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Morphology and Synaptic Organization of Non-Dopaminergic Nigral Projections to the Medio Dorsal Thalamic Nucleus of the Rat, a Study by Anterograde Transport of PHA-L

Parichehr Pasbakhsh[1] Mehdi Mehdizadeh[*2] and Gila Behzadi[3]

[1]Dept. of Anatomy, Tehran University of Medical sciences; [2]Dept. of Anatomy ,Cellular and Molecular Research Center, Iran University of Medical sciences, P. O. Box: 15875-1454 and [3]Dept. of Physiology, Shaheed Beheshti University of Medical sciences, Tehran, Iran

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ABSTRACT

Background: Mediodorsal (MD) thalamic nucleus, which is considered to take place between extra pyramidal and limbic feedback circuit, receives projective fibers from ventrolateral neurons of reticular part of substantia nigra (SNr). In order to better understand the influence and chemical reaction of these fibers upon MD nucleus, the morphology and synaptology of them were examined in the present study. **Methods:** Phaseolous vulgaris-leucoagglutin (PHA-L) was injected into substantia nigra pars reticulate. After 3-4 days, the sections of SNr injection site and MD nucleus were prepared. Then, we examined organization, morphology and, synaptology of PHA-L labeled SNr fibers that go to caudal and lateral part of MD thalamic nucleus. **Results:** At the electron microscopic level, the SNr terminals made synapses predominantly with the medium to small dendrites and far less frequently with soma and large dendrites. These terminals were packed with polymorphic synaptic vesicles and formed symmetrical synapses; furthermore, it has been already recognized that cortico straital fibers from sensory-motor cortex go to region of the SNr that give rise to the nigrothalamic fibers. **Conclusion:** This data suggest that upon the synaptic organization, morphology and chemical nature of GABAergic, SNr fibers may have different inhibitory influence on MD neurons regulating the thalamic output from MD to cerebral cortex in the control of limbic and extra pyramidal feedback system. *Iran. Biomed. J. 12 (4): 209-215, 2008*

Keywords: Mediodorsal (MD) nucleus, Substantial nigra, Pars reticulate, Synapse

INTRODUCTION

The mediodorsal (MD) thalamic nucleus of the rat, which is one of the thalamic nuclei, sends its fibers to the striatum [1-3], reticular thalamic nucleus [4], cerebellum [5], sub thalamic nucleus [6] and cerebral cortex [7, 8]. The observation indicated that the cerebellum influences several area of prefrontal cortex via the thalamus [9]. On the other hand, MD receives afferent fibers from the somatosensory and motor area of cortex [10]. Our previous retrograde horse radish peroxides study has also indicated that the MD thalamic nucleus received projective fibers directly or indirectly from ventrolateral part of substantia nigra pars reticulates (SNr) [11]. SNr is a major link between the cores of the nucleus accumbens, the prefrontal cortex, and provides further evidence for concept of a parallel architecture in the basal ganglia, thalamocortical circuits of the ventral striatum [12].

On the basis of these hodological data and according to the behavioral disorders relating to basal ganglia system, the nigrothalamic pathway plays an important role in motor behavioral mechanisms [3, 13].

In spite of these reports, the precise morphology and synaptic organization of MD afferent fibers and chemical reaction from SNr and precise sites of neurons termination of SNr to MD nucleus have not been considered.

*Corresponding Author; Tel. & Fax: (+98-21) 8805 8689; E-mail: maranaoo@iums.ac.ir

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In order to better understand the influence and chemical nature of SNr fibers upon MD nucleus, in the present study, we first demonstrate the distribution and morphology of efferent neurons from SNr to MD thalamic nucleus by injection of phaseolous vulgaris-leucoagglutin (PHA-L) and using an anterograde tracing method and then observe morphology and synaptology of SNr axon terminals by electron-microscopy.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (n = 10, weighting 220-280, Pasteur Institute of Iran, Tehran) were housed three to four per cage in temperature controlled colony room under light-dark cycle with food and water ad libitum. All procedures of the study were according to the Guidelines of Animal Experiments of Research Council of Shaheed Behesti and Tehran University of Medical Sciences (Tehran, Iran). All surgical procedures were performed under general anesthesia with intraperitoneal injection of Nembutal-Na (40 mg/kg).

Anterograde labeling. Ipsilateral injections of PHA-L (vector) into the ventrolateral parts of SNr [11] were made stereotaxically by using Lab Standard Dual Stereotaxic (Stoelting 51600, USA) in ten rats based on atlas of Paxinos and Watson [14]. Depending on anterior and posterior distance from Bregma, midline plane and height (AP, L and H), the location of MD was found and 2.5% solution of PHA-L dissolved in 0.01 M phosphate buffer (PB) and Tris buffer (pH 7.6) were injected into the SNr by using Hamilton syringe (1 µl) and a glass micropipette (30 µm). The photomicrograph of injection site by using microprojector (Ken-A-Vision) confirmed injection of PHA-L in the SNr. After 3-4 days of survival, the animals were deeply re-anesthetized and perfused transcardially with 200-300 ml of 0.9% NaCl followed by 500 ml of 25% glutaraldehyde plus 1% Para formaldehyde in 0.1M PB and then with 100-200 ml of 10% phosphate-buffered sucrose. Next, the brains were removed and placed in 20% sucrose in the same buffer overnight, then blocked and cut serially in the coronal plane at a thickness of 50 µm using a cryostat microtome. The sections of SNR injection site and MD nucleus were then washed in 0.2 M phosphate-buffered sucrose and incubated overnight in PBS containing 1.5% normal goat serum, 0.2% Triton X-100 and rabbit anti-PHA-L (EY lab 1:1000, USA). The sections were washed in 0.2 M PBS and respectively incubated in PBS containing ABC complex, then in biotinylated rabbit anti-goat IgG (Vector, Canada 1:200) and in 25 ml of 0.1 M PB (pH 7.3) containing 10 mg diaminobenzidine (DAB) and 10 ml of 30% H_2O_2.

Finally, PHA-L-labeled axons were visualized as brown reaction products.according to the method of Tsumori *et al* [3] After several washing with PBS, the sections were mounted on gelatinized slides, air-dried and cleared in xylene. PHA-L-labeled axons were traced by using a camera Lucida (Olympus DP11, Japan) attached to the microscope (Olympus AX70, Japan). Cover slips were then removed and the sections were counterstained with1% cresol violet for cytoarchitectural landmarks (Figs. 1 and 2).

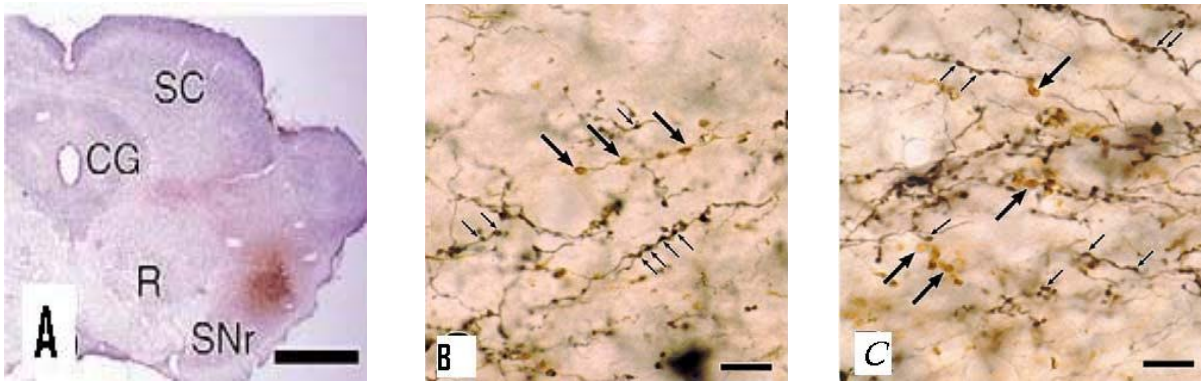

Fig. 1. PHA-L injection into the ventrolateral part of the SNr **(A)**, and resulting intergraded labeling in the caudal and lateral part of the MD **(B** and **C)**. Button-like varicosities labeled with PHA-L is indicated by large and small arrows, respectively. Bar = 0.5 mm in (A), 20 µm in (B and C). SC, superior colliculus; CG, central gray matter; R, red nucleus; SNr, substantial nigra pars reticulate.

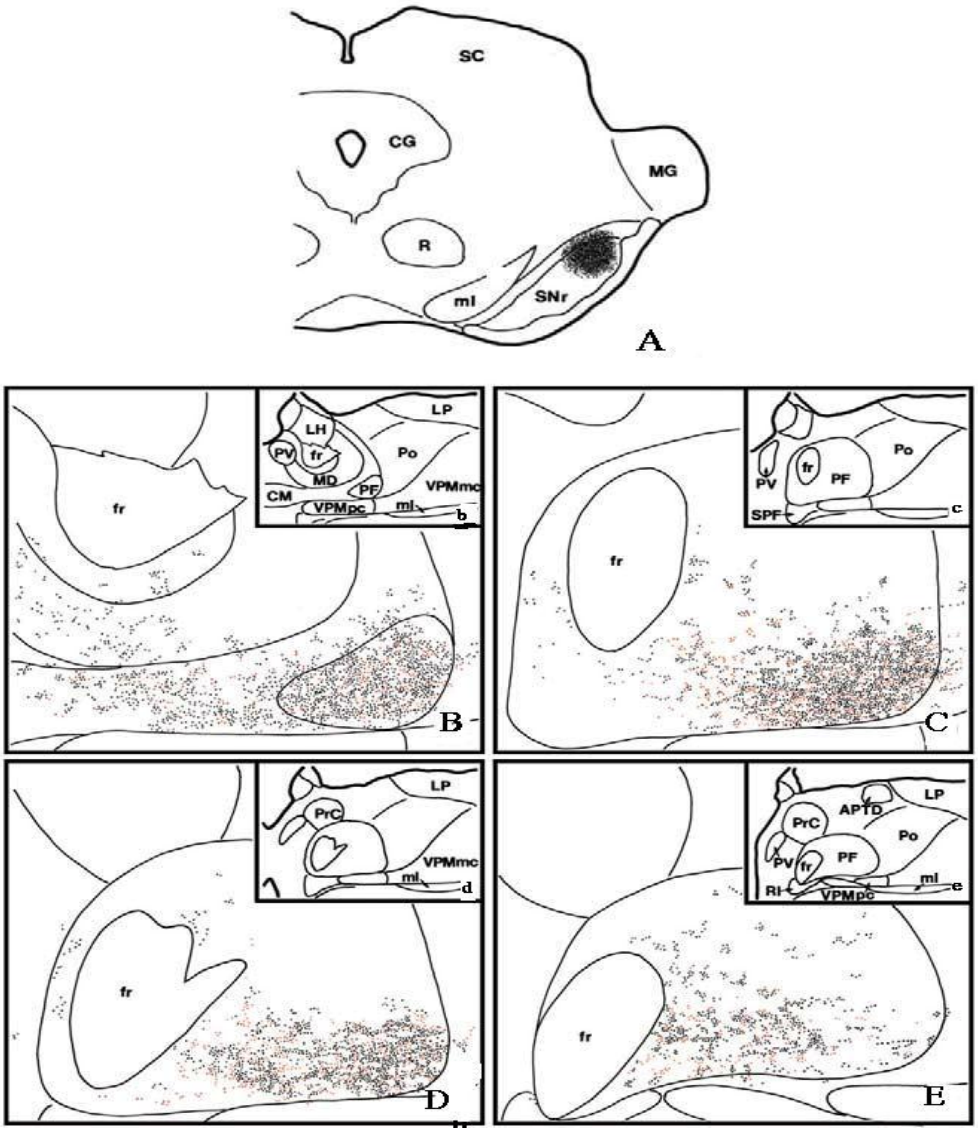

Fig. 2. Line drawings showing the sites of PHA-L injection into the ventrolateral part of the SNr (shaded area in A), and resulting distributions of PHA-L-labeled terminals (black and red dots, respectively) in the caudal and lateral part of the MD (B-E, rostra to caudal). Each MD region in the thalamic planes (b-e) is enlarged in (B)-(D), respectively. APTD, dorsal part of the anterior pretectal nucleus; CM, central medial nucleus; fr, fascicules retroflex us; LH, lateral habenular nucleus; LP, lateral posterior nucleus; MD, mediodorsal nucleus; MG, medial geniculation nucleus; ml, medial lemniscuses; PF, Para fascicular nucleus; Po, posterior nuclear group; PrC, precommissural nucleus; PV, para ventricular thalamic nucleus; RI, rostra interstitial nucleus; SPF, subparafascicular nucleus; VPMmc, magnocellular division of the ventral poster medial nucleus; VPMpc, parvicellular division of the ventral poster medial nucleus. Other abbreviations are as in Figure 1.

In the sections for electron microscopic observation, the specimens of MD nucleus in which there was a good distribution of PHL-label axons were cut and collected in 0.1 M PB (pH 7.3) then post fixed in a solution of 2% osmium tetroxide in 0.2 M PBS for 1.5 h and rinsed twice in 0.2 M PBS for 15 min at room temperature. After washing in distilled water, the specimens were dehydrated in a graded series of acetone and then embedded flat in Epon (TAAB 812, Araldite 502 Resin Kit, Canada). Subsequently, several resin sections were cut at 0.5 micron as semithin and then was stained with 1% toluidine blue in 1% borate solution for 1min at 80°C. Finally, serial ultra thin sections (50 nm) were cut on an ultra microtome (LEICA ultra cut). The sections were transferred to the 100 mesh grid and stained with uranyl acetate and lead citrate, collected on collodion-coated grids and stained with lead

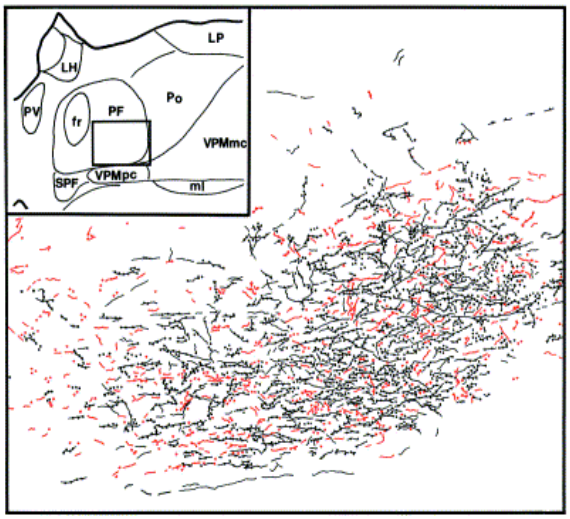

Fig. 3. Line drawing showing a distribution of PHA-L-labeled fibers with bouton-Iike varicosities (red, respectively) is illustrated at a higher magnification. An area enclosed by a small rectangle in the caudal and lateral part of the MD indicates a region enlarged. Abbreviations are as in Figure 2.

nitrate. Finally, the sections were examined under an electron transmission microscope (Zeiss, EM 900, Germany).

To obtain the size and distribution of postsynaptic dendrites, the minor diameters of postsynaptic dendrites with PHA-L-labeled terminals and synaptic membrane specializations were measured on the photomicrographs using an image analyzing computering system (Olympus AX70, Olysia soft ware). The cross-sectional areas of these terminals were also measured in the same manner. Furthermore, the synapses with labeled terminals were counted and classified by their origin and postsynaptic target.

RESULTS

Distribution and morphology of SNr terminals:

Light microscopic observations. After ipsilateral injections of PHA-L into ventrolateral part of the SNr (PHA-L-labeled SNr fibers stained brown), distribution of PHA-L labeled axon terminals were found in the caudal and lateral part of the MD (Figs. 2 and 3). The SNr fibers formed a less dense plexus and their terminal buttons were generally large (Figs. 1B and 1C).

Electron microscopic observations. When the caudal and lateral part of the MD was examined under the electron microscope, the SNr axon terminals labeled with PHA-L were packed with electron-dense DAB reaction product filling up the entire space between the vesicles and the mitochondria (Fig. 4A and 4C).

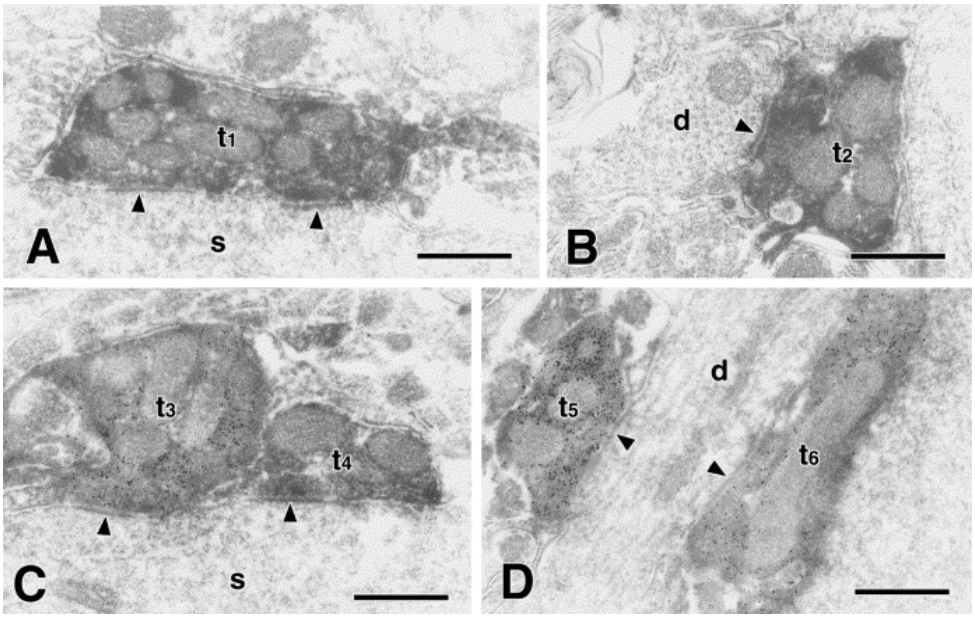

Fig. 4. Electron photomicrographs of PHA-L-labeled terminals making symmetrical synaptic contacts with MD neurons after Injections of PHA-L into the ventrolateral part of the SNr. **(A)** A PHA-L-labeled SNr terminal (t1) makes synaptic contact With a somatic profile (s). **(B)** A PHA-L-labeled SNr terminal (t2) makes synaptic contact with a dendritic profile (d). (C) PHA-L-labeled SNr terminal (t3 and t4) form synapses with a somatic profile (s). Arrowheads indicate the synaptic sites. Bar = 0.5 µm.

Table 1. Cross-sectional area and postsynaptic distribution of the SNr terminals in the MD.

Source of terminals	Mean area ± SD (μm^2)	Postsynaptic distribution			
		Soma (%)	Dendrites >2 µm (%)	Dendrites 1-2 µm (%)	Dendrites <1 µm (%)
SNr	1.09 ± 047 (n = 101)	12 (11)	3 (3)	65 (58)	32 (29)

The SNr terminals formed synapses with medium to small dendrites more than somata and large dendrites (Table 1), the terminals that were rich in mitochondria and dense pleomorphic synaptic vesicles formed symmetrical synapses (Gray 3Type II) and the others that had round vesicles formed asymmetrical synapses. The relation of labeled terminals and their target structure in the MD are summarized in Table 2. Most of the postsynaptic dendrites received SNr terminals, although in some cases, single dendritic profiles were seen to receive both SNr and some were else. The majority of the postsynaptic somata also received SNr terminals, although approximately most of the somata observed here were found to receive convergent synaptic inputs from SNr.

DISCUSSION

The results of this study have confirmed our previous study which there is a strong relation between substantia nigra and MD nucleus of thalamus [11]. It is clear that multipolar neurons of ventrolateral part [11, 13, 15] of SNr send a lot of efferents to the caudal and lateral part of MD nucleus of thalamus (Figs. 2 and 3). The overall morphological organization of individual nigral axons is complex and allows single axons to influence thalamic neurons via the combination of divergent, convergent and amplification processes [16] and the SNr terminals have been examined electron microscopically in several thalamic nuclei and species [6, 17]. According to Sakai *et al.* [6], a large proportion of the SNr terminals in the rat forms axosomatic synapses in the ventroanterio-lateral complex (VAL), whereas the SNr terminals in the ventromedial nucleus (VM) are rarely in contact with somata and about two-thirds of them make synapses with thin dendrites and axon terminal from brain stem structure to the MD is mostly of the En passant [3].

In monkey, the SNr terminals tend to target the somata and proximal dendrites of thalamocortical projection neurons in the magnocellular part of the ventral anterior nucleus [17]. The SNr terminals of the cat have been reported to make synaptic contacts mainly with dendrites [18] or to be frequently in contact with secondary and tertiary dendrites of VM neurons [19]. In the present study, we examined for the first time the SNr terminals in the MD under the electron microscope, and revealed that these terminals formed synapses predominantly with medium to small dendrites and far less frequently with somata and large dendrites. Thus, it seems likely that the pattern of distribution of the SNr terminals in the MD of the rat is almost identical with that in the VM of the rat and cat and parafasiculus (PF) thalamic nucleus in the rat. With respect to the basic morphological feature, most of the previous studies indicated that SNr terminals contained pleomorphic synaptic vesicles and formed symmetrical synapses. Sakai *et al.* [6] described that the synapses between the SNr terminals and the VAL and VM neurons were of asymmetrical or intermediate type. In the present study, we observed that a large number of SNr terminal buttons contain polymorphic synaptic vesicles and form symmetrical synapses, although only a small number of SNr terminals containing round vesicles form asymmetrical synapses.

Table 2. PHA-L-labeled SNr terminals and their target structures in the MD.

Postsynaptic target structure						
Soma (n = 123)				Dendrites (n = 285)		
Source of Terminals	Number of labeled terminals contacted			Source of terminals	Number of labeled terminals contacted	
	1	2	3<		1	2
	24	9	2	SNr	114	5

Tsumori *et al.* [20] indicated GABAergic neuron situated in the ventro-latral part of SNr recent tract tracing and immunohistochemical studies [21] confirm the GABAergic nature of SNr efferent to the thalamic nuclei [22]. It is interesting to note that ending place of inhibitory fibers from substantial nigra to MD nucleus is the same area of ending stimulating efferent from dorsal segmental region. Such (push-pull) organization may represent an important difference between MD and other principal thalamic nuclei [23]. Llinsky *et al.* [24] reported that the parts of MD which receive some efferent fibers from substantial nigra send in turn their efferent fibers to prefrontal cortex.

Recent electrophysiological study with the intra cellular recording in MD nucleus of thalamus after stimulation of SNr efferent shows that SNr has an inhibitory effect on the same neurons of MD which send their ending to prefrontal cortex [25].

In this regard, it has been suggested that the SNr exert its inhibitory action on the MD from its lateral, ventro-lateral and dorsomedial parts. It has been shown that the inhibitory action of SNr on MD is controlled via pallido-SNr GABAergic pathway [13]. On the other hand, corticostriatal projection from sensory-motor cortex to that region of the SNr giving rise to the nigrothalamic projection has been already recognized [22].

In this regard and upon the behavioral experiments, in combined motor and behavioral disorders relating basal ganglia system, and because of reciprocal connection between perifrontal cortex and MD nucleus [24] the nigrothalamic pathway play an important role between extra pyramidal and limbic feedback circuit. In gray type II synapses, the vesicle are 40 nm in diameter and ellipsoidal in shape and there are thickening of both presynaptic and post synaptic membranes. The small synaptic vesicles an gray type II synaptic boutons contain GABA [26], at least 70% of the afferent to substantia nigra dopaminergic neuron are GABAergic [27], it indicated that in addition to the effects that nigral GABAergic output neurons have on their target nuclei outside of the basal ganglia, local interaction between GABAergic projection neurons and dopaminergic neurons are crucially important to the functioning of the nigral dopaminergic neurons.

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