Efferent Projections of the Retrorubral Nucleus to the Substantia nigra and Ventral Tegmental Area in Cats as Shown by Anterograde Tracing

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ABSTRACT: The aim of the present study was to determine whether the retrorubral nucleus projects to the dopaminergic nuclei in the ventral midbrain of the cat. For this purpose, injections of biotinylated dextran-amine or Phaseolus vulgaris-leucoagglutinin were placed into the retrorubral nucleus under stereotaxic guidance. The tracers were visualized by means of (immuno) histochemical procedures. In addition, tyrosine hydroxylase immunohistochemistry was used to evaluate the location of the injection sites and the distribution of the anterogradely labeled fibers. Both tracers reveal the same topography of labeled fibers in the ventral mesencephalon. Labeled fibers with varicosities were found ipsilaterally in the substantia nigra pars compacta, the substantia nigra pars lateralis, the ventral tegmental area and, contralaterally, in the substantia nigra pars compacta, the ventral tegmental area, and the retrorubral nucleus. A considerable number of labeled axons with varicosities were observed to be wrapped around the dendrites and perikarya of tyrosine hydroxylase-positive neurons in these areas. The present results are discussed in view of the possible role of the A8 dopaminergic cell group in the coordination of A9 nigrostriatal and A10 mesolimbic systems, as well as in the progressive pathophysiology seen in patients suffering from Parkinson's disease.

KEY WORDS: A8, A9, A10, Dopamine, Tyrosine hydroxylase immunohistochemistry, Phaseolus vulgaris-leucoagglutinin, Biotinylated dextran-amine.

INTRODUCTION

Parkinson's disease is a progressive neurological disorder, characterized by the degeneration of dopaminergic neurons in the midbrain. This degeneration of neurons appears to proceed, despite the improvement of neurological symptoms following treatment with routine anti-Parkinson medication. Why the dopaminergic neurons cease to function and eventually die is as yet unknown.

The dopaminergic neurons in the midbrain are distributed over three major cell groups: the substantia nigra pars compacta (SNC, A9), the ventral tegmental area (VTA, A10), and the retrorubral nucleus (RRN, A8). The RRN contains a large number of dopaminergic neurons in the tegmentum, located dorsally and caudally to the SNC [1,3,11,25,32].

The A8 cell group differs in a number of aspects from the other dopaminergic cell groups. First, the striatal projection areas of A8 cells play a role in the control of facial musculature in cats [42]. In this context, it is relevant to note that an early sign in Parkinson's disease is masking of facial expression [7]. If the A8 neurons in humans have a function similar to that in cats, this may imply that A8 cells degenerate early in the disease. Second, it has been reported [12] that, in primates, A8 dopaminergic neurons have a higher vulnerability to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as compared to the other dopaminergic cell groups [cf. refs. 40, 47]. Animals intoxicated with this drug display an unequivocal parkinsonian syndrome. This, again, suggests an important role for A8 cells in the degeneration seen in Parkinson patients. Third, the three mentioned cell groups collectively give rise to the mesotelencephalic dopaminergic pathway which displays a broad topographical organization [5,13,26,43,45]. The A9 cell group primarily innervates the dorsolateral "motor" part of the caudate nucleus and putamen, whereas the A10 cell group gives rise to fibers which predominantly terminate in the ventral, "limbic" striatum, i.e., nucleus accumbens and olfactory tubercle [15,35]. In contrast, the A8 dopaminergic efferents innervate both the "motor" and the "limbic" striatum [6,13,14,17,26,35,44,45]. As such, the A8 cell group can control the activity in these two striatal domains. Fourth, both the projections from the SNC to the dorsal striatum and the projections from the VTA to the ventral striatum are reciprocal [24,27,35], suggesting that the A9 and A10 nuclei receive direct feedback from their respective target areas in the striatum. In contrast, the dorsal striatal A8-innervated region has no projection back to the A8 cell group, implying that there does not exist such a feedback loop [19,20,27,30,37, see however ref. 39]. The ventral striatal target region of the A8 cell group has relatively sparse projections to A8 cells [2,19,22,35]. Finally, it

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has been suggested that A8 cells directly innervate A10 cells, at least in rats [13]. If the latter could be confirmed, it is evident that degeneration of A8 cells will have far-reaching consequences for the functioning of A10 cells.

The aim of the present study was to determine whether there exist projections from the RRN to the A9 and A10 dopaminergic cell groups in the cat. For this purpose, injections of the tracers biotinylated dextran-amine (BDA) and Phaseolus vulgaris-leucoagglutinin (PHA-L) were placed in the retrorubral nucleus.

MATERIALS AND METHODS

Thirteen adult male cats (13–16 months old, 4.3–5.2 kg) were used in the present study. Two cats were perfused for tyrosine hydroxylase (TH)-immunohistochemical analysis of the ventral mesencephalon. Two cats received an injection of the anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) [18], and nine cats received an injection of the tracer biotinylated dextran-amine (BDA) [46]. In addition, these nine cats were also processed for a TH-immunohistochemical staining.

Surgery

Prior to surgery, the animals were anaesthetized with a mixture of O2 and 5% Éthane® (Abbott BV, The Netherlands) and subsequently intubated. The animals received an injection of Albopen® LA (Mycopharm Nederland BV, The Netherlands; 20 mg/kg SC) to prevent infection and Atropin Sulfa® (Pharmachem BV, The Netherlands; 0.5 mg/4.5 kg IM), to prevent mucous secretion. Surgical levels were maintained with a mixture of O2, 3–3.5% Éthane® and N2O.

The animals were unilaterally injected with either 0.3 µl BDA (Bruuschwig Chemie, The Netherlands; 1% solution in distilled water) or 0.3 µl PHA-L (Bruuschwig Chemie, The Netherlands; 1% solution in distilled water) aimed at the RRN. Coordinates with respect to the interaural line were: anterior (+) 2.8 mm (with an angle of 10°, tip pointing caudal), lateral 2.8 mm, and ventral (−) 5.7 mm according to the atlas of Snider and Niemer [41]. The tracers were delivered by means of a 1 µl syringe (Hamilton Bonaduz AG, Switzerland) at a speed of 0.02 µl/min. Survival times ranged between 6 and 9 days.

General Fixation Procedure

The cats were anaesthetized with a lethal dose of pentobarbital, IP (Narcovet®, Aphrmo, The Netherlands) and perfused transcardially with 500 ml saline followed by 1 l fixative, which consisted of 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4), either with (in case of TH-staining alone) or without (in case of double staining) 0.2% of a saturated picric acid solution. After this fixation procedure, the cats were finally perfused with 500 ml of a 10% sucrose solution in the same fixative. The brains were removed and stored in a 30% sucrose solution in PB for 3 days at 4°C. Thereafter, transverse sections were cut at either 60 µm (BDA tracer) or 40 µm (PHA-L tracer) on a freezing microtome and collected in 8 vials (providing 8 parallel series of sections) containing a 30% sucrose solution in PB to store at −20°C or in PB for further (free-floating) processing (modified procedure from Spooren et al.) [42].

Immunohistochemical Staining

Sections from the mesencephalon of the non-operated cats (n = 2) were stained for tyrosine hydroxylase-immunoreactivity (TH) according to Spooren et al. [42]. Following repeated rinses in PB and subsequently in Tris-buffered saline (TBS; pH 7.4, 0.05 M), the sections were treated with a 1% H2O2 in TBS to reduce endogenous peroxidase. After rinsing (standard procedure: 3 × 15 min), the sections were incubated for 18 h in the primary antibody (a monoclonal mouse anti-TH-antisera, Incstar Corp. Nijmegen, MN, USA, 1:750), diluted in supernoxin (0.05 M TBS, 0.5% Triton-X-100, 0.25% gelatine, pH 7.4). Thereafter, the sections were rinsed with TBS, and incubated for 1 h in the secondary antibody (goat anti-mouse IgG, Dakopatts, Denmark; 1:50) diluted in supernoxin. Subsequently, the sections were again rinsed in TBS and incubated for 1 h in rat peroxidase anti-peroxidase complex (PAP, Nordic Immunology, The Netherlands; 1:100 in supernoxin). After rinsing the sections three times in TBS and lastly in PB, the sections were treated with nickel-enhanced diaminobenzidine (DAB, 12.5 mg in 25 ml 0.1 M PB, pH 7.4 with 1 ml 2% ammonium-Ni-sulphate and 6.6 µl H2O2) for 11 min. Finally, the sections were mounted and coverslipped with Entellan® (Merek, The Netherlands).

BDA and PHA-L Staining

In the tracing experiments, 1 out of 4 sections were stained for either BDA (n = 9; sections 240 µm apart) or PHA-L (n = 2; sections 160 µm apart). Following a pretreatment with a 1% H2O2 solution in TBS for 10 min, the sections containing BDA were incubated using an avidin biotin complex-staining (ABC, Elite PK-6100, Brunschwig Chemie, The Netherlands) for 1 hour. Prior to the ABC-staining, the sections containing PHA-L tracer were incubated with a biotinylated anti-PHA-L antibody (affinity purified, raised in goat, Brunschwig Chemie, The Netherlands; 1:2000 in supernoxin) at 4°C overnight. Thereafter, the sections were treated with nickel-enhanced DAB to stain the labeled fibers and injection site, resulting in a black reaction product. These sections were further processed for the double-staining with antibodies against tyrosine hydroxylase. The tyrosine hydroxylase-immunoreactive neurons were visualized as described above (see Immunohistochemical Staining); however, the end-product was turned brown by means of the DAB reaction (12.5 mg DAB in 20 ml Tris-HCl, 6.6 µl 30% H2O2). Throughout the staining procedures, the rinses between the incubations were in TBS.

RESULTS

Tyrosine Hydroxylase-Positive Cell Groups

A brief overview will be presented of the different tyrosine hydroxylase-immunoreactive midbrain areas as identified in this study (Fig. 1A–D).

Retrorubral nucleus. The A8 cell group is located within the RRN as designated by Berman [3]. Rostrally, this nucleus is situated medial to the medial lemniscus and ventral to the red nucleus. At this level, the A8 cells appear as a cell-bridge joining the TH-positive neurons of the A10 cell group and the A9 neurons of the SNC (Fig. 1B). More caudally, two subdivisions are distinguished within the RRN [16]; a dorsal (A8d) and a ventral part (A8v; Fig. 1C,E). The dorsal subdivision appears as a rather diffuse population of neurons, while the ventral subdivision contains a more densely packed neurons. The A8v extends more caudally than the SNC(dz) (Fig. 1D).

Substantia nigra. The substantia nigra is composed of three divisions: pars compacta (SNC), pars lateralis (SNL), and pars reticulata (SNR) [3]. The pars compacra consists of at least two parts [26], a densocellular zone (SNC(dz)), which is characterized by its densely packed neurons and finger-like extensions, and a cell-sparse region (SNC(cs)), which is located rostrally, dorsolateral to the SNC(dz) [17A–C,F].
FIG. 1. (A)–(D): Photomicrographs of transverse sections taken at three different rostrocaudal levels through the feline ventral mesencephalon, illustrating the distribution of TH-positive neurons. (A) rostral section; (D) caudal section. The distance between the sections shown in (A) and (B), and (C) and (D) is 1.4 mm, and between (B) and (C) is 1.7 mm. The primary subdivisions are the VTA, the SNC(cs) and SNC(dz), SNL, and the A8d and A8v. (E) High-power photomicrograph of the section shown in (C), illustrating the difference in distribution of TH-positive neurons in the dorsal and ventral part of the RRN. (F) High-power photomicrograph of the section shown in (C). The SNC(dz) and A8v are separated by the medial lemniscus. (G) Photomicrograph of a double staining, consisting of a TH staining with a representative BDA injection site (93-11), located in the dorsolateral part of the RRN. III, oculomotor nerve; A8d, dorsal subdivision of the A8 cell group; A8v, ventral subdivision of the A8 cell group; IP, interpeduncular nucleus; I.C, linearis centralis; LR, linearis rostralis; ML, medial lemniscus; NIF, nucleus interfascicularis; PBP, parabrachialis pigmentous; PN, nucleus paranigralis. RN, red nucleus; RRN, retrornubral nucleus; SNC(cs), cell-sparse zone of the SNC; SNC(dz), densocellular zone of the SNC; SNL, substantia nigra pars lateralis; SNR, substantia nigra pars reticulata. Bar in (D) = 750 μm and also applies to (A)–(C). Bar in (E) = 100 μm and also applies to (F). Scale bar in (G) = 250 μm.
The pars lateralis of the substantia nigra (SNL) forms a cluster of rather loosely packed cells and is located ventral to the medial lemniscus and dorsolateral to the SNC (Fig. 1A,B). The SNR comprises a large area located ventral to the SNC and SNL (Fig. 1A,B). This area is almost devoid of TH-stained neurons; however, it contains many TH-positive dendrites and axons, originating from the TH-immunoreactive cells of the SNC.

**Ventral tegmental area.** The A10 cell group is distributed over five nuclei, collectively referred to as ventral tegmental area (VTA) [23,36]. Three nuclei are arranged along the midline: the nucleus linearis rostralis (LR), the nucleus linearis centralis (LC), and the more ventrally located nucleus interfascicularis (NIF). Two groups are located more laterally: the nucleus parabrachialis pigmentosus (PB) dorsally and the nucleus paranigralis (PN) ventrally (Fig. 1A-C).

**Anterograde Tracing Experiments.**

The location and extent of the injection sites of the BDA (n = 9; 93-11, 93-30, 93-31, 93-35, 93-36, 93-37, 93-43; controls: 93-14, 93-42) and PHA-L (n = 2; 94-343; control: 93-316) in different parts of the RRN and adjacent structures are schematically represented in Figure 2. The majority of BDA injection sites are ovoid in shape with an intensely stained centre varying between 300 and 600 µm in diameter (Fig. 1G, corresponding to Fig. 3E). In a few cases in which the centre of the BDA injection site is located in the reticular formation dorsal to the RRN, the peripheral part of the injection site extends medially and ventrally into the RRN (cases 93-36 and 93-43). The BDA injections in the rostromedial RRN result in labeling of nearby dendrites and perikarya located in the SNC (Fig. 3). The PHA-L injection site in the RRN has a more compact appearance, with a smaller peripheral zone. No labeled perikarya were seen outside the PHA-L injection site.

Following all injections of BDA and PHA-L in the RRN, three types of labeled fibers can be distinguished in the ventral mesencephalon. The first type of fibers is smooth and very thin (with a diameter less than 1 µm; Fig. 4A). The second type of labeled fibers, with approximately the same diameter as the first type, carries numerous varicosities (Fig. 4A). These varicosities have diameters slightly larger than the interconnecting axonal segments and are 1.7–2.1 µm long. The distance between the varicosities ranges from 5–20 µm. The third type of fibers is rather coarse and smooth (Fig. 4B). These fibers are ribbon-shaped (approximately 4–6 µm in width and 2.5 µm thick). Most of these fibers traverse the midline and bend ventrally and caudally following crossing.

**RRN-Efferents Within the Ventral Mesencephalon.**

Following the PHA-L injection in the RRN, a pattern of labeled fibers is found in the mesencephalon that does not differ from the pattern seen following the BDA experiments.

In view of the fact that several of the injection sites primarily located in the RRN also involve parts of the overlying reticular formation, the pattern of labeling following injections in the reticular formation just dorsal to the RRN will be briefly described first. Three cats received such an injection in the reticular formation, dorsal to the RRN (cats 93-14, 93-42, and 94-316). In cat 93-14, sparse labeling of the fibers with varicosities is seen in the ipsilateral A9 and A10 cell groups, whereas the A8, A9, and A10 cell groups contralateral to the injection site are devoid of labeled axons. In cats 93-42 and 94-316, no terminal labeling is seen ipsi- or contralaterally in the three TH-positive areas. In these three cases, however, the above-mentioned coarse fibers are most prominent. These fibers are directed primarily in a caudal direction and do not appear to terminate in the SNC. Since the present study focuses on the afferents of the dopaminergic cell groups in the ventral mesencephalon, the following description will concentrate on the distribution of the thin fibers with or without varicosities, ignoring the coarse fibers (Fig. 4A-B).

Cat 93-11, with a BDA injection in the most caudal part of the RRN, displays a representative fiber pattern in the ventral mesencephalon (Fig. 3). From the injection site, fibers run either in a medial direction through the RRN to the VTA, or they traverse the medial lemniscus and extend ventrally into the ipsilateral A9. At caudal levels, thin fibers are present predominantly in the SNC(dz). An example of a labeled fiber with varicosities oriented along the dendrites of a TH-positive neuron located in the most caudal part of the SNC is given in Figure 5A. More rostrally, fibers can be distinguished in the medial and lateral regions of the SNC and in the substantia nigra pars lateralis (SNC; Figs. 4A, 5B). Fiber and terminal labeling display similar patterns in the ipsi- and contralateral A10 cell groups. In the most caudal part of the A10 cell group, densest labeling is found in the central linear nucleus and the nucleus paranigralis (Fig. 5C,D). At more rostral levels, fibers are confined to the nucleus paranigralis, parabrachialis pigmentosus, and nucleus rostralis linearis (Fig. 5E,F) of the A10 cell group. Contralaterally to the injected side, terminal labeling has the highest density in the caudomedial SNC. More rostrally, axons with varicosities are present in the contralateral medial and lateral SNC, but not in the SNC. A few labeled fibers can be traced to the contralateral A8 (Fig. 5G,H). Most fibers are concentrated in the ventral part, namely A8v.

Following smaller injections in the RRN (e.g., cat 93-43), the overall pattern of termination in the ventral mesencephalon is similar to that in cases 93-11 and 94-343. However, the number of labeled fibers is smaller.

Compared with the injections described above, a small injection of BDA (case 93-30), which was placed in the rostral part of the RRN, results relatively in the largest number of labeled fibers. These fibers traverse the medial lemniscus and distribute over the entire ipsilateral SNC, including the SNL (Fig. 4A). Moreover, the dorsolateral part of the A10 cell group, both ipsi- and contralaterally, is densely labeled, whereas the medial A10 contains only sparse labeling. Contralaterally, labeled fibers are found in the RRN, both in its caudal and rostral parts. No fibers can be traced to the lateral part of the A9 cell group.

Considering all injections in which the RRN is involved, the total number of anterogradely labeled fibers with varicosities is rather small, however, they bear a specific relationship with dendrites and cell bodies of TH-positive neurons. In most cases, these axons appear to have close appositions with the dendrites and cell bodies of the TH-positive neurons. In some cases, the labeled axons have the same orientation as the dendrites (Fig. 5A,B,H). This relationship between the retrorubral varicose axons with somata and proximal dendrites of TH-positive neurons is most prominent at the ipsilateral side in the SNC(dz) and SNL. At the contralateral side in the A8, similar phenomena are seen (Fig. 5G,H). In the ipsi- and contralateral VTA, labeled fibers with varicosities are present in the neuropil, however less frequently in close apposition with TH-positive neurons (Fig. 5C-F).

**RRN-Efferents to the Tenecephalon.**

Several labeled, thin fibers with or without varicosities are found in the lateral part of the caudal nucleus, the nucleus accumbens, olfactory tubercle, hypothalamus, and thalamus (data not shown).
FIG. 2. Schematic drawings of transverse sections to show the location of the injection sites, ordered from rostral (A) to caudal (D). On the left side of each section, the BDA (A–D) and PHA-L (B’,C’) injection sites are depicted. On the right side of each section, the different TH-positive cell groups are delineated. The number of each cat is abbreviated in the drawings (316 corresponds with 94-316, and 11 corresponds with 93-11). A8, A8 cell group; ML, medial lemniscus; RN, red nucleus; SNC(cs), cell-sparse zone of the SNC; SNC(dz), densocellular zone of the SNC; SNL, substantia nigra pars lateralis; SNR, substantia nigra pars reticulata; VTA, ventral tegmental area.

DISCUSSION

Until now, little has been reported on the efferent connections of the retrombular nucleus (RRN) to the other dopaminergic cell groups within the ventral mesencephalon. One indication that such connections exist in rats is the description by Lindvall and Björklund [32] of the course of the ascending catecholaminergic fibers in the mesencephalon. Tegmental radiations from the dor-
FIG. 3. Schematic representation of the distribution of anterogradely labeled thin fibers with varicosities in the ventral mesencephalon following an injection of BDA in the RRN (cat 93-11), illustrating the BDA labeling, seen at six different rostrocaudal levels. (A) rostral section; (F) caudal section. The injection site is also depicted. Dots mark the location of a presumptively retrogradely labeled neurons outside the injection site. III, oculomotor nerve; ML, medial lemniscus; RN, red nucleus.
sal tegmental bundle run through the A8 cell group and are at this level supplemented by catecholaminergic fibers originating in the A8. Together, these fibers run in a ventromedial direction through the medial lemniscus and the SNC and, subsequently, bend in rostromedial direction to run through the VTA. However, Lindvall and Björklund [32] did not describe a pattern of terminating fibers. Experimental evidence for efferents of the RRN to the VTA has been reported by Deutch et al. [13]. Their study in rats shows that fibers originating in the RRN and ascending to the forebrain travel through the SNC and VTA. Moreover, they described terminal specializations in the VTA and contralateral RRN.

The results of the present study show that also in cats there are neurons in the RRN that give rise to fibers traversing the medial lemniscus and the SNC, and terminating in the VTA and contralateral RRN. In addition, the present results clearly demonstrate the existence of terminal fibers in the ipsilateral SNL and the ipsi- and contralateral SNC. Moreover, the labeled fibers in these areas appear to establish a rather frequent close apposition on dendrites and cell bodies of TH-positive neurons.

Methodological Considerations

Not all injections used in the present study are strictly confined to the RRN, but in a number of cases, the injection site extends into the dorsally located reticular formation. However, injections which are largely confined to the reticular formation almost exclusively give rise to the coarse fibers which traverse the midline without terminating in the TH-positive cell groups of the ventral mesencephalon. By contrast, injections that, in addition to the reticular formation involved the RRN, result in labeling of the three types of fibers described in the Results, thus including the very thin axons with or without varicosities. Accordingly, primarily the latter two types are considered as efferent fibers of the RRN.

Following BDA injections in the RRN, a few labeled neurons are found in the SNC, which was included in the peripheral zone of the injection site. Theoretically, these labeled neurons may have contributed to the pattern of anterograde labeling seen in the present study. However, given the small number of labeled perikarya in the SNC, this is unlikely. This conclusion is supported by the fact that following the PHA-L injection in the RRN, which did not include any part of the SNC, the pattern of anterograde labeling in the dopaminergic cell groups is not different from that following BDA injections.

Uptake by passing fibers cannot be fully excluded for either of the tracers used in the present study [8,21,38,46]. However, for PHA-L, which is mainly transported in the anterograde direction, this phenomenon of uptake by passing fibers has been considered a minor phenomenon [18,21]. Since the pattern of labeled fibers and terminals in the areas of interest of the present study resulting from a BDA injection did not differ significantly from those following PHA-L injections, we tentatively conclude that uptake of BDA by passing fibers also poses a minor problem in the interpretation of the results of the present study.

Efferents of the Retrorubral Nucleus

The location of the dopaminergic cell groups in the ventral mesencephalon is consistent with previous observations in cats [27,29,42]. Following injections of either BDA or PHA-L into the RRN, labeled fibers with or without varicosities are found in the ipsilateral SNL, the ipsi- and contralateral SNC, VTA, and RRN. Although the resulting number of anterogradely labeled fibers is relatively small, these fibers appear to be close to, or even wrapped around, the dendrites and perikarya of TH-positive neurons in these areas. Therefore, it is concluded that there exists a highly specific relationship between the RRN efferent fibers and the dopaminergic cells in the SNC, VTA, and contralateral RRN.

The present experimental approach does not allow the conclusion that RRN efferents to the latter nuclei are dopaminergic. As indicated by Swanson [43], the RRN in rats is heterogeneous with respect to the neurotransmitter identity of the neurons that compose this nucleus; certainly not all neurons are dopaminergic [31,34]. Cholinergic afferents to the substantia nigra appear to have also quite close contacts with the proximal dendrites and the cell bodies of the dopaminergic neurons [4]. It must be further noted that cholinergic neurons have been located within the lateral parts of the nigral complex in rats [28,33].

Although the RRN contributes to the dopaminergic containing innervation of the caudate nucleus and also to other parts of the ascending mesencephalic dopaminergic projection, only a relatively small number of labeled fibers could be traced to the
FIG. 5(A)-(H) High magnification photomicrographs of double stained sections showing examples of the relationships between anterogradely labeled fibers from the RRN and TH-immunostained perikarya and dendrites in different parts of the ventral mesencephalon (cat 94-343). (A) a TH-positive neuron in the ipsilateral, and most caudal part of the SNC(du), which is apposed by a PHA-L labeled fiber. (B) a neuron located in the ipsilateral SNL. (C)-(H) The left photomicrographs provide an overview to show the location of the PHA-L fibers which are shown by a higher magnification in the right photomicrographs. (C)-(D) PHA-L labeled axons and terminals in the nucleus paranigralis of the A10 cell group. (E)-(F) PHA-L labeled fibers in the nucleus rostralis linears of the A10 cell group. (G)-(H) a neuron located in the contralateral RRN (indicated by arrow head), just bordering the A10 cell group, which is apposed by a PHA-L labeled fiber with varicosities. Bar in (A), (B), (F), (H) = 40 µm. Bar in (C), (G) = 500 µm. Bar in (D) = 125 µm. Bar in (E) = 75 µm.
caudate nucleus. However, it should be noted that previous descriptions of these projections have been based on different tracing techniques, i.e. the anterograde tritiated amino acid tracing [26, 27] or retrograde tracing [42]. It is possible that PHA-L and BDA used in the present study, are less suitable to visualize small calibre mesostriatal fiber systems over such long distances in cats. This implies that the present study does not answer the question whether and how the pattern of labeling in the TH-positive cell groups in the ventral mesencephalon, originating in the RRN, is related to the projections ascending to the forebrain.

Functional Considerations

One can only speculate on the function of these RRN efferents to the SNc and VTA. The present data suggest that the RRN can directly influence the activity of the A9 and A10 cells. This has two far-reaching consequences. First, the RRN may play a crucial role in coordinating the function of the dopaminergic A9 and A10 systems, each of which have their own specific function in regulating cognitive and motor behavior [9, 10]. As discussed elsewhere in detail [9], there appears to exist a delicate balance and interplay between both systems. The present study implies that the RRN, including the A8 cells, may be involved in the modulation of this balance and interplay. Second, degeneration of A8 cells may have a strong impact on the functioning of the dopaminergic cell groups. Once A8 cells start to degenerate, there are no, or only slight, feedback mechanisms from the striatal regions to counteract these disturbances (see Introduction). If this is indeed true, degeneration of A8 cells may play a key role in the progression of the pathology seen in Parkinson's disease.

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