

## The Organization of Non-Dopaminergic Nigral Projections to the Thalamic Nucleus of the Rat

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### Abstract

**Introduction:** Nigrothalamocortical tract is one of the important outputs of the basal ganglia, but its role in motor disturbances is still vague, because there is little information on its connection structure. Some electrophysiological and pharmacological studies report that non-dopaminergic outputs originate from reticular part of substantia nigra to thalamus. In this study the topographical and organization connection of substantia nigra pars reticulata (SNR) with thalamic MD nucleus was investigated using HRP tracer.

**Materials and Methods:** 25 male rats received injection of 33% HRP into the ipsilateral MD nucleus of thalamus stereotactically. 48 hours later the animals were perfused transcardially, and the brain tissue was fixed. 40 micrometer sections were prepared from diencephalon and midbrain. Following enzymatic reactions of TMB, the sections were stained by neutral red.

**Results:** The light microscopic study showed that there is a high concentration of neurons which project to the MD nucleus from the rostro-lateral part and middle parts of pars reticulata (SNR) and the number of labeled cells decrease in the caudal parts. Other labeled neurons are located at the border of SNC, SNR and VTA, specially close to the passage of III cranial nerve. In general, the size of neurons was mostly medium; and they were multipolar in shape. Any labeled cells were observed in the SNR contralateral to injection site.

**Conclusion:** Our findings show that the SNR connection to the MD arise mainly from lateral and dorsomedial part of SNR. It seems that these connection may influence the limbic system in orientating behaviour.

**Key words:** substantia nigra, HRP tracer, mediodorsal, thalamus, non-dopaminergic

## Introduction

Substantia nigra is part of basal ganglia complex that has an important role in controlling of the movements via its relationship with thalamus and neocortex. Dopaminergic cells are mostly located in compact part and GABAergic neurons are placed in the reticular part of the substantia nigra. Electrophysiological and biochemical reports indicate the existence of non-dopaminergic connections from the pars reticulata to the parts of thalamus (1) which in return project to association limbic-motor cortex (1, 2). Recent tract tracing and immunohistochemical studies (3) confirm the GABAergic nature of SNR efferents to the thalamic nuclei (4,5).

In spite of these reports, the precise topographical and quantitative relationship between SNR and nonsensory nuclei of thalamus has not yet been identified. As the role of SNR is predominantly related to conditioned behavioral movements (6), the present study was undertaken to further classifying the SNR projection to prefrontal related thalamus (mediodorsal nucleus, MD) using HRP tracing method.

## Materials and Methods

### \* Animals

25 adult male Sprague-Dawley rats (Pasteur's Institute, Tehran, Iran) weighing 220-280g 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 this study were according to the guidelines of animal experiments of Research Council at Iran and Shaheed Beheshti University of Medical Sciences (Tehran, Iran).

### \* Retrograde tracing experiments

Following intraperitoneal injection of Nembutal-Na 40 mg/kg, anesthetized rats were placed in stereotaxic apparatus (Stoelting, USA). Unilateral injection of 0.3 to 0.5  $\mu$ l of HRP (33% Sigma) in MD nucleus of thalamus were performed using a glass micropipette (30  $\mu$ m) attached to the 1- $\mu$ l Hamilton Syringe. After 48h survival, the animals were deeply re-anesthetized and perfused transcardially with 200-300 ml of 0.9% NaCl followed by 500ml 25% glutaraldehyde plus 1.0%

paraformaldehyde in 0.1 M phosphate buffer and then with 100-200 ml of 10% phosphate-buffered sucrose solution. Next the brains were removed, blocked and cut in the coronal plane at a thickness of 40  $\mu$ m using a cryostat microtome or vibratome. Sections were immediately processed for HRP reaction according to the tetramethylbenzidine protocol of Mesulam (7). Sections were then counterstained with 0.1% neutral red, dehydrate and cover slipped.

### \* Neuronal Counting

One from each five consecutive sections containing substantia nigra entirely at their length were selected. Labeled neurons were counted according to Königsmark (8) formula:  $Nt = Ns \cdot St / Ss$

Nt = total number of counted labeled cells in SNR

Ns = number of counted labeled cells in each selected sections

St = total number of sections through SNR

Ss = total number of selected sections

The area of SNR was drawn by a Camera Lucida (Wild, Switzerland, x10), and representative sections were photomicrographed

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## Results

### \* Definition of injection site

The MD nucleus that is in anterior part of thalamus is limited from its dorsal part by stria medullaris and from the ventral part by IML (InterMedulary Lamina).

The medial border of this nucleus is separated from the other side by IMD (Intermediodorsal thalamic nucleus). According to Paxinos and Watson (9), the co-ordinations of MD are as follow: AP -3.4, L 0.6, H 5.8. To reach this nucleus the needle trace has been passed through the cortex, hippocampus, and habenulla (Fig. 1,2).

### \* Labeled Neurons

HRP labeled neurons were observed ipsilateral to injection site. Some of the lightly labeled cells were scattered in the length of the ventral part of SNR (Fig.1 micrographs A-D) but, the more densely labeled neurons are mostly gathered in the lateral and medial parts of SNR (Fig.2 B,C).

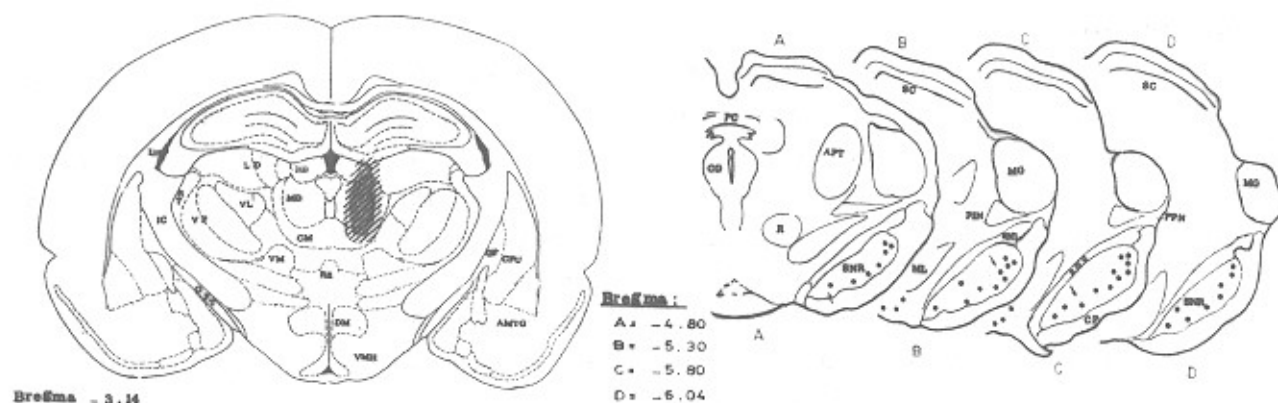


Fig. 1: Ipsilateral distribution of labeled cell in substantia nigra after injection of HRP to MD nucleus (shaded). The bregma of substantia nigra is from cephalic to caudal part of nucleus. The mass of the labeled neurons is located in the middle part of nucleus (section B, C) and it decreases in caudal part.

Consider to the labeling site of VTA. Any labeled cells were seen in SNC part (Scale bar A, D= 1mm)

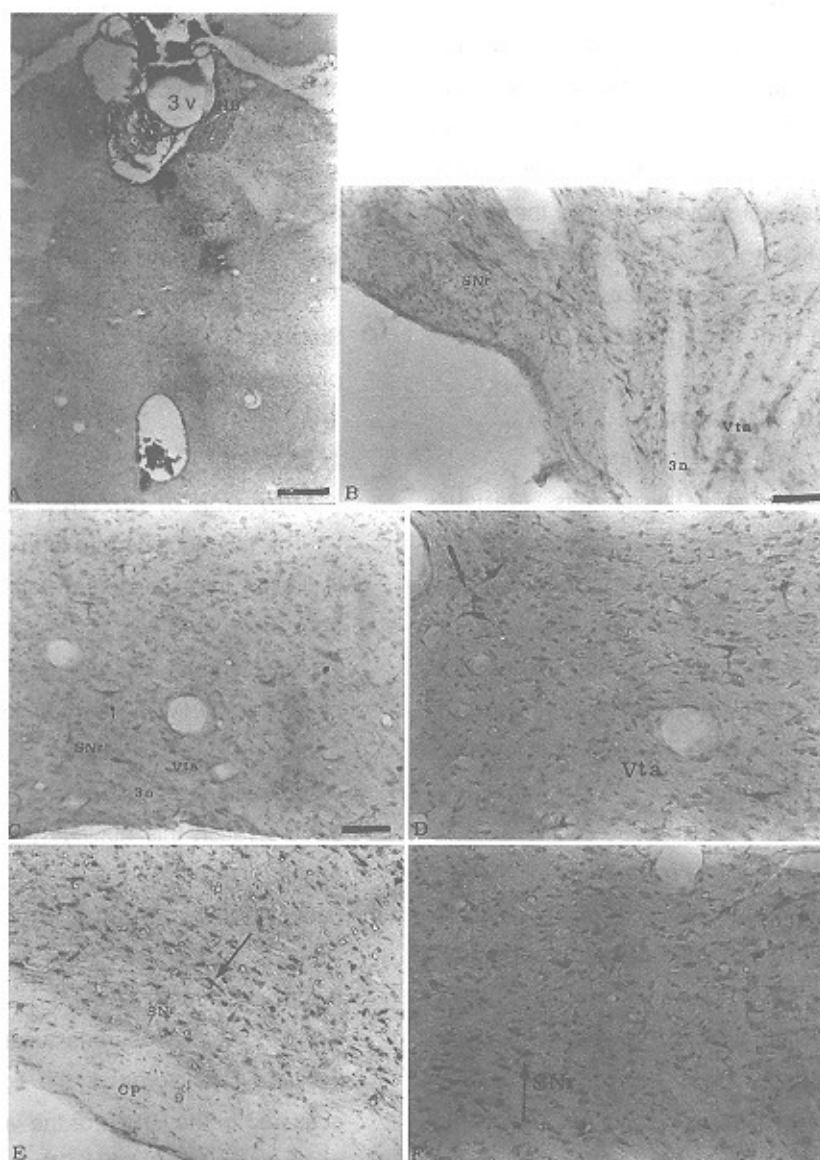


Fig. 2: A photomicrograph from injection site of HRP in MD nucleus of thalamus. Creyl Violet Staining. B labeled neurons in VTA in the exit site of nerve III (small arrows). C,F labeled neurons in SNr (long arrows) Scale bar= 100µm

In addition some cells in VTA (Ventral Tegmental Area) around interpeduncle nucleus at the exit site of III cranial nerve were also labeled (Fig.2 B,C). Any labeled cells were observed in the SNC as well as contralateral part of SNR. The average number of labeled neurons by HRP was 168, mostly medium in size (8-15 micron), and the cells were pyramidal, triangular; usually multipolar in shape.

## Discussion

The results of this study have revealed a strong connection between substantia nigra and MD nucleus of thalamus. It is clear that ventrolateral neurons of reticular part of substantia nigra (SNR) send a lot of efferents to this nucleus. The neurons of the origin of this relation are multipolar and generally small or medium in size. These result correspond with the other studies in rat (3, 5, 10, 11, 12), cat (1, 13) and monkey (14, 15). However it should be noted that in these reports the precise sites of population neurons connecting MD nucleus from SNR has not been considered. Because of strong reciprocal connections between prefrontal cortex and MD nucleus of thalamus, SNR can be regarded as relay site connecting limbic and extra pyramidal system (15). Ultrastructural studies have shown that the synaptic pattern of SNR-MD connection is mostly of the *en passant*, axosomatic and axodendritic synapses (16). The efferent fibers from substantia nigra to MD nucleus are generally

distributed in caudal and lateral part of this nucleus and less fibers are terminated in the middle part (17). It is interesting to note that ending place of inhibitory fibers from substantia nigra to MD nucleus is the same area of ending stimulating efferent from dorsal tegmental region. Such (push-pull) organization may represent an important difference between MD and other principal thalamic nuclei (16). Ilinsky et al. 85 reported that the central, caudal and lateral parts of MD which receive some efferent fibers from substantia nigra, send in turn their efferent fibers to prefrontal cortex (15). 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 (18). In this regard we can conclude that the SNR exerce its inhibitory action on the MD from its lateral and dorsomedial parts. It has been shown that the inhibitory action of SNR on MD is controlled via pallido-SNR GABAergic pathway (19). On the other hand, cortico-striatal projection from sensory-motor cortex to that region of the SNR giving rise to the nigrothalamic projection has been already recognized (20). In this regard and upon the behavioral experiments, in combined motor and behavioural disorders relating basal ganglia system, the nigrothalamic pathway play an important role between extra pyramidal and limbic feedback circuit.

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