ANATOMICAL CONNECTIONS OF THE VENTRAL, BUT NOT THE DORSAL PART OF THE STRIATUM WITH THE PARVICELLULAR RETICULAR FORMATION: IMPLICATIONS FOR THE ANATOMICAL SUBSTRATE OF ORAL MOVEMENTS


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SUMMARY
Using a double labelling procedure by combining injections of the anterograde tracer Phaseolus vulgaris leucoagglutinin into different parts of the striatum with injections of the retrograde tracer cholera toxin subunit B into the parvicellular reticular formation of rats (pcRF), it was found that the ventrolateral part of the striatum (vIS) projects onto neurons in the dorsolateral part of the substantia nigra pars reticulata (SNR), a region that, in turn, was found to project to the pcRF. In contrast, the dorsomedial part of the striatum (dMS) projects onto neurons in the ventrolateral part of the SNR, a region that was found not to project to the pcRF. These differential striato-nigro-reticular pathways are discussed in view of the differential role of the vIS and dMS in the expression of oral movements.

KEY WORDS: striato-nigro-reticular pathways; parvicellular reticular formation; PHA-L; CTB; oral movements.

INTRODUCTION
It has been shown that neurons arising in the ventrolateral part of the striatum (vIS) primarily converge onto neurons in the dorsolateral part of the substantia nigra pars reticulata (dMSNR) which projects to the medullary reticular formation (RF), a region whose neurons are directly connected with orofacial motor nuclei such as the trigeminal motor nucleus (Vmo, 1–3). Based upon available literature, these pathways are suggested to serve as anatomical substrates for the expression of oral movements (1). Indeed, we (4,5) and others (6–8) have found that activation of dopamine D, and/or...
D₂ receptors in the vlS elicits jaw movements in the rat, showing that the vlS plays a critical role in the generation of oral movements. In contrast, neurons arising in the dorsomedial part of the striatum (dmS) primarily converge onto neurons in the ventrolateral part of the SNR (vlSNR; 9–11). It is unknown whether this vlSNR also projects to the RF. In this context, it is relevant to note that the dmS appears to play only a regulatory role in the expression of oral movements (4,5,12): it does certainly not play a critical role in the generation of these movements, since direct activation or blockade of dopamine D₁ and/or D₂ receptors in the dmS cannot elicit oral movements (4). These pharmacological data suggest that the vlS and the dmS are differentially connected with the RF. Given the extensive studies of von Krosigk et al. (1) on the vlS-dlSNR-RF connections, we primarily focused our attention on the possible dmS-vlSNR-RF connections. Thus, the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA–L; 13,14) was injected into the dmS or vlS, namely regions delineated previously in our pharmacological studies as regions that have a differential effect in the expression of oral movements (fig. 1; 4,5,12), and the retrograde tracer cholera toxin subunit B (CTB; 15) was injected into the RF. In comparison with von Krosigk et al. (1), we used CTB instead of horseradish peroxidase in order to limit the diffusion of the injections in the RF to the parvicellular part of the RF (pcRF), since that part contains neurons which are connected with Vmo (3).

Fig. 1. Schematic drawing of the dorsal (A) and the ventral (B) regions in the striatum that have been found to have a differential role in the expression of oral movements.
MATERIALS AND METHODS

12 Male Sprague-Dawley rats weighing 350 – 400 g were used. The rats were anaesthetized with sodium pentobarbitone (40 mg/kg i.p.) and placed in a stereotactic frame. Following surgical exposure, PHA-L was unilaterally injected into either the dmS or the vlS, and CTB was injected into the pcRF. The injections (0.05 – 0.1 µl) were made with glass micropipettes (tip diameter, 20 – 30 µm) attached to a 1 µl syringe filled with either 2.5 % PHA-L (Vector Lab., Burlingame, CA) or 1 % CTB (Vector Lab., Burlingame, CA) in 0.01 M sodium phosphate-buffered saline (PBS, pH 8.0 for PHA-L and pH 7.3 for CTB, respectively). The coordinates for the dmS and vlS were based on our above-mentioned pharmacological studies (4,5,12), i.e. dmS, anterior = 8.6 ~ 10.0, lateral = 2.0 ~ 3.5, vertical = 5.0 ~ 6.5; vlS, anterior = 7.2 ~ 9.7, lateral = 3.0 ~ 4.5, vertical = 3.0 ~ 4.5; and the pcRF, anterior = -0.2 ~ -0.8, lateral = 1.0 ~ 2.0, vertical = 1.0 ~ 2.5 (16). After 14 days of survival, the rats were given an overdose of sodium pentobarbitone (80 mg/kg i.p.) and subsequently transcardially perfused with PBS (pH 7.4) followed by 4 % paraformaldehyde in 0.1 M phosphate buffer. Serial coronal sections (50 µm) were cut and processed with PHA-L and CTB immunohistochemistry using the ABC (Vector Lab., Burlingame, CA) staining technique according to previously described procedures (17). Goat anti-PHA-L (1 : 2000; Vector Lab., Burlingame, CA) was used as the primary anti-serum to PHA-L, and when double staining with PHA-L and CTB

Fig. 2. Schematic drawings of the injection sites of (A) phaseolus vulgaris leucoagglutinin (PHA-L; shaded areas) and (B) choleratoxin subunit B (CTB; hatched areas). Planes are modified to a series of two or three sections for each injection from the atlas of Paxinos and Watson (16); approximate coordinates are indicated in mm anterior to the interaural line. ac: anterior commissure; CPU: caudate putamen; GP: globus pallidus; ic: internal capsule; LSO: lateral superior olive; Vme: mesencephalic trigeminal nucleus; MPB: medial parabrachial nucleus; PnC: pontine reticular nucleus, caudal part; PnO: pontine reticular nucleus, oral part; Vpr: principal sensory trigeminal nucleus; py: pyramidal tract; Vs: sensory root trigeminal nerve; scp: superior cerebellar peduncle; tz: trapezoid body.
axon collaterals from the anteromedially labeled striatonigral fibers are illustrated by dots. 

Fig. 3: Low-power photomicrographs of PHA-L injection sites in the dmSo (v) and CTh (p) in the neostriatum. These sites were analyzed to determine the number of PHA-L-positive fibers extending into that region. 

Results

The PHA-L immunoreactivity was primarily found in the dmSo of rats (Fig. 2A: nos. 1-8), while it was only faintly detected in the CTh (Fig. 2B: nos. 1-3 and 6).
SNR.

Note that CTB-positive cells with axonal connections are mainly distributed in the dorsal half of the

SNR. L. Injection into the thalamus and ventrolateral nucleus of the thalamus into the SNR. C. Injection into the SNR. D. Injection into the SNR. E. Injection into the SNR. F. Injection into the SNR. G. Injection into the SNR. H. Injection into the SNR. I. Injection into the SNR. J. Injection into the SNR. K. Injection into the SNR. L. Injection into the SNR. M. Injection into the SNR. N. Injection into the SNR. O. Injection into the SNR. P. Injection into the SNR. Q. Injection into the SNR. R. Injection into the SNR. S. Injection into the SNR. T. Injection into the SNR. U. Injection into the SNR. V. Injection into the SNR. W. Injection into the SNR. X. Injection into the SNR. Y. Injection into the SNR. Z. Injection into the SNR.

2. Injection of PHA-L into the thalamus and ventrolateral nucleus of the thalamus into the SNR. A similar labeling pattern was seen in cases no. 1, 2, and 6. No contralateral labeling was observed in the thalamus. The labeled cells in the SNR (Fig. 3B) were also similar to those observed in the thalamus. The labeled cells in the SNR (Fig. 3B) were also similar to those observed in the thalamus. The labeled cells in the SNR (Fig. 3B) were also similar to those observed in the thalamus. The labeled cells in the SNR (Fig. 3B) were also similar to those observed in the thalamus. The labeled cells in the SNR (Fig. 3B) were also similar to those observed in the thalamus.
axons were running ventrolaterally from the vlS, penetrated the ic and the middle portion of the substantia nigra pars compacta, and terminated predominantly in the dlSNR. A similar labelling pattern was seen in rats no. 9, 10 and 12. Following CTB injection into the pcRF around Vmo (fig. 4B), CTB positive cells were found in the dorsomedial and dorsolateral part of the SNR. Figure 4a–f illustrates the distribution of CTB positive cells in the SNR with and without apparent contact by anterogradely labelled PHA–L immunoreactive varicosities. The dlSNR contained both a large number of PHA–L anterogradely labelled fibres with varicosities and a large number of CTB positive retrogradely labelled cells. Many CTB positive cells were surrounded by PHA–L positive varicosities (fig. 5A, B and C). Numerous cells of this type were found predominantly in the dlSNR, but some of these CTB positive cells missed contact with PHA–L positive varicosities (fig. 5D). Most of the contacts between PHA–L positive varicosities and CTB positive cells in the dlSNR were located on the soma and proximal dendrites. A similar labelling pattern was seen in rat no. 9.

DISCUSSION

The present study was guided by the outcome of the pharmacological studies performed in the same laboratory (4,5,12). This approach had the advantage that the anatomical experiments could be matched with the earlier reported pharmacological experiments in all aspects. Apart from using the same type of techniques, methods and animals, we purposely directed the PHA–L injections at striatal regions which have been found to have a differential effect upon the expression of oral movements, namely the vlS and the dmS as delineated in fig. 1.

In line with earlier studies (1,2,8,10), we found that the vlS primarily projects onto neurons in the dlSNR, a region that, in turn, projects to the medullary reticular formation. Given the fact that our CTB injections into the RF were far smaller than those described earlier (1) and were actually restricted to the pcRF which embeds the Vmo, this study provides direct evidence in favour of the suggestion of von Krosigk et al. (1) that the nigroreticular neurons terminate in the pcRF. Given our data together with the known electron microscopic data about the nature of the contacts between striato–nigral neurons and nigroreticular neurons in the dlSNR (1), it is concluded that there indeed exists a monosynaptic connection between the vlS and the pcRF which contains neurons that are directly connected with the Vmo (3).

Apart from the finding that the dmS primarily projects onto neurons in the vlSNR, a finding
Fig. 5. High-power photomicrographs of CTB-positive cells (open stars) with axonal contacts (arrows) and without contacts (closed). (A) and (C) illustrate cells that are positive for CTB and from the raphe, respectively. (B) shows a micrograph of a cell that is negative for CTB but has axonal contacts (open stars) with CTB-positive cells from the raphe. Scale bar = 20 μm.
consistent with earlier reported data (9,11), we found now that this vlSNR does not project to the pcRF: no CTB positive cells could be found in this region following CTB injections into the pcRF. In other words, this study provides evidence that the vlS and dmS are differentially connected with the pcRF.

As mentioned in the Introduction, the vlS plays a critical role in the generation of oral movements, whereas the dmS plays at best a regulatory role in this respect. This study shows that this differential role of the vlS and the dmS in the expression of oral movements can be directly related to their differential connections with the pcRF.

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REFERENCES