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A re-examination of the striatal input from the mediodorsal thalamic nucleus in the rat

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SUMMARY

The projection from the mediodorsal thalamic nucleus to the striatum in the rat was studied by placing injections of the retrograde tracer Fluoro-Gold into the caudate putamen and small deposits of biotinylated dextran amine (BDA) into the mediodorsal thalamic nucleus. The relationship of thalamic afferent fibers with the compartmental organization of the striatum was studied by combining (BDA) tracing and enkephalin immunohistochemistry. The medial segment of the mediodorsal thalamic nucleus projects to the nucleus accumbens whereas the lateral segment projects sparsely to the medial regions of the caudate putamen. The caudal pole of the mediodorsal thalamic nucleus was found to project abundantly to ventrolateral regions of the precommissural caudate putamen, reaching areas of strong enkephalin immunoreactivity. The dorsolateral and caudal regions of the striatum did not receive projections from the mediodorsal thalamic nucleus. The present findings demonstrate a topographical organization of the thalamostriatal projections originating in the mediodorsal thalamic nucleus of the rat.

Key Words: Basal ganglia – Thalamus – Thalamostriatal projections – Fluoro-Gold – BDA – Enkephalin – Immunohistochemistry

INTRODUCTION

The thalamic midline and intralaminar nuclei have long been recognized as the main source of the thalamostriatal projections (Newman and Winans, 1980; Veening et al., 1980; Hazlett and Bagley, 1983; Beckstead, 1984; Phillipson and Griffiths, 1985; Jarayaman, 1985; Takada et al., 1985; Christie et al., 1987; Berendse and Groenewegen, 1990; Nakano et al., 1990). However, there is some discrepancy concerning the existence of thalamostriatal projections from the mediodorsal thalamic nucleus (MD) in different animal species. Using retrograde tracers, projections from the MD to the ventral striatum were described in *Macaca Mulatta* and in *Macaca Nemestrina* (Giménez-Amaya et al., 1995) whereas labeled neurons were rarely seen in the MD after HRP injections into the dorsal striatum of *Macaca Fuscata* (Nakano et al., 1990). Several studies have revealed the existence of a projection from the MD to the caudate nucleus in the cat (Royce, 1978; Sato, 1979; Heras de las et al., 1998), which has been verified in physiological experiments (Kunze, 1979). By contrast, other anatomical studies have failed to demonstrate this projection (Beckstead, 1984; Jarayaman, 1985). The reports about the existence of this projection in the rat are contradictory. Retrograde and anterograde tract-tracing studies have described the projection from the MD to the nucleus accumbens (Phillipson and Griffiths, 1987; Veening et al., 1980; Groenewegen, 1988; Deschênes et al., 1995), although this could not be confirmed in other studies (van der Kooy, 1979; Berendse and Groenewegen, 1990).

The purpose of the present study was to re-examine the existence of the striatal input from the MD in the rat. For this purpose, to characterize the population of the MD cells projecting to the striatum injections of the retrograde tracer Fluoro-Gold (FG) were placed in different sites of the caudate putamen (CPu). Small injections

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of the anterograde tracer biotinylated dextran amine (BDA) were placed in the MD in an attempt to confirm the previous projection. Furthermore, in order to determine the relationship of thalamic fibers and the compartmental organization of the striatum, BDA tracing was combined with immunohistochemistry for enkephalin (ENK).

Materials and Methods

The present report derives from a broader experimental series (n = 30 rats) aimed at determining the organization of the thalamostriatal projections. Experiments were carried out on female adult rats (Wistar) deeply anaesthetised (0.1 ml/100g) with an intramuscularly injected mixture of 4:3 parts of Ketaset® (1% of a solution of Ketamine) and Rompun® (2% of a solution of xylacine) respectively, and placed in a stereotaxic frame. A 2% solution of the retrograde tracer FG (Fluorochrome, Englewood, CO) in 0.1 M cacodylate buffer, pH 7.3, was injected iontophoretically into different sites of the CPu (Figure 1A, C, E and G). In three animals, a 10% solution of biotinylated dextran amine (BDA, Molecular Probes Europe, Leiden, The Netherlands) in 0.01 M phosphate buffer, pH 7.25, was injected in the same way into the MD (Figure 2A-B). All the stereotaxic coordinates were taken from the atlas of Paxinos and Watson (1998). After a survival time of seven days, the animals were perfused transcardially, the brain was removed, and coronal sections (40 µm thick) were obtained with a freezing microtome. At all times, animals were handled according to the Society for Neuroscience Policy on the Use of Animals in Neuroscience Research as well as to the European Communities Council Directive 86/609/EEC. Transported BDA was detected with an avidin-biotin-peroxidase complex (ABC) stained with nickel-enhanced diaminobenzidine solution (DAB-Ni) as chromogen, whereas transported FG was detected via a peroxidase-antiperoxidase (PAP) method using the chromogen DAB. In one series from each individual case, and once the tracers had first been visualised with DAB, an additional counterstain with thionin was performed. This procedure afforded an unequivocal localization of the injection sites as well as the structures displaying labeling. BDA tracing was combined with immunohistochemistry for enkephalin (Figure 3). After the procedure for the visualisation of BDA had finished, sections were incubated in a mouse anti-ENK antiserum (1:100, Medicorp, Montreal, Quebec, Canada) for 60 h. at 4° C, followed by incubation in a biotinylated antiserum raised in horse (Vector Laboratories, Burlingame, CA), then in an ABC solution, and finally they were stained with DAB.

Results

The main findings of this study are (Figure 1): (1) After FG injections into the rostral-most regions of the CPu, a low number of loosely arranged retrogradely labeled neurons were found in the three divisions of the MD (Figure 1H). (2) Clusters of retrogradely labeled neurons within the medial portion of the MD, adjacent to the paraventricular and intermediodorsal thalamic nuclei, were obtained after FG injections into the nucleus accumbens (Figure 1D). (3) When FG injections were located in the medial regions of the CPu, adjacent to the lateral ventricle, retrogradely labeled neurons were observed in the lateral portion of the MD (Figure 1F). (4) A high number of retrogradely labeled neurons was found in the caudal pole of the MD following FG injections into ventrolateral regions of the precommissural CPu (Figure 1A). FG injections into the dorsal- and caudal-most regions of the CPu did not result in neuronal labeling in the MD.

After the two BDA injections into the centre of the MD (Figure 2A), few anterogradely labeled fibers were only found in the medial portions of the precommissural CPu and also in the nucleus accumbens (Figure 2C-E). By contrast, after BDA injection into the caudal pole of the MD (Figure 2B), abundant anterogradely labeled fibers were found in the ventral regions of the precommissural CPu, ascending until its centrolateral part. These fibers branched extensively into the ventral striatum and displayed high number of varicosities (Figure 2D-F). As regards the rostral striatum, the dorsal part of this was devoid of labeled fibers. BDA-labeled fibers were also absent in the caudal striatum. In this case, the agranular insular cortex showed a high density of BDA terminal fields.

The immunoreactivity for ENK in the striatum, preferentially in the ventral striatum, displayed areas of different staining density. Patches of strong ENK-immunoreactivity were surrounded by a moderately immunoreactive matrix. BDA labeled fibers overlapped extensively with patches of strong ENK-immunoreactivity (Figure 3).

Discussion

Before discussing our results, some methodological considerations should be taken into account. The sparse and widespread labeling found in the MD after rostral injections into the CPu is probably due to uptake by passing fibers, because these rostral injections had a central area of necrosis, unlike the rest of our FG injections. Furthermore, BDA injections at the centre of the MD did not reproduce these findings.

We believe that the projection from the MD to the nucleus accumbens probably does exist. We

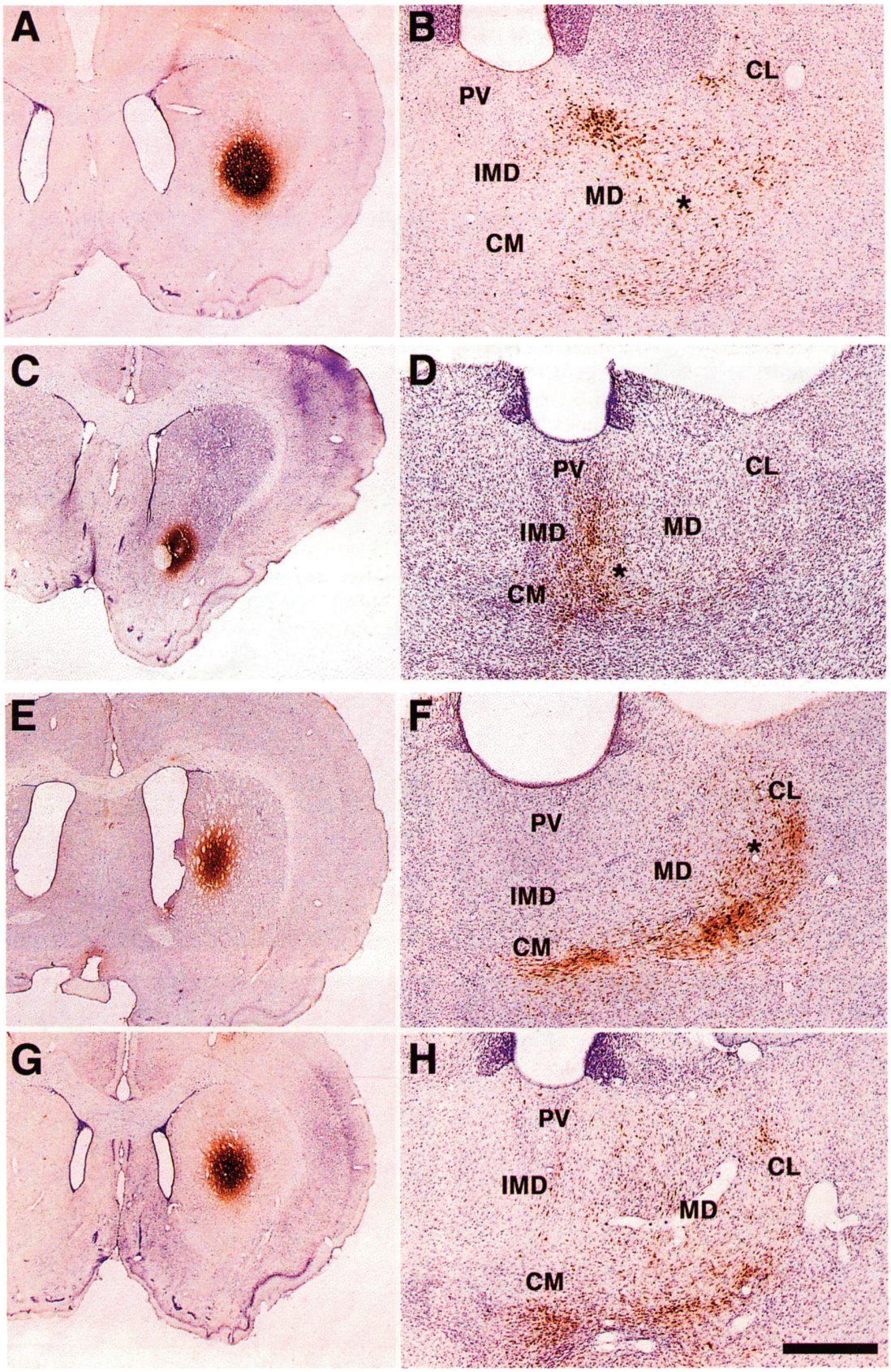

Fig. 1.- The right column (A, C, E and G) shows low magnification photomicrographs of different FG injections within the CPu stained with DAB and counterstained with thionin. The left column (B, D, F and H) shows low magnification photomicrographs of coronal sections through the thalamus, showing the different distribution of the FG-labeled neurons within the MD. (A) FG injection site into the ventrolateral CPu. (B) FG-labeled neurons within the caudal pole of the MD. (C) FG injection site into the nucleus accumbens. (D) FG-labeled neurons within the medial segment of the MD. (E) FG injection site into the medial CPu. (F) FG-labeled neurons within the lateral segment of the MD. (G) FG injection site into the rostral CPu. (H) FG-labeled neurons sparsely distributed within the three subdivisions of the MD. Abbreviations: **CL**; centrolateral thalamic nucleus; **CM**, central medial thalamic nucleus; **IMD**, intermediodorsal thalamic nucleus; **MD**, mediodorsal thalamic nucleus; **PV**, paraventricular thalamic nucleus. Scale bar: 2000 μm A, C, E and G; 500 μm B, D, F and H.

constantly observed clusters of retrogradely labeled neurons in the medial subdivision of the MD after FG injections into the nucleus accumbens. This projection was confirmed with the BDA injections in our study, and it has also been described in several earlier studies (Phillipson and Griffiths, 1987; Veening et al., 1980; Groenewegen, 1988; Deschênes et al., 1995). It would be no surprise that the medial subdivision of the MD, which is associated with the limbic system, could project to the limbic striatum. However, we think that these groups of neurons adjacent

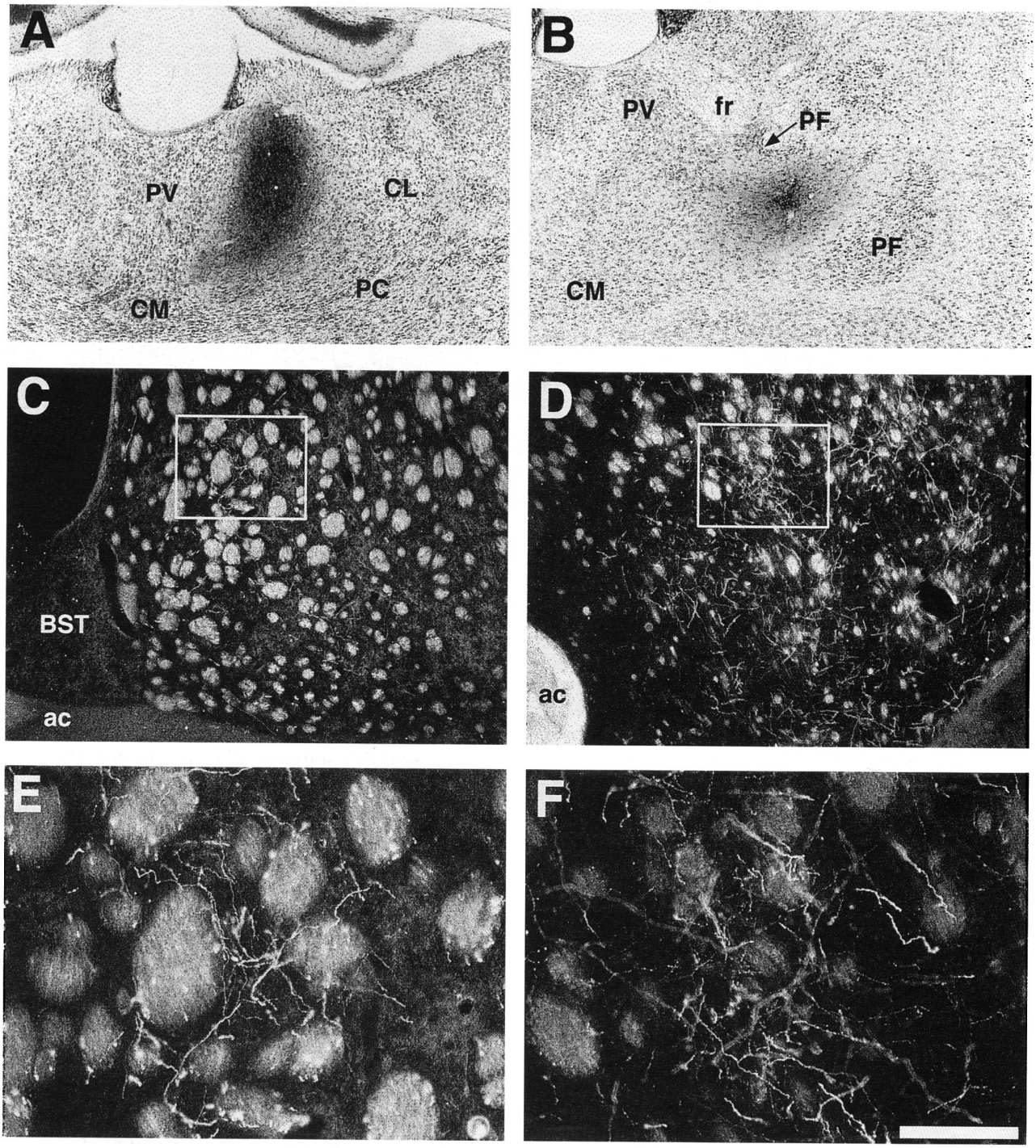

Fig. 2.- (A) Low magnification photomicrograph illustrating a BDA injection site within the MD, stained with DAB and counterstained with thionin. (B) Low magnification photomicrograph illustrating a BDA injection site within the caudal pole of the MD, stained with DAB and counterstained with thionin. (C) Dark field photomicrograph taken from a coronal section through the pre-commissural CPu, showing a terminal field of BDA-labeled fibers, corresponding to the case illustrated in A. (D) Dark field photomicrograph taken from a coronal section through the pre-commissural CPu showing a terminal field of BDA-labeled fibers corresponding to the case illustrated in B. (E) Inset taken from C showing the former BDA terminal field at higher magnification. (F) Inset taken from D showing the former BDA terminal field at higher magnification. This axonal plexus was composed by ramifying fibers with many varicosities. Abbreviations: **ac**, anterior commisssure; **BST**, bed nucleus of the stria terminalis; **CL**, centrolateral thalamic nucleus; **CM**, central medial thalamic nucleus; **fr**, fasciculus retroflexus; **PC**, paracentral thalamic nucleus; **PF**, parafascicular thalamic nucleus; **PV**, paraventricular thalamic nucleus. Scale bar: 670 μm A and B; 536 μm C and D; 134 μm E and F.

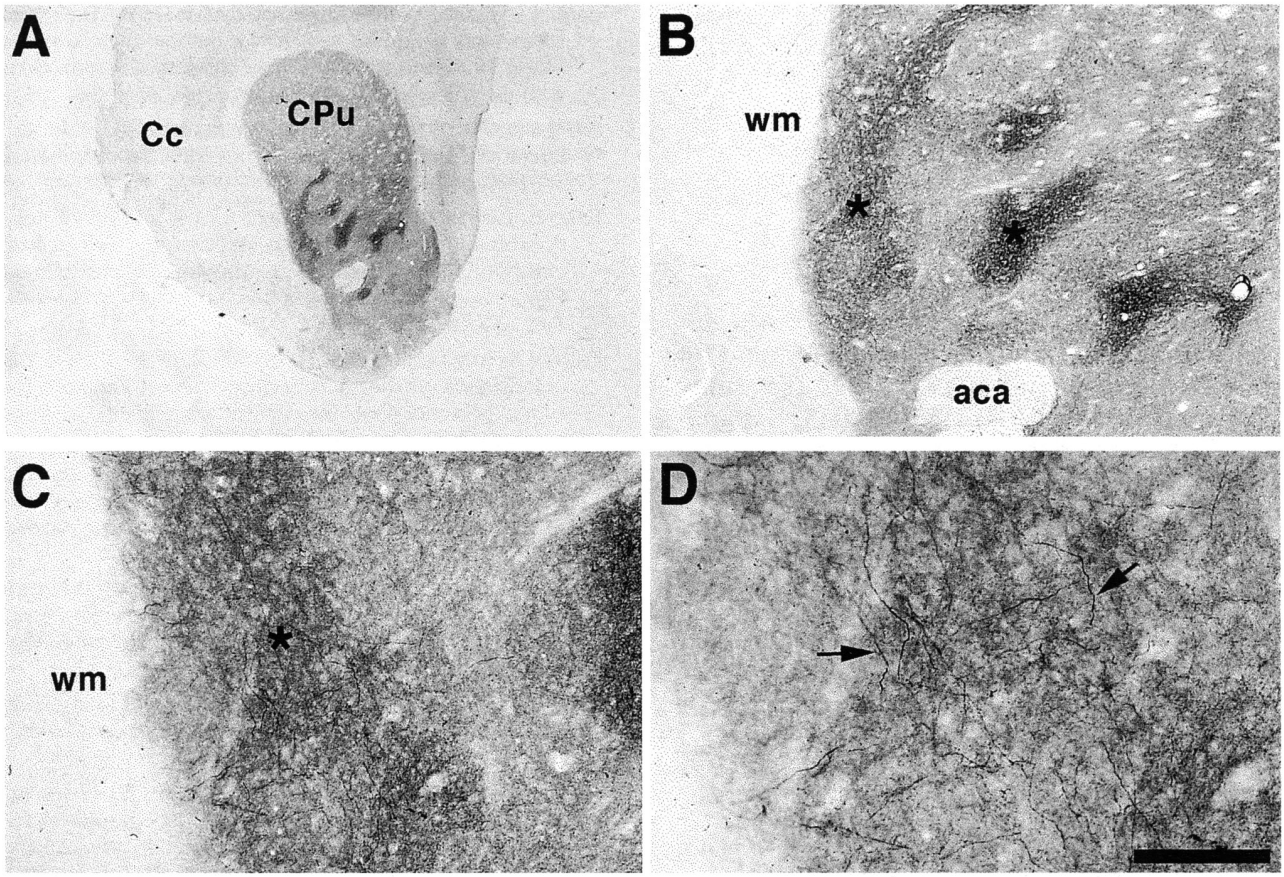

Fig. 3.- (A-B) Low magnification photomicrographs illustrating a coronal section of the ventral CPu processed for ENK-immunoreactivity and BDA histochemistry. Patches of strong ENK-immunoreactivity (() are surrounded by a moderately immunoreactive matrix. (C-D) BDA terminal fields stained with nickel-enhanced DAB overlap extensively with patches of strong ENK-immunoreactivity at higher magnification. Abbreviations: **aca**, anterior commissure, anterior part; **Cc**, cerebral cortex; **CPu**, caudate putamen; **wm**, white matter. Scale bar: 2000 μm A; 500 μm B; 200 μm C; 100 μm D.

to the midline thalamic nuclei could be ectopic cells, really belonging to the paraventricular or intermediodorsal thalamic nuclei. The same explanation could be applied to the group of retrogradely labeled neurons found in the MD adjacent to the paracentral and centrolateral thalamic nuclei after medial injections into the CPu close to the lateral ventricle.

Finally, a high number of retrogradely labeled neurons was found at the caudal pole of the MD after ventrolateral injections in the CPu. Berendse and Groenewegen (1990) reported this previously, but they considered it to be due to uptake of the retrograde tracer (cholera toxin ß subunit) by fibers crossing the striatum. However, we consider that this projection from the caudal pole of the MD to the striatum does in fact exist. The results from our BDA injections support this finding (for technical considerations about the FG and BDA techniques, see Lanciego et al., 1998 and Erro et al., 1999). Two interpretations can be drawn. First, the caudal pole of the MD may have a different projection pattern as compared to the main body of the nucleus, in agreement with the rostrocaudal differences described in the structural organization and connectivity of the MD (Groenewegen, 1988). This caudal pole of the MD shows a poor acetylcholinesterase staining, like the medial and central subdivisions of the nucleus, and it projects to the insular cortex (Krettek and Price, 1977; Clascá et al., 1997), suggesting a possible relationship with the vegetative and limbic systems. Another possibility is that the presence of neurons at the caudal edge of the MD may be due to the gradual decrease in size of this nucleus along the rostrocaudal axis. This would lead to a concentration of eccentrically placed neurons adjacent to the midline and intralaminar nuclei in more rostral levels.

The relationship between the thalamostriatal projections and the neurochemical compartmentation of the striatum in the rat has been studied in the ventral striatum (Herkenham and Pert, 1981; Berendse et al., 1988; Berendse and Groenwegen, 1990) and after anterograde injections in the lateral posterior thalamic nucleus (Funaki et al., 1997). The paraventricular and rhomboid thalamic nuclei were found to project to the striosomal compartment whereas the intralaminar nuclei projected to the matrix. We observed that the projections from the caudal

pole of the MD reach the striosomal compartment defined as the areas of rich enkephalin content. Therefore, this part of the MD does share the termination pattern of the midline nuclei in the striatum, supporting its relationship with the limbic system.

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Abbreviations

ABC	avidin-biotin-peroxidase complex
ac	anterior commissure
aca	anterior commissure, anterior part
BDA	biotinylated dextran amine
BST	bed nucleus of the stria terminalis
Cc	cerebral cortex
CL	centrolateral thalamic nucleus
CM	central medial thalamic nucleus
CPu	caudate putamen
DAB	diaminobenzidine solution
DAB-Ni	nickel-enhanced diaminobenzidine solution
ENK	enkephalin
fr	fasciculus retroflexus
FG	Fluoro-Gold
IMD	intermediodorsal thalamic nucleus
MD	mediodosal thalamic nucleus
PAP	peroxidase-antiperoxidase
PF	parafascicular thalamic nucleus
PV	paraventricular thalamic nucleus
wm	white matter

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