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DIFFERENTIAL PROJECTIONS TO THE DORSAL AND VENTRAL STRIATUM FROM THE DOPAMINERGIC, NORADRENERGIC, SEROTONERGIC AND CHOLINERGIC NEURONAL GROUPS OF THE BRAIN STEM. A RETROGRADE TRACING STUDY IN THE RAT

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Abstract

The striatum is divided into two parts: dorsal striatum, implicated in the motor neuronal circuitry of the basal ganglia, and ventral striatum (nucleus accumbens), affiliated to the limbic system. We investigated the differential afferent connections to both striatal divisions from the dopaminergic, noradrenergic, serotonergic and cholinergic neuronal groups of the rostral brain stem by means of retrograde axonal transport of the fluorescent tracer Fluoro-Gold. The dopaminergic neurons of the ventral midbrain tegmentum emit a mighty projection to either of the striatum parts. The dorsal striatum is innervated by numerous neurons in the ventral tier of substantia nigra, pars compacta and pars lateralis and by only few scattered neurons in the ventral tegmental

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area. Conversely, numerous neurons in the ventral tegmental area send axons to the nucleus accumbens, whilst few cells in the dorsal tier of pars compacta innervate the ventral striatum. Only occasional noradrenergic neurons in locus ceruleus project to the dorsal striatum, whereas the ceruleal projection to the ventral striatum is substantial. The serotonergic dorsal raphe nucleus innervates the entire striatum. The dorsal striatum receives axons from cells concentrated near the midline in the rostral sector of the dorsal raphe nucleus. The projection to the ventral striatum is stronger and is emitted by numerous neurons throughout the dorsal raphe nucleus. In contrast to the profuse innervation of substantia nigra, subthalamic nucleus and globus pallidus by the cholinergic reticular formation of the mesopontine tegmentum, the projection to the striatum is moderate. The cells of origin are mainly located in pars dissipata of the pedunculopontine tegmental nucleus that innervates the dorsal striatum, and in the laterodorsal tegmental nucleus that sends axons to the nucleus accumbens.

Key words: dorsal raphe nucleus, Fluoro-Gold, locus ceruleus, nucleus accumbens, pedunculopontine nucleus, substantia nigra, ventral tegmental area

Introduction. Traditionally the striatum is described as containing three structures. First two of them are the caudate nucleus and putamen which are separated by the internal capsule. The basal portions of these two nuclei fuse ventrally by the internal capsule and form the third striatal portion, the nucleus accumbens, also called "fundus striati". Through the last few decades a considerable body of evidence from tract-tracing, physiological, and neurological studies suggests another subdivision of the striatum. The caudate nucleus and putamen comprise the dorsal (extrapyramidal) striatum, implicated in the motor neuronal circuitry of the basal ganglia, whilst the nucleus accumbens appears to be affiliated to the limbic system and is regarded as ventral (limbic) striatum [1, 2]. The largest accumulations of dopaminergic, noradrenergic and serotonergic neurons are located in the rostral brain stem, as initially described by DAHLSTRÖM and FUXE ([3]; for comprehensive reviews see [1, 4–6]). In addition, in the pontomesencephalic tegmentum two prominent cholinergic groups are situated ([7]; broadly reviewed in [8]). Here we report that these four neuronal populations with different transmitter characteristics have differential projections to the dorsal and ventral striatum.

Material and methods. All procedures were carried out according to a standard protocol established by the Ethic Commissions at the Medical University of Sofia, and at the University of Rostock, Germany. Eight male Wistar Albino rats weighing 220–260 g were used. The deeply anaesthetized animals were mounted in a David Kopf stereotaxic apparatus in the flat skull position. Using a Hamilton syringe the rats received 0.5–0.6 µl 2% Fluoro-Gold (FG) in the dorsal striatum ($n = 4$) and in the nucleus accumbens ($n = 4$). The coordinates were obtained from the atlas of PAXINOS and WATSON [9]. After survival time of 4 days, the animals were deeply anaesthetized and perfused transcardially with

100 ml phosphate buffered saline, followed by 500 ml 4% paraformaldehyde in phosphate buffer (PB; pH 7.2), and finally with 100 ml of the same fixative to which 5 g sucrose was added. The brains were removed and stored in 20% sucrose in the same fixative for 2–30 days at 4 °C until they sunk. Serial sections were cut at a thickness of 30 μm on a Reichert Jung freezing microtome and stored in PB overnight at 4 °C. The sections were mounted on chrome alum gelatin coated slides and allowed to dry overnight at room temperature. Thereafter, they were completely dehydrated in 100% ethanol, cleared in xylene and coverslipped with Entellan. The sections were observed in Nikon and Leitz Aristoplan fluorescent microscopes, equipped with filter set with excitation length of 350–395 nm. Photomicrographs of selected fields were taken with a digital camera (7.3 three Shot Colour, Visitron Systems, Diagnostic Instruments) and saved in TIF format.

Results. The FG injections in the dorsal striatum were successfully placed in four experiments. In one experiment the injection focus invaded ventrally the nucleus accumbens, and this case was completely excluded. In a second experiment there was a light spillage of the fluorescent dye upon the overlying cortex. This case was also excluded from systematic examination, although it provided a useful additional control concerning the projection of locus ceruleus to the cerebral cortex. In two experiments the injection focus was limited to the dorsal striatum, and only the periphery of the injection halo reached ventrally the nucleus accumbens (Fig. 1*a*). Similarly, in all cases with FG injections in the nucleus accumbens the focus was placed successfully. However, two cases with larger injection foci were excluded due to involvement of the dorsal striatum and the bed nucleus of stria terminalis. In the remaining two cases the injection focus in the nucleus accumbens was selective (Fig. 1*b*), and only the periphery of the injection halo reached the dorsal striatum and the septum (dorsally), and the olfactory tubercle (ventrally).

In both experimental groups a strong retrograde labelling in the dopaminergic nuclei of the ventral mesencephalon was observed though the pattern of labelling was clearly different. Following the tracer injection in the dorsal striatum the great majority of nigrostriatal neurons was encountered in the ventral, larger tier of substantia nigra, pars compacta (Fig. 1*c*). Many of these neurons emitted robust dendrites that descended ventrally in substantia nigra, pars reticulata. Retrogradely labelled neurons, organized in discrete patches, were observed in substantia nigra, pars lateralis as well. Immediately dorsally to the medial part of substantia nigra, pars compacta, a substantial number of fluorescent neurons was present in the parabrachial pigmented nucleus. Substantia nigra, pars reticulata contained no labelled neurons, except for solitary fluorescing neurons in its ventral border, adjacent to the cerebral peduncle. In the ventral tegmental area relatively few, scattered retrogradely labelled neurons were visible. Labelled neurons were not seen in the ventromedial part of the ventral tegmental area. Most of the cells

in the ventral tegmental area appeared with smaller dimensions compared with the cells of substantia nigra, pars compacta. Few retrogradely labelled neurons appeared in the ventral tier of substantia nigra, pars compacta contralateral to the injection. Their number was approximately 4% of the neurons in the ipsilateral substantia nigra, pars compacta. Only occasionally labelled neurons (hardly more than 0.5%) were revealed in substantia nigra, pars lateralis, and in the ventral tegmental area, contralateral to the striatal injection. Following injection of FG in the nucleus accumbens the entire ventral tegmental area contained retrogradely labelled neurons (Fig. 1*d*). Such were also present in the interfascicular nucleus located in the midline. Few labelled neurons were seen in the medial part of the ventral tegmental area, contralateral to the injection in the nucleus accumbens (to the left in Fig. 1*d*). Their number was approximately 3% of the labelled neurons in the ipsilateral ventral tegmental area. In substantia nigra, pars compacta only few labelled neurons were seen, mainly in its medial portion, adjacent to the ventral tegmental area. The labelled cells were located in the dorsal portion of pars compacta, and often the elongated perikarya extended in mediolateral direction.

Fig. 1*a*. A selective injection focus in the medial portion of the dorsal striatum. The necrotic tissue in the centre of the focus was removed during the histological preparation. Ventrally the periphery of the injection halo reaches the nucleus accumbens (Acb). CC – corpus callosum; LV – lateral ventricle; Spt – septum. Scale bar – 500 μm →

Fig. 1*b*. A selective injection focus in the nucleus accumbens. The injection halo reaches but not infiltrates the septum, striatum (Str) and substantia innominata, and only slightly infiltrates the dorsomedial portion of the olfactory tubercle (OT). Aco – anterior commissure. Scale bar – 500 μm

Fig. 1*c*. Pattern of retrograde neuronal labelling in the ventral mesencephalic tegmentum following injection of FG in the dorsal striatum, as shown in Fig. 1*a*. Numerous strongly fluorescing cells in the ventral tier of substantia nigra, pars compacta (SNc). Their dendrites descend ventrally in substantia nigra, pars reticulata (SNr). Only few neurons in the dorsal tier of substantia nigra, pars compacta are retrogradely labelled (*arrows*). Several labelled neurons are also present in substantia nigra, pars lateralis (SNl). SNr contains no labelled neurons, except for a single cell at its ventral border. In the ventral tegmental area (VTA) comparatively few labelled neurons are present. The aggregation of labelled neurons dorsal to the medial part of SNc represents the parabrachial pigmented nucleus. Scale bar – 300 μm

Fig. 1*d*. Pattern of retrograde neuronal labelling in the ventral mesencephalic tegmentum following injection of FG in the nucleus accumbens, shown in Fig. 1*b*. Numerous fluorescing neurons fill the entire ventral tegmental area. The accumulation of small, intensively fluorescing neurons in the midline, at the ventral brain surface, is the interfascicular nucleus (If) that remains unlabelled in Fig. 1*a*. In substantia nigra only few labelled neurons are present in the dorsal tier of substantia nigra, pars compacta. To the left – several fluorescing neurons in the VTA contralateral to the injection. Scale bar – 300 μm

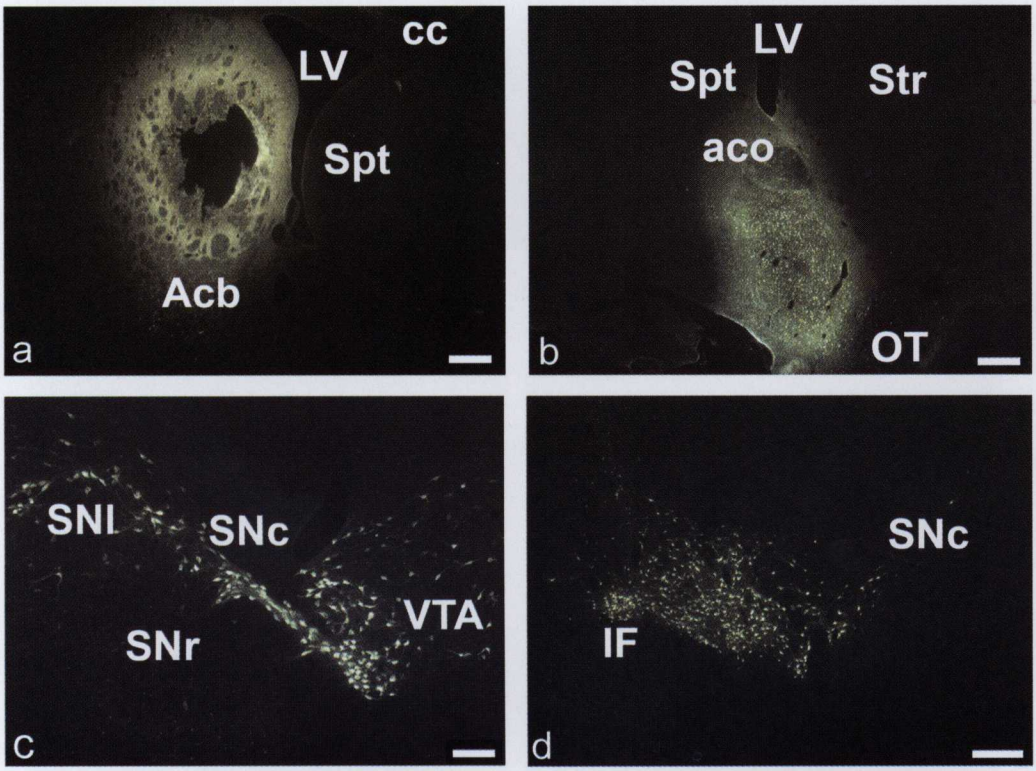
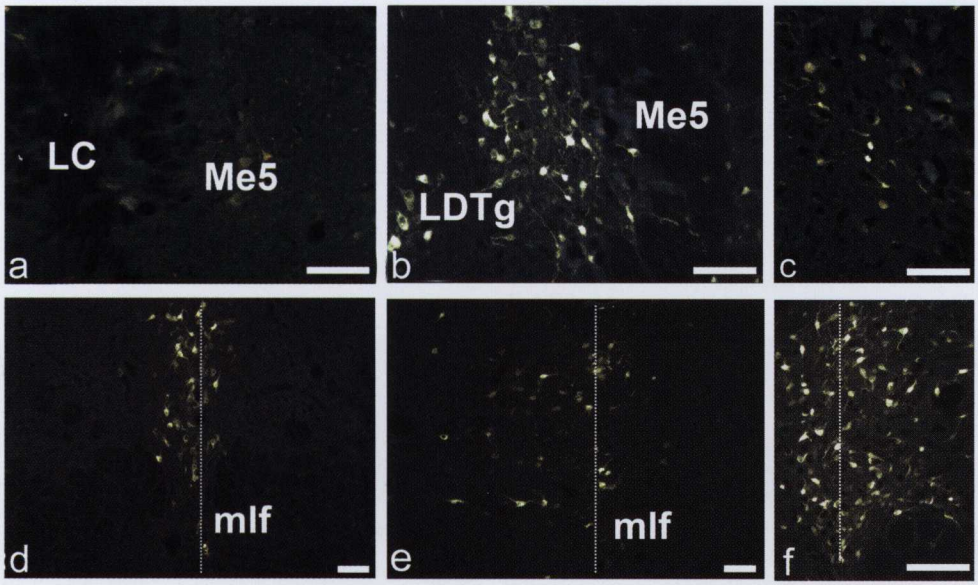


Fig. 1


LC
Me5
a
Me5
LDTg
b
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mlf
e
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Fig. 2

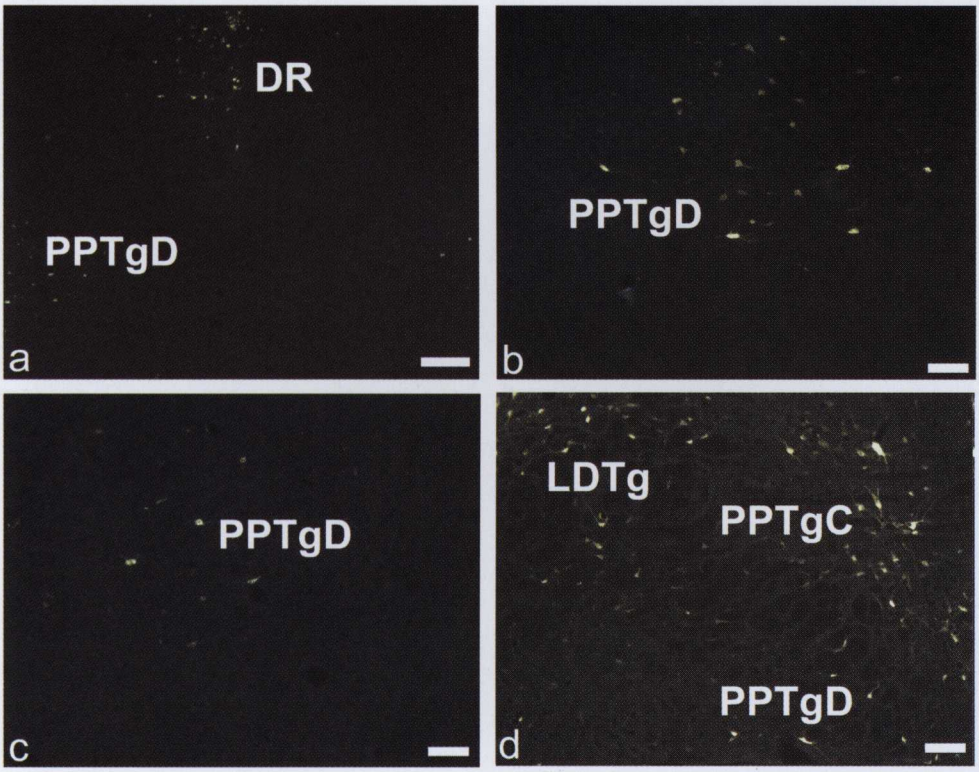
DR
PPTgD
a
PPTgD
b
PPTgD
c
LDTg
PPTgC
PPTgD
d

Fig. 3

← Fig. 2*a*. Few weakly fluorescing neurons in locus ceruleus, ipsilateral to the injection of FG in the dorsal striatum. Me5 – mesencephalic nucleus of the trigeminal nerve. Scale bar – 100 μm

Fig. 2*b*. A substantial number of retrogradely labelled neurons in locus ceruleus, ipsilateral to the injection of FG in the nucleus accumbens. The group of fluorescing neurons in the lower left corner belongs to the laterodorsal tegmental nucleus (LDTg) (see also Fig. 3*d*). Scale bar – 100 μm

Fig. 2*c*. Scattered fluorescing neurons in the locus ceruleus, contralateral to the injection of FG in the nucleus accumbens. Scale bar – 100 μm

Fig. 2*d*. Retrogradely labelled neurons in the most rostral part of the dorsal raphe nucleus following FG injection in the dorsal striatum. The fluorescing neurons are located close to the midline (the dashed line), and are slightly more numerous ipsilateral to the injection (to the left). mlf – medial longitudinal fasciculus. Scale bar – 100 μm

Fig. 2*e*. A detail from Fig. 3*a*. Scattered retrogradely labelled neurons in the central (most voluminous) part of the dorsal raphe nucleus following FG injection in the dorsal striatum. The dashed line indicates the midline. Ipsilateral to the injection (to the left) the labelled neurons reach the lateral border of the nucleus, whereas contralateral to the injection the fluorescing cells remain in the medial part of the dorsal raphe nucleus. Scale bar – 100 μm

Fig. 2*f*. Numerous retrogradely labelled neurons in the dorsal raphe nucleus following a tracerinjection in the nucleus accumbens. Scale bar – 100 μm

Fig. 3*a*. Low magnification microphotograph of the caudal mesencephalic tegmentum. In the upper part of the figure is the dorsal raphe nucleus (DR), shown at higher magnification in Fig. 2*e*. In the lower part of the figure the moderate bilateral retrograde labelling in pars dissipata of the pedunculopontine nucleus (PPTgD) is seen. Scale bar – 300 μm

Fig. 3*b*. A detail from Fig. 3*a*. Retrogradely labelled neurons in pars dissipata of the pedunculopontine nucleus, ipsilateral to the injection in the dorsal striatum. Scale bar – 100 μm

Fig. 3*c*. A detail from Fig. 3*a*. Retrogradely labelled neurons in pars dissipata of the pedunculopontine nucleus, contralateral to the injection in the dorsal striatum. Scale bar – μm

Fig. 3*d*. Retrograde labelling in the cholinergic neuronal groups of the rostral pontine tegmentum following injection of the tracer in the ipsilateral nucleus accumbens. Medial is to the left. PPTgC – pars compacta of the pedunculopontine nucleus. Scale bar – 100 μm

No labelled cells were seen in substantia nigra, pars lateralis, as well as in the contralateral substantia nigra.

The findings in locus ceruleus (the largest accumulation of noradrenergic neurons in the brain) were also very different. In the cases with selective injection in the dorsal striatum only very few labelled neurons were seen in the ipsilateral locus ceruleus: 1–3 per section, and not in every one (Fig. 2*a*). Moreover, all retrogradely labelled neurons were pale. Notably, in the case with the spillage of FG upon the overlying cortex an appreciable number of retrogradely labelled neurons appeared in locus ceruleus (see the Discussion). Following the tracer injection in the nucleus accumbens an unexpectedly large number of retrogradely labelled neurons was encountered in the ipsilateral locus ceruleus (Fig. 2*b*), and an appreciable number of labelled cells was present in locus ceruleus contralateral to the injection in the nucleus accumbens (Fig. 2*c*).

The selective injections of the tracer in the dorsal striatum were followed by a characteristic labelling in the dorsal raphe nucleus (the largest accumulation of serotonergic neurons in the brain). In the rostral pole of the dorsal raphe nucleus the retrogradely labelled neurons were concentrated towards the midline (Fig. 2*d*), and ipsilaterally to the injection, the number of fluorescing cells was larger. Moving caudally, at the level of the maximum extent of the dorsal raphe nucleus (Fig. 2*e*), the labelled neurons were scattered over the entire territory of the nucleus ipsilateral to the injection, whereas contralateral to the injection their number was small and the cells were located near the midline. The caudal portion of the dorsal raphe nucleus was free of retrograde labelling. The projection to the nucleus accumbens appears to be stronger. All sectors of the dorsal raphe nucleus contained retrogradely labelled neurons. The largest number of fluorescing neurons was encountered in the central portion of the nucleus (Fig. 2*f*). Ipsilateral to the injection a significant number of retrogradely labelled neurons was evenly distributed over the entire territory of the dorsal raphe nucleus. An appreciable number of labelled neurons was also observed contralaterally, although here the tendency of concentration of fluorescing cells towards the midline was present as well.

Following injection of FG in the dorsal striatum a moderate to scant number of retrogradely labelled neurons was seen bilaterally in the pedunculopontine nucleus (the cholinergic Ch5 group of Mesulam, first described in [7]). The fluorescing cells were located in pars dissipata of the pedunculopontine nucleus (Figs. 3*a*, *b*, *c*). The ipsilateral connection (Fig. 3*b*) was stronger than the contralateral one (Fig. 3*c*), although the contralaterally projecting neurons reached approximately 35–40% of the pedunculopontine neurons projecting to the ipsilateral dorsal striatum. In pars compacta of the pedunculopontine nucleus few labelled neurons were seen ipsilateral to the injection, and only occasional cells were found to project to the contralateral dorsal striatum. In the laterodorsal

tegmental nucleus (the cholinergic Ch6 group of MESULAM [7]) no labelled neurons were encountered. Unexpectedly strong cholinergic projection to the nucleus accumbens was found. Ipsilateral to the injection an appreciable number of labelled neurons was seen in pars compacta of the pedunculopontine nucleus (Fig. 3*d*) and in the laterodorsal tegmental nucleus (Figs 2*b*, 3*d*), while in pars dissipata of the pedunculopontine nucleus the number of fluorescing neurons was moderate (Fig. 3*d*). The contralateral cholinergic groups also contained labelled neurons (not shown) that accounted for approximately 20% of the neurons innervating the ipsilateral nucleus accumbens.

Discussion. The present results clearly indicate that the projections of the monoaminergic and cholinergic neuronal groups of the mesopontine tegmentum towards the dorsal (extrapyramidal) and ventral (limbic) striatum are differential, indeed sometimes significant.

The differential projections of the dopaminergic groups are already known. Our prior Nauta and Fink-Heimer silver impregnation study [10] on the nigrostriatal connection in the cat was among the first investigations suggesting that substantia nigra, pars compacta (the A9 group of Dahlström and Fuxe [3]) projects to the caudate nucleus and putamen, whereas the ventral tegmental area (group A10) projects to the nucleus accumbens. In the years to come the highly effective anterograde and retrograde axonal transport tracing methods were introduced, and the differential nigrostriatal and mesolimbic connections were elucidated ([11, 12], see [1, 2, 4] for comprehensive reviews). Thus, the first part of the present study is largely confirmatory, and the original results concern mainly details. However, we found it reasonable to present these data because they unequivocally demonstrate the reliability of the results to be discussed below.

It is a common belief that the striatum is the only structure that receives no noradrenergic innervation. In 1995 ASTON-JONES et al. [13] stated "Of all the major structures of the brain the striatum is uniquely immune to noradrenergic innervation". In 2004 ASTON-JONES [5] slightly modified the previous explicit negation: "Noradrenergic innervation is very sparse or absent in most areas of the striatum. In fact, the dorsal striatum stands out as perhaps the only major brain region essentially devoid of noradrenergic fibres". Only very recently CASTELINO et al. [14] presented data on the noradrenergic innervation of area X of the medial striatum in male songbirds. The parallel between the avian and mammalian basal ganglia is difficult, and the mentioned "area X" combines both striatal and pallidal features. Here we report evidence for a weak projection of locus ceruleus (the noradrenergic group A6 of Dahlström and Fuxe [3]) to the dorsal striatum. Only very few cells of the densely packed neuronal population of locus ceruleus emit striopetal axons. As a rule, locus ceruleus neurons were faintly fluorescing. Probably, the scant striatal innervation is carried out by collaterals of cerulean axons that profusely innervate the cerebral cortex [1, 5, 13]. The noradrenergic in-

nervation of the ventral striatum has relatively recently been demonstrated [5, 15]. These authors stated that the primary source of the noradrenergic innervation of the nucleus accumbens is the A2 group [3], located on the territory of nucleus solitarius in medulla oblongata, with lesser contributions of locus ceruleus and the A1 group [3] located in the ventrolateral medulla oblongata. We presently report a significant innervation of the ventral striatum by locus ceruleus that has also a moderate contralateral component.

Since the first immunohistochemical mapping of serotonergic systems [6] it is well known that the entire striatum is profusely innervated by serotonergic axons. However, there are only few data on the topical distribution of the raphe nuclei of origin and the striatal zones of termination [16, 17]. The present data suggest a differentiation of the cells of origin giving rise to striopetal serotonergic axons. The projection to the dorsal striatum is emitted mainly from the rostral sector of the dorsal raphe nucleus and the striopetal neurons are concentrated in the median region. More caudally these neurons are dispersed throughout the dorsal raphe nucleus, and the caudal pole of the nucleus does not emit axons to the dorsal striatum. All sectors of the dorsal raphe nucleus contain neurons that innervate the nucleus accumbens, and their number is very large in the central part of the dorsal raphe nucleus, bilaterally. The mighty innervation of the limbic striatum is expected since the dorsal raphe nucleus serotonin system has been implicated in acute responses to stress and stress-related psychiatric disorders such as anxiety and depression ([17] and references therein).

The reticular formation of the mesopontine tegmentum contains two cholinergic nuclei: pedunculopontine nucleus, Ch5 group and laterodorsal tegmental nucleus, Ch6 group [7]. The pedunculopontine nucleus is presently considered as a member of the basal ganglia family because it has prominent, often – reciprocal connections with substantia nigra, the subthalamic nucleus and the pallidum, and is implicated in the motour neuronal circuitry (broadly reviewed in [8]). The possible innervation of the striatum was persistently searched but the results were nearly negative. Only following the introduction of highly effective anterograde tracers a moderate number of axons emitted by neurons in the pedunculopontine nucleus was demonstrated in the caudate nucleus and putamen of monkeys [18]. We expand the data of LAVOIE and PARENT [18], reporting that the sites of origin of the connection to the dorsal striatum are located bilaterally in pars dissipata of the pedunculopontine nucleus.

Our data on the substantial innervation of the nucleus accumbens by the laterodorsal tegmental nucleus are, however, unexpected. Previous investigations [19, 20] indicated that this cholinergic nucleus innervates several structures of the limbic forebrain, but a projection to the nucleus accumbens was not mentioned. Our injections in the nucleus accumbens reach the medial forebrain bundle. Thus, some of the labelled neurons in the laterodorsal tegmental nucleus might emit

axons to several structures adjacent to the nucleus accumbens: the lateral septal nucleus, medial prefrontal cortex, and the preoptic area.

Further, more refined experiments might help us to unequivocally define the newly described projection.

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