PROJECTIONS OF DORSAL AND MEDIAN RAPHE NUCLEI TO DORSAL AND VENTRAL STRIATUM

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Abstract- The ascending serotonergic projections are derived mainly from mesencephalic raphe nuclei. Topographical projections from mesencephalic raphe nuclei to the striatum were examined in the rat by the retrograde transport technique of HRP (horseradish peroxidase). In 29 rats stereotaxically injection of HRP enzyme were performed in dorsal and ventral parts of striatum separately. The extent of the injection sites and distribution of retrogradely labeled neuronal cell bodies were drawed on representative sections using a projection microscope. Following ipsilateral injection of HRP into the dorsal striatum, numerous labeled neurons were seen in rostral portion of dorsal raphe (DR) nucleus. In the same level the cluster of labeled neurons were hevier through caudal parts of DR. A few neurons were also located in lateral wing of DR. More caudally some labeled neurons were found in lateral, medial line of DR. In median raphe nucleus (MnR) the labeled neurons were scattered only in median portion of this nucleus. The ipsilateral injection of HRP into the ventral region of striatum resulted on labeling of numerous neurons in rostral, caudal and lateral portions of DR. Through the caudal extension of DR on 4th ventricle level, a large number of labeled neurons were distributed along the ventrocaudal parts of DR. In MnR, labeled neurons were observed only in median part of this nucleus. These findings suggest the mesencephalic raphe nuclei projections to caudo-putamen are topographically organized. In addition dorsal and median raphe nuclei have a stronger projection to the ventral striatum.

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Key words: Striatum, dorsal and median raphe nuclei, ascending projection

INTRODUCTION

In its most general form, the term “corpus striatum” refers to two subcortical nuclei which play a critical role in the expression of normal movements. The structures included in the corpus striatum were originally considered to be the striatum (caudate nucleus and putamen) and globus pallidus (1).

The cell territories that send afferents to the neostriatum appear to be rather *well* known. The cells of origin of neostriatal afferents were found in the neocortex, the thalamus, substantia nigra pars compacta and dorsal raphe nucleus (2). Neostriatum contains large quantities of serotonin (5HT), therefore, this area is innervated by projections originating in cell bodies in the raphe nuclei (3).

The ascending serotonergic projections are derived largely from the midbrain median and dorsal raphe nuclei (4). The raphe nuclei are distributed near the midline of the brainstem along its entire rostro-caudal extension. The mesencephalic neurons within the rostral raphe complex project primarily to the forebrain (5). The raphe nuclei play important roles in mediating the effects of serotonin on sleep,

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neuroendocrine regulation, sexuality, aggression and pain responses (6).

In this study we have used the horseradish peroxidase (HRP) retrograde transport technique to determine topographical projections from the dorsal and median raphe nuclei to the dorsal and ventral striatum.

MATERIALS AND METHODS

A total of 29 male Sprague-Dawley rats weighing 200-250 gr were used. The rats were controlled with a 12:12 h light-dark cycle. Food and water were available ad libitum.

The animals were deeply anaesthetized with a mixture of ketamine (40 mg/kg) and xylazine (5 mg/kg). The rats received single stereotaxically injections of horseradish peroxidase (HRP) (Sigma type VI) into the dorsal and ventral striatum separately. HRP (30%) in a volume of 0.5 µl was injected over a period of 15 min. The injections were delivered by a 1 µl Hamilton syringe which was mounted on a stereotaxic apparatus. After a 48 hours of survival time, the animals were anaesthetized and perfused through the heart with normal saline followed by 500 ml of fixative solution (paraformaldehyde 1% and glutaraldehyde 1.25%) in 0.1 M phosphate butter pH=7.4 , over 30 min. the brains were removed and postfixted overnight (4°C). Frozen sections (40 µm) were taken in the frontal plane and treated with tetramethylbenzidine (TMB) to reveal HRP activity. Reacted sections were mounted on gelatinized slides and counterstained with 1% neutral red and were studied with bright-field microscope. The extent of the injection sites and distribution of retrogradely labeled neuronal cell bodies were drawed on representative sections by using a projection microscope and Image tool 2 software.

RESULTS

Following ipsilateral injection of HRP into the dorsal striatum (dorsal caudoputamen), the numerous labeled neurons were seen in dorsal raphe nucleus (Fig. 1). In rostral portion of dorsal raphe nucleus, labeled neurons were seen numerously in comparison to caudal portion of this nucleus. There was a significant reduction in the number of labeled neurons at the caudal part of nucleus from that seen further rostrally in the dorsal raphe nucleus (Fig. 2).

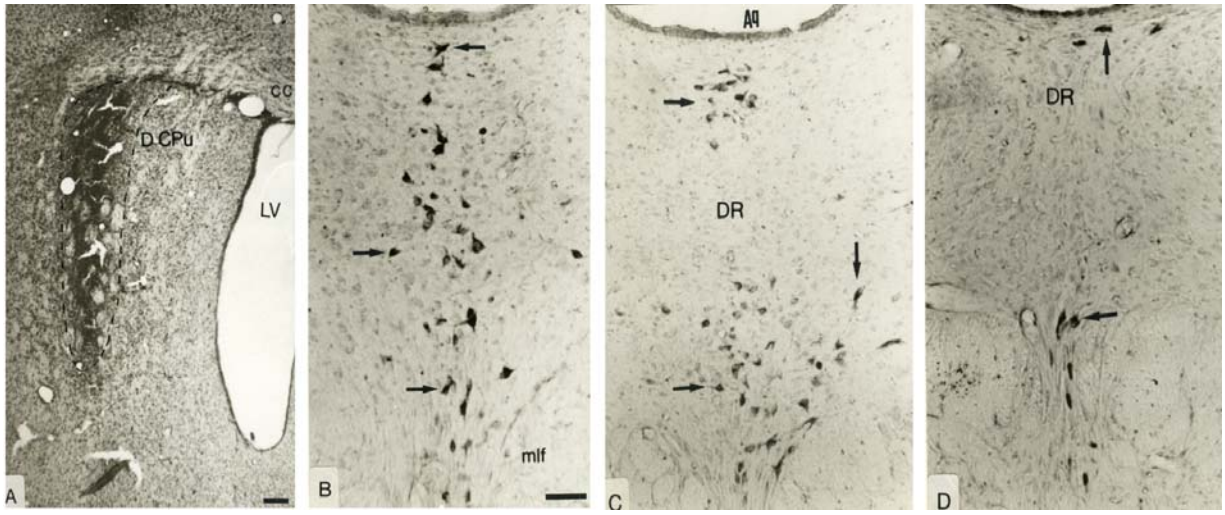

Fig. 1. Photomicrograph of HRP injection site in the dorsal striatum (A) and labeled neurons in the dorsal raphe nucleus from rostral to caudal portions (B, C, D). Scale bar A= 300 µm ; Scale bar B, C, D= 78 µm.

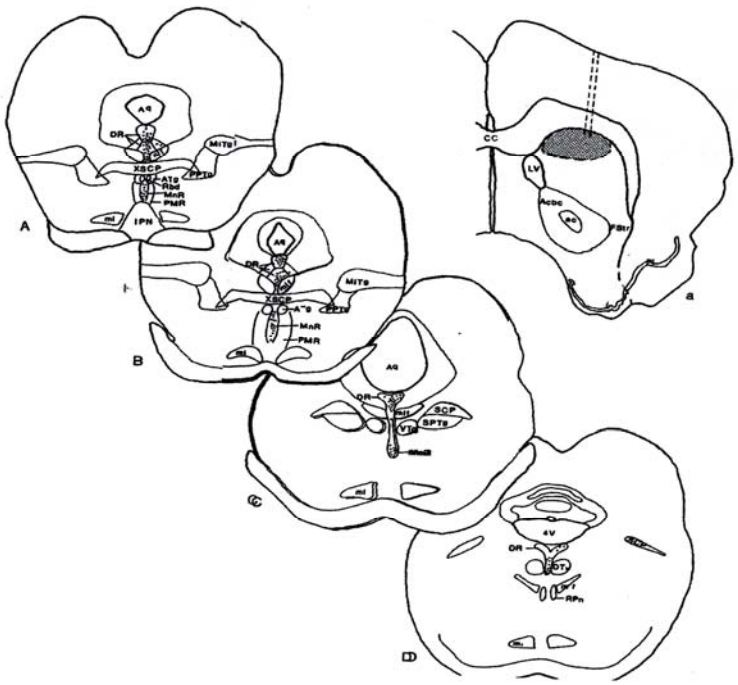

Fig. 2. Schematic representation of the injection site in the dorsal striatum (A) and retrogradely labeled neurons in dorsal and median raphe nuclei from rostral to caudal portions of these nuclei (A,B,C,D).Scale bar= 1 mm.

Labeled neurons distributed widely throughout the medial part of dorsal raphe nucleus. A few neurons were also located in lateral wing of dorsal raphe nucleus (DR). Some labeled neurons were found in lateral-medial line of DR more caudally (Fig. 2).

In median raphe nucleus (MnR), the labeled neurons were scattered only in median portion of this nucleus (Fig. 3). The labeled neurons were organized in a topographical manner in MnR (Fig 2). In caudal portions of MnR labeled neurons were less than rostral parts.

The ipsilateral injection of HRP into the ventral striatum resulted on labeling of numerous neurons in rostral, caudal and lateral portions of dorsal raphe nucleus (Fig 4).

The labeled neurons were significantly numerous in DR after injection of HRP into the ventral striatum and rostro-caudal reduction were also seen (Fig. 5). Through the caudal extension of DR on 4th ventricle level, a large number of labeled neurons were distributed along the ventral-caudal parts of DR (Fig. 5). In median raphe nucleus, a few labeled neurons were observed only in median part of this nucleus (Fig. 5, 6).

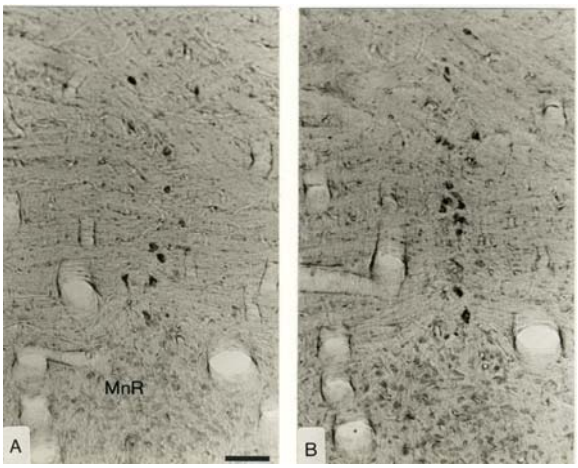

Fig. 3. Photomicrograph of labeled neurons in the median raphe nucleus following injection of HRP to dorsal striatum (A, B).Scale bar= 78 µm.

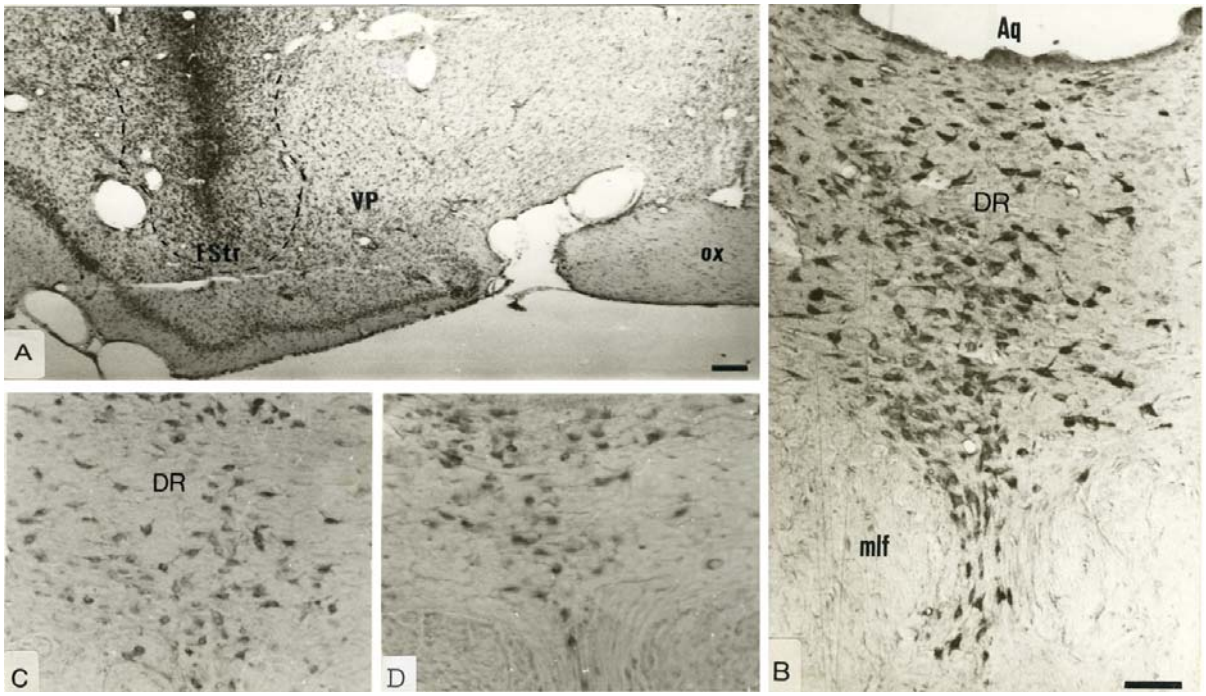

Fig. 4. Photomicrograph of HRP injection site in the ventral striatum (A) and labeled neurons in the dorsal raphe nucleus from rostral to caudal portions (B, C, D). Scale bar B, C, D = 75 µm.

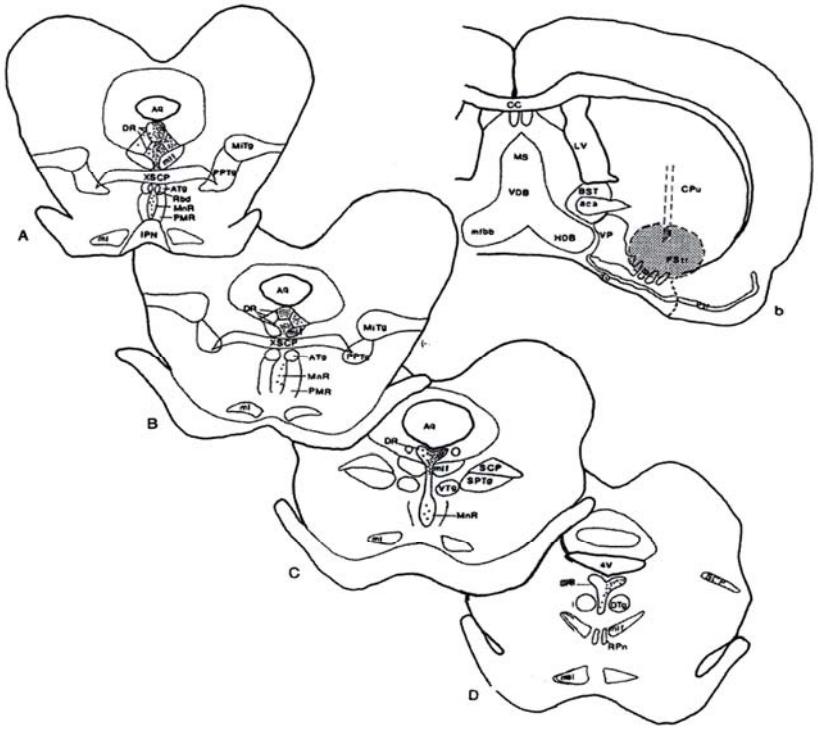

Fig. 5. Schematic representation of the injection site in the ventral striatum (B) and retrogradely labeled neurons in dorsal and median raphe nuclei from rostral to caudal portions of these nuclei (A, B, C, D). Scale bar= 1 mm.

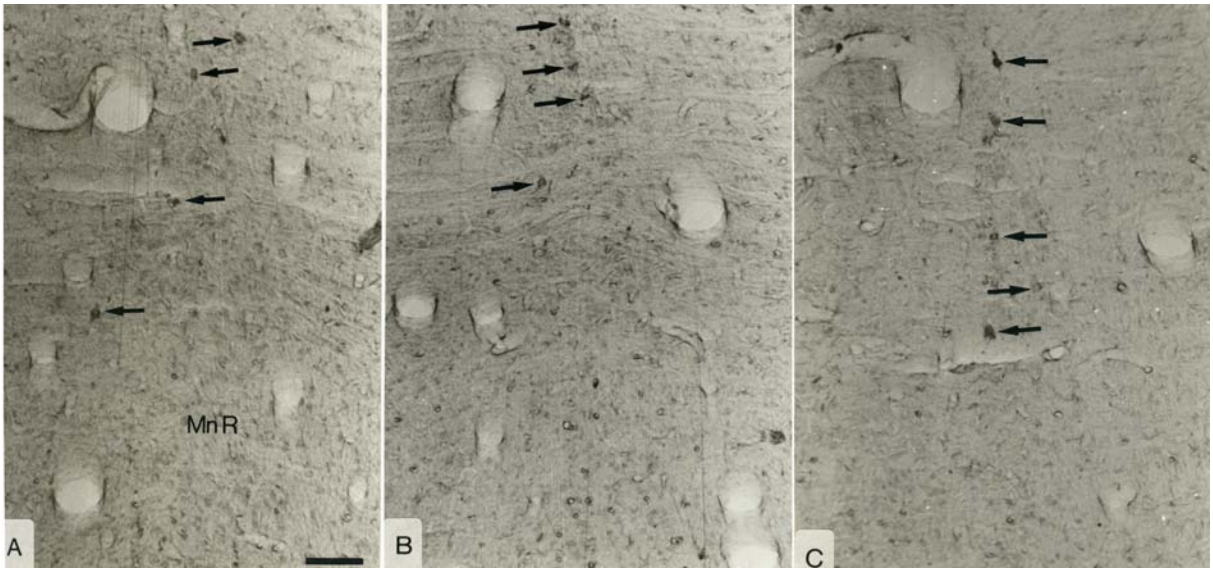

Fig. 6. Photomicrograph of labeled neurons in the median raphe nucleus following injection of HRP to ventral striatum (A, B, C). Scale bar= 78 µm.

DISCUSSION

Our results demonstrate that the neurons of mesencephalic raphe nuclei are topographically organized and projections of these nuclei to caudo-putamen of rat have topographical organization.

From a comparative point of view, after injection of HRP into the dorsal and ventral striatum, the labeled neurons in dorsal raphe nucleus were more numerous than median raphe nucleus. This finding is suggested by several previous studies (7-9). Anatomical studies have shown the presence of a major serotonergic projection from the dorsal raphe nucleus to the striatum, although the function of this pathway is unknown (10). Dorsal raphe nucleus project to both dorsal and ventral striatum but more significantly to ventral striatum. This finding confirms by previous studies (11-13), but Veening (1980) suggested that after injection of HRP to dorsal striatum no labeled neurons were observed in the dorsal raphe nucleus (2). In this study, we observed that the labeled neurons in rostral portions of DR are numerous than caudal portions of this nucleus. This finding was suggested previously (14, 15). Our findings indicate that a small number of retrogradely labeled neurons were observed in the median raphe nucleus following injections in the dorsal and ventral striatum. These findings suggest the mesencephalic raphe nuclei projections to caudoputamen are topographically organized. In addition dorsal and median raphe nuclei have a stronger projection to the ventral striatum. On the basis of their connections, the dorsal striatum is considered to be involved in sensorimotor and cognitive functions, where as the ventral striatum is suggested to be the main gate for limbic information to enter the basal nuclei (6).

Further retrograde double labeling studies are indicated to determine the possible axonal collateralization of raphe nuclei to different striatal portions (17, 18). It is now generally accepted that the dorsal and median raphe nuclei differentially innervate distinct forebrain regions (19-21). A comparison of MnR with DR projections shows that these two major serotonin-containing cell groups of the midbrain distribute to essentially nonoverlapoing regions of the forebrain (22). While many researcher believe that DR and MnR nuclei provide parallel and overlapping projections to many forebrain structures (4, 5). Therefore, the projections of dorsal and median raphe nuclei are organized in a topographical manner in all principal planes.

Conflict of interests

The authors declare that they have no competing interests.

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