal, M., Behavioral effects of lesions in the A10 dopaminergic area of the rat, *Brain Research*, 124 (1977) 83–97.
32 Graybiel, A. M., Wallerian degeneration and anterograde tracer methods. In W. M. Cowan and M. Cuenod (Eds.), *The Use of Axonal Transport for Studies of Neuronal Connectivity*, Elsevier, New York, 1975, pp. 173–216.
33 Hedreen, J. C. and McGrath, S., Observations on labeling of neuronal cell bodies, axons and terminals after injection of horseradish peroxidase into rat brain, *J. comp. Neurol.*, 176 (1977) 225–246.

34 Herkenham, M. and Nauta, W. J. H., Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study with a note on the fiber of passage problem, *J. comp. Neurol.*, 173 (1977) 127–146.
35 Hökfelt, T., Elde, R., Fuxe, K., Johansson, O., Ljungdahl, A., Goldstein, M., Luft, R., Efendic, S., Nilsson, G., Terenius, L., Ganten, D., Jeffcoate, S. L., Rehfeld, J., Said, S., Perez de la Mora, M., Possani, L., Tapia, R., Teran, L. and Palacios, R., Aminergic and peptidergic pathways in the nervous system with special reference to the hypothalamus. In S. Reichlin, R. J. Baldessarini and J. B. Martin (Eds.), *The Hypothalamus*, Raven Press, New York, 1978, pp. 69–135.
36 Hökfelt, T., Johansson, O., Fuxe, K., Goldstein, M. and Park, D., Immunohistochemical studies on the localization and distribution of monoamine neuron system in the rat brain. II. Tyrosine hydroxylase in the telencephalon, *Med. Biol.*, 55 (1977) 21–40.
37 Jones, B. E. and Moore, R. Y., Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study, *Brain Research*, 127 (1977) 23–53.
38 Kizer, J. S., Palkovits, M. and Brownstein, M. J., The projections of the A8, A9 and A10 dopaminergic cell bodies: evidence for a nigral-hypothalamic-median eminence dopaminergic pathway, *Brain Research*, 108 (1976) 363–370.
39 König, J. F. R. and Klippel, R. A., *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*, Williams and Wilkins, Baltimore, md., 1963.
40 Kristensson, K. and Olsson, Y., Retrograde transport of horseradish peroxidase in transected axons. I. Time relationships between transport and induction of chromatolysis, *Brain Research*, 79 (1974) 101–109.
41 Le Moal, M., Cardo, B. and Stinus, L., Influence of ventral mesencephalic lesion on various spontaneous and conditioned behaviours in the rat, *Physiol. Behav.*, 4 (1969) 567–573.
42 Le Moal, M., Stinus, L. and Galey, D., Radiofrequency lesion of the ventral mesencephalic tegmentum: neurological and behavioural considerations, *Exp. Neurol.*, 50 (1976) 521–535.
43 Le Moal, M., Stinus, L., Simon, H., Tassin, J. P., Thierry, A. M., Blanc, G., Glowinski, J. and Cardo, B., Behavioral effects of a lesion in the ventral mesencephalic tegmentum: evidence for involvement of A10 dopaminergic neurons, In E. Costa and G. L. Gessa (Eds.), *Nonstriatal Dopaminergic Neurons. Advanc. Biochem. Psychopharmacol., Vol. 16*, Raven Press, New York, 1977, pp. 237–245.
44 Lindvall, O., Mesencephalic dopaminergic afferents to the lateral septal nucleus of the rat, *Brain Research*, 87 (1975) 89–95.
45 Lindvall, O. and Björklund, A., The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method, *Acta physiol. scand.*, Suppl. 412 (1974) 1–48.
46 Lindvall, O. and Björklund, A., Anatomy of the dopaminergic neuron systems in the rat brain. In P. J. Roberts, G. N. Woodruff and L. L. Iversen (Eds.), *Dopamine. Advanc. Biochem. Psychopharmacol., Vol. 19*, Raven Press, New York, 1978, pp. 1–23.
47 Lindvall, O. and Björklund, A., Organization of catecholamine neurons in the rat central nervous system. In L. L. Iversen, S. D. Iversen and S. H. Snyder (Eds.), *Chemical Pathways of the Brain. Handbook Psychopharmacol., Vol. 9*, Plenum, 1978, pp. 139–222.
48 Lindvall, O., Björklund, A. and Divac, I., Organization of mesencephalic dopamine neurons projecting to neocortex and septum. In E. Costa and G. L. Gessa (Eds.), *Nonstriatal Dopaminergic Neurones. Advanc. Biochem. Psychopharmacol., Vol. 16*, Raven Press, New York, 1977, pp. 39–46.
49 Lindvall, O., Björklund, A. and Divac, I., Organization of catecholamine neurons projecting to the frontal cortex in the rat, *Brain Research*, 142 (1978) 1–24.
50 Lindvall, O., Björklund, A., Moore, R. Y. and Stenevi, U., Mesencephalic dopamine neurons projecting to neocortex, *Brain Research*, 81 (1974) 325–331.
51 MacLean, P. D., Cerebral evolution and emotional processes: new findings on the striatal complex, *Ann. N.Y. Acad. Sci.*, 193 (1972) 137–149.
52 Matthies, H., *Learning and Memory, 7th Int. Congr. Pharmacol. (Paris)*, Pergamon Press, 1978.
53 Miliaressis, E., Thoa, N. B., Tizabi, Y. and Jacobowitz, D. M., Catecholamine concentration of discrete brain areas following self-stimulation in the ventromedial tegmentum of the rat, *Brain Research*, 100 (1975) 192–197.
54 Moore, R. Y., The catecholamine innervation of the basal forebrain. I. Septal area, *J. comp. Neurol.*, 77 (1978) 665–683.
55 Moore, R. Y. and Bloom, F. E., Central catecholamine neuron systems: anatomy and physiology of the dopamine systems, *Ann. Rev. Neurosci.*, 1 (1978) 129–169.

56 Moore, R. Y., Halaris, A. E. and Jones, B., Serotonin neurons of the midbrain raphe: ascending projections, *J. comp. Neurol.*, 180 (1978) 417–438.
57 Nauta, W. J. H. and Haymaker, W., Hypothalamic nuclei and fiber connections. In W. Haymaker, E. Anderson and W. J. H. Nauta (Eds.), *The Hypothalamus*, Thomas, Springfield, Ill., 1969, pp. 136–209.
58 Ochi, J. and Shimizu, K., Occurrence of dopamine-containing neurons in the midbrain raphe nuclei of the rat, *Neurosci. Lett.*, 8 (1978) 317–320.
59 Pasquier, D. A., Anderson, C., Forbes, W. B. and Morgane, P. J., Horseradish peroxidase tracing of the lateral habenular-midbrain raphe nuclei connections in the rat, *Brain Res. Bull.*, 1 (1976) 443–451.
60 Pasquier, D. A., Kemper, T. L., Forbes, W. B. and Morgane, P. J., Dorsal raphe substantia nigra and locus coeruleus: interconnections with each other and the neostriatum, *Brain Res. Bull.*, 2 (1977) 323–339.
61 Pickford, M., Neurohypophysis-antidiuretic (vasopressor) and oxytocic hormones. In W. Haymaker, E. Anderson and W. J. H. Nauta (Eds.), *The Hypothalamus*, Thomas, Springfield, Ill., 1969, pp. 463–505.
62 Reichlin, S., Baldessarini, R. J. and Martin, J. B., (Eds.), *The Hypothalamus*, Raven Press, New York, 1978, 490 pp.
63 Saavedra, J. M., Setler, P. E. and Kebabian, J. W., Biochemical changes accompanying unilateral 6-hydroxydopamine lesions in the rat substantia nigra, *Brain Research*, 151 (1978) 339–352.
64 Simon, H. and Le Moal, M., Demonstration by the Fink–Heimer impregnating method of a ventral mesencephalic-locus coeruleus projection in the rat, *Experientia (Basel)*, 33 (1977) 614–615.
65 Simon, H., Le Moal, M., Galey, D. and Cardo, B., Silver impregnation of dopaminergic systems after radiofrequency and 6-OHDA lesions of the rat ventral tegmentum, *Brain Research*, 115 (1976) 215–231.
66 Simon, H., Le Moal, M., Stinus, L. and Calas, A., Anatomical relationships between the ventral mesencephalic tegmentum A10 region and the locus coeruleus as demonstrated by anterograde and retrograde tracing techniques, *J. Neurol. Transmiss.*, 44 (1979) 77–86.
67 Simon, H., Le Moal, M., Stinus, L. and Calas, A., Afferents and efferents of the ventral mesencephalic tegmentum A10 region studied by using tracing techniques. In E. Usdin, I. J. Kopin and J. Barchas (Eds.), *Catecholamines: Basic and Clinical Frontiers*, Pergamon Press, New York, 1979, pp. 1322–1324.
68 Simon, H., Stinus, L., Christolomme, A. et Le Moal, M., Etude radioautographique préliminaire des efférences des neurones du mésencéphale ventral et du groupe dopaminergique A10, *J. Physiol. (Paris)*, 73 (1977) 56.
69 Smialowski, A., The effect of intrahippocampal administration of dopamine or apomorphine on EEG of limbic structures in the rabbit brain, *Pol. J. Pharmacol. Pharm.*, 28 (1976) 579–585.
70 Snider, R. S., Maiti, A. and Snider, S. R., Cerebellar pathways to ventral midbrain and nigra, *Exp. Neurol.*, 53 (1976) 714–728.
71 Snider, S. R. and Snider, R. S., Alterations in forebrain catecholamine metabolism produced by cerebellar lesions in the rat, *J. Neural. Transmiss.*, 40 (1977) 115–128.
72 Somogyi, G., Hadju, F., Tömböl, T. and Madarász, M., Limbic projections to the cat thalamus. A horseradish peroxidase study, *Acta anat. (Basel)*, 102 (1978) 68–73.
73 Stinus, L., Gaffori, O., Simon, H. and Le Moal, M., Small doses of apomorphine and chronic administration of D-amphetamine reduce locomotor hyperactivity produced by radiofrequency lesions of dopaminergic A10 neurons area, *Biol. Psychiat.*, 12 (1977) 719–732.
74 Stinus, L., Thierry, A. M., Blanc, G., Glowinski, J. and Cardo, B., Self-stimulation and catecholamines. III. Effect of imposed or self-stimulation in the area ventralis tegmenti on catecholamine utilization in the rat brain, *Brain Research*, 64 (1973) 199–210.
75 Storm-Mathisen, J., Localization of transmitter candidates in the brain: the hippocampal formation as a model, *Progr. Neurobiol.*, 8 (1977) 119–181.
76 Swanson, L. W. and Hartman, B. K., The central adrenergic system: an immunofluorescence study of the location of cell bodies and their afferent connections in the rat utilizing dopamine-β-hydroxylase as a marker, *J. comp. Neurol.*, 163 (1975) 467–506.
77 Taber Pierce, E., Foote, W. E. and Hobson, J. A., The efferent connection of the nucleus raphe dorsalis, *Brain Research*, 107 (1976) 137–144.
78 Tassin, J. P., Cheramy, A., Blanc, G., Thierry, A. M. and Glowinski, J., Topographical distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. I. Micro-

estimation of [^{3}H]dopamine uptake and dopamine content in microdiscs, *Brain Research*, 107 (1976) 291–301.

79 Tassin, J. P., Lavielle, S., Blanc, G. and Thierry, A. M., Decreased activity and increased arborizations of the rat mesocortical DA neurons after destruction of ascending NE bundles. In E. Usdin, I. J. Kopin and J. Barchas (Eds.), *Catecholamines: Basic and Clinical Frontiers*, Pergamon Press, New York, 1979, pp. 1762–1764.

80 Tassin, J. P., Stinus, L., Simon, H., Blanc, G., Thierry, A. M., Le Moal, M., Cardo, B. and Glowinski, J., Distribution of dopaminergic terminals in rat cerebral cortex: role of dopaminergic mesocortical system in ventral tegmental area syndrome. In E. Costa and G. L. Gessa (Eds.), *Nonstriatal Dopaminergic Neurons, Advanc. Biochem. Psychopharmacol., Vol. 16*, Raven Press, New York, 1977, pp. 21–28.

81 Thierry, A. M., Blanc, G., Sobel, A., Stínus, L. and Glowinski, J., Dopamine terminals in the rat cortex, *Science*, 182 (1973) 499–501.

82 Ungerstedt, U., Stereotaxic mapping of the monoamine pathways in the rat brain, *Acta physiol. scand.*, Suppl. 367 (1971) 1–48.

83 Versteeg, D. M. G., Van Der Gugten, J., De Jong, W. and Palkovits, M., Regional concentrations of noradrenaline and dopamine in rat brain, *Brain Research*, 113 (1976) 563–574.