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A Morphological and Electrophysiological Study of Nigrotectal Pathway in the Rat*

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After horseradish peroxidase injections in the superior colliculus of the rat, labelled cells were found in the pars reticulata of the substantia nigra. Nigrotectal cells are organized in a band beginning in the ventromedial area of the rostral part of pars reticulata, while at caudal levels they occupy a more lateral position, closely packed near the cerebral peduncle. In another series of experiments, the effects of substantia nigra electrical stimulation on collicular unitary activity was studied in anaesthetized rats. Electrical stimulation of the substantia nigra decreased the spontaneous and pharmacologically induced activity of superior colliculus neurons. Inhibition took place with short latencies (1-4 msec). Inhibited cells were localized in deep layers of the superior colliculus. In addition, long latency activation and inhibition were also obtained. It is concluded that nigrotectal pathway is mainly inhibitory in character.

Early observations had suggested the existence of a pathway connecting the substantia nigra (SN) with the superior colliculus (SC) (2, 11, 14). With the advent of retro- and anterograde axon tracer techniques this nigrotectal connection has been clearly demonstrated in the rat, cat and monkey (5, 7-10, 13). At the same time, these morphological findings have been corroborated by electrophysiological studies in the SC and SN of the rat (3, 4, 15) and cat (1).

The reasons to further study the nigrotectal pathway in the rat using both morphological and electrophysiological techniques have been: Firstly, previous injections of the retrograde tracer horseradish peroxidase (HRP) in the SC of the rat were rather large (5) resulting in the spread of the enzyme to the neighbouring dorsal mesencephalic tegmentum. In experiments reported here, an attempt was made to obtain HRP injections restricted to SC boundaries, but, at the same time, reaching enough diffusion to impregnate SN termi-

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nals in the nucleus. Secondly, there is a controversy about the electrophysiological characteristics of nigral projection to SC, since YORK and FABER (15) have proposed that it is excitatory, while DENIAU *et al.* (3) reported it as inhibitory. Therefore, the effect of SN stimulation on the activity of SC neurons has been reinvestigated in acute rats paying special attention to those collicular unitary responses that, because of their short latencies, could be accepted as direct (monosynaptic) result of nigral activation.

Materials and Methods

Morphological experiments were carried out on 10 albino rats. Animals were anesthetized with ether preceded by an injection of athropine sulphate (0.1 mg/kg, i.m.), the scalp sagittally opened, the left parieto-occipital bone partially removed and the underlying cortex suctioned. This surgical approach allowed the direct visualization of SC surface. HRP (Sigma VI, 30% in saline) was delivered by means of a mechanically-driven Hamilton syringe so that needle shaft entered SC in an anterior-posterior direction and parallel to its dorsal surface. With this procedure a single HRP injection (0.1-0.3 μl) was enough to reach by diffusion the two posterior thirds of the SC. Twenty-four to 48 hours after the injection, animals were perfused by a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were removed, potsfixed in the perfusate overnight and, lately, cut in 1 cm thick pieces and sliced at 50 μm in a freezing microtome. Sections were treated with a modification (12) of GRAHAM and KARNOVSKY (6) method to visualize HRP labelled neurons in SN. Alternative sections were lightly counterstained with 0.1% cresyl violet. Once mounted and coverslided, sections were examined microscopically under bright-field and dark-field illumination. Selected slices were photographed and the more interesting morphological details drawn using a camera lucida.

Electrophysiological experiments were carried out on 26 albino rats anesthetized with urethane (1.3 g/kg, i.p.). In each animal a bipolar stimulating electrode was stereotaxically implanted in SN. Stimulation consisted of cathodic rectangular pulses of 10 μsec at 1-4 Hz. At the end of the experiments stimulation sites were marked by electrocoagulation using a DC anodal current of 100 μA for 5 min and their positions checked histologically. The electrical activity of ipsilateral SC neurons was recorded extracellularly with glass microelectrodes (2-4 Mohms) filled with 2M NaCl saturated with pontamine sky blue (PSB). In those cases in which the recorded units showed a low rate of spontaneous activity neuronal firing was increased by injecting iontophoretically DL-homocisteic acid through a micropipette attached to the recording microelectrode. Recording sites were marked at the end of the experiment by iontophoretic injections of PSB (20 μA for 10 min).

Results

Morphological experiments. In 3 animals in which HRP injections were restricted into the caudal two-thirds of SC (fig. 1 and 2) the pars reticulata of the ipsilateral SN was densely labelled. Distribution pattern of SN cells containing HRP reaction products is shown in the serial coronal diagrams in figure 3. The highest density of labelled cells appeared at the level of coronal plane 2.8 (taking bregma as a reference), although marked cell bodies extended anteriorly to coronal plane P 2.2 and caudally to plane P 4.3 In the rostral part of SN labelled somata were found within the ventromedial region of the pars reticulata, while at caudal levels, labelled nigrotectal neurons appeared in a more lateral position, closely packed in the ventral aspect of the

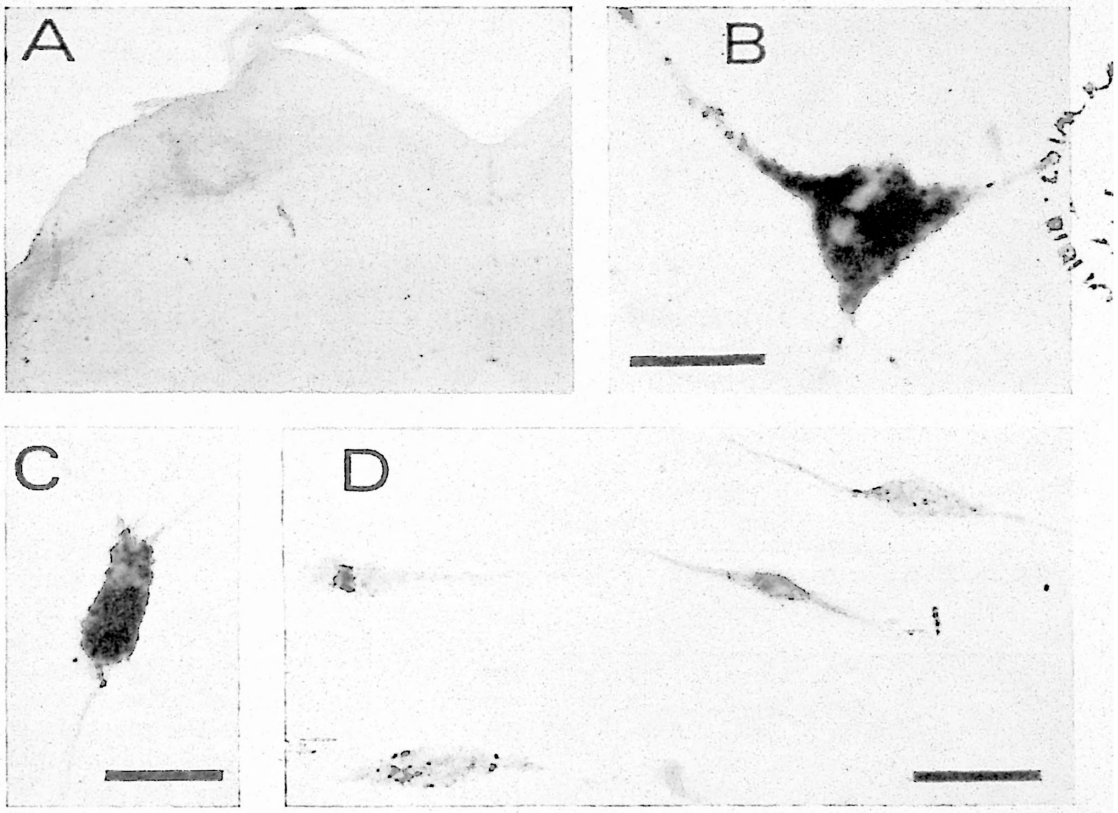

Fig. 1. *Examples of labelled cells in substantia nigra (SN) after horseradish peroxidase (HRP) injection in the superior colliculus (SC) of the rat.*
A: HRP injection in the left SC in animal #4. *B* and *C:* Two examples of multipolar cells labelled in the medial region of the SN pars reticulata after the HRP injection showed in *A*. *D:* A group of fusiform SN cells labelled after the injection showed in *A*. Calibration bar: 20 μm.

pars reticulata adjoining the cerebral peduncle (fig. 3). In normal and Nissl counterstained sections no labelled cells were found in the pars compacta of the SN. Furthermore, only a few cells were marked in the pars reticulata of the contralateral SN, mainly at the level of coronal planes P 2.8 to 3.4.

Labelled cell bodies in the pars reticulata showed two different shapes, fusiform and multipolar, with many intermediate types (fig. 1). Fusiform somata were usually situated with their major axis parallel and near cerebral peduncle fibers, especially at caudolateral levels, while multipolar cells were found in rostromedial areas of the pars reticulata at some distance from the cerebral peduncle.

Labelled cells were also found in an area situated between the brachium of the inferior colliculus and the cerebral peduncle. This area is not well defined in the rat, but it could correspond to the parabigeminal nucleus of cat and monkey. Another areas showing HRP reaction products were the temporal and parietal cor-

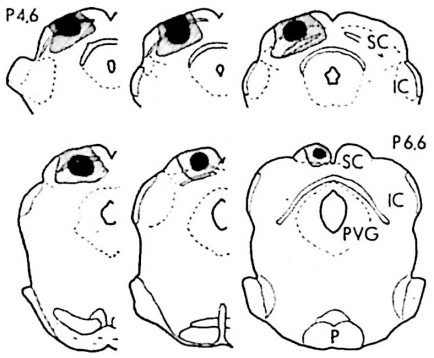

Fig. 2. *Schematic diagram showing the horseradish peroxidase injection in the superior colliculus (SC) in animal #4.*
The dark area corresponds to maximal enzyme concentration and the stripped area indicates the difusion zone in the nucleus. IC: inferior colliculus; PVG: periventricular gray; P: pyramidal fibers.

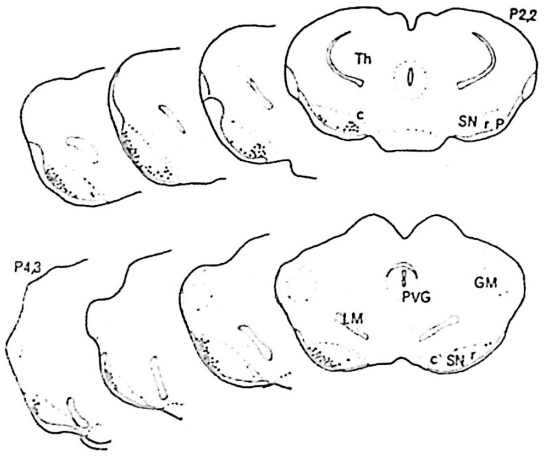

Fig. 3. *A composite diagram showing the location of substantia nigra (SN) labelled cells after the horseradish peroxidase injection in the superior colliculus showed in figures 1 and 2.*
Note that SN labelled cells occupy a ventromedial position in the anterior part PVC: periventricular gray, GM: medial geniculate nucleus; LM: medial lemniscus; r: pars reticulata of SN; c: pars compacta of SN; Th: thalamus.

tices, caudate nucleus, frontal eye field, lateral geniculate nucleus and medial regions of pontine reticular formation. A more detailed account of above mentioned projections will be published elsewhere (RIBAS and DELGADO-GARCÍA, in preparation).

In the remaining 7 animals HRP injections spread to SC neighbouring areas, mainly to periventricular gray matter, dorsal tegmemtum and inferior colliculus; the results of these large injections will not be considered here, but in relation to SN, distribution pattern of labelled cells in the reticulata was similar to the above described for HRP injections restricted to the SC.

Electrophysiological experiments. The activity of 73 neurons recorded within the boundaries of SC showed some kind of modification by the electrical stimulation of the SN. The types of recorded responses could be classified as follows: *Short latency inhibition.* The spontaneous activity of 13 SC cells was inhibited with a very short latency (1-4 msec) by SN stimulation. The inhibitory period lasted for 26 $\pm$ 6 ms being followed by a slight increase in the spontaneous activity of the cell. In figure 4 it is shown an example of this type of response. The histological analysis of recording electrode positions showed that inhibited cells were located in the intermediate and deep layers of SC (fig. 5 *A*). These SC cells were inhibited from SN sites showed in figure 5 *B*. Inhibitory effects were not longer induced when the stimulating electrode was located outside the limits of the SN. Inhibition of the SC neurons remained present even during the increase in their spontaneous firing induced by the iontophoretic injection of DL-homocisteic acid; *Long latency inhibition.* Twenty nine collicular cells were inhibited with latencies ranging from 10 to 16 ms. In these cells the inhibitory period lasted for 11.5 $\pm$ 5 ms. The blockage of cell activity was sometimes

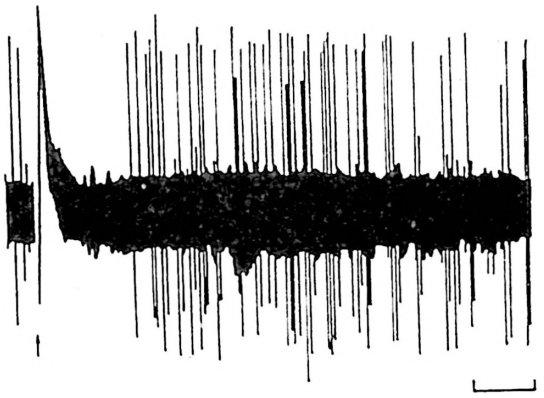

Fig. 4. *Extracellular recording of a superior colliculus unit inhibited by the electrical stimulation of the substantia nigra (superposition of 20 successive sweeps).*
Calibration bar: 20 msec. The arrow indicates the beginning of the stimulation artifact.

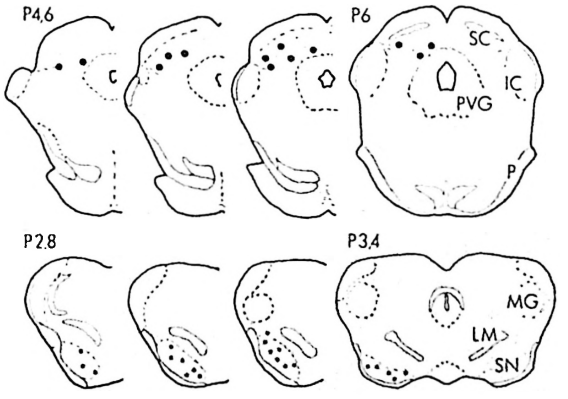

Fig. 5. *Anatomical location of superior colliculus (SC) neurons inhibited by substantia nigra (SN) electrical stimulation.*
Top part: A composite diagram showing the location (black dots) of 13 SC neurons inhibited with short latencies by SN stimulation. Bottom part: A composite diagram showing the location (asterisks) of stimulation sites from which short latency inhibition was induced in SC cells. MG: medial geniculate nucleus; LM: medial lemniscus; PVG: periventricular gray.

preceded and/or followed by a small activation. This group of cells was located in the deeper layers of SC; *Orthodromic activation.* A total of 26 SC neurons were activated with long latencies (18-32 ms) showing in most cases a burst of activity lasting for 32 ± 6 ms. No SC cell was activated with a latency similar to the short latency inhibitory effect; finally, another group of recorded responses was *Antidromic activation.* Only 5 cells were antidromically activated in the SC by SN stimulation. Latency for antidromic activation was 1.5-3 ms. These units could follow a high rate of SN stimulation (up to 100 Hz) without changing their activation latencies and showed all or no responses at threshold stimulation intensities.

Discussion

Results obtained in present experiments confirm previous reports (5, 10) about the existence of a nigrotectal projection in the rat. The same pathway has also been described in the cat and monkey by using retro- and anterograde tracer techniques (7-10, 13). According to findings reported here, this nigrotectal projection appears to be mainly ipsilateral, but a few labelled cells were also found in the pars reticulata of the contralateral side, especially in the more lateral portions of the nigra. Similar findings have been proposed elsewhere (5, 7, 9, 13).

The present study shows that cells giving origin to the nigrotectal pathway are located in the two anterior thirds of SN pars reticulata. This result is in opposition to previous reports (5) in which it was suggested that nigral cells projecting to SC are located in the medial third of SN pars reticulata. A likely reason to explain these different results is that in present experiments HRP injections in SC were made using an anterior-posterior approach thereby allowing the enzyme to spread to most parts of SC; since Faull

and MEHLER (5) used a lateral approach their HRP injections probably spread to more limited parts of SC.

In agreement with previous findings (5) present experiments have confirmed that SN neurons projecting to SC form a longitudinal band beginning rostrally in the medioventral aspect of the pars reticulata and shifting caudally to a more lateral position in the ventral part of the same nigral structure. A similar regional distribution of SN cell bodies projecting to the ipsilateral SN has also been described in cats and monkeys (7, 10).

Above mentioned morphological findings have been complemented by a second series of experiments in which the effects of SN stimulation on collicular unitary activity were studied. According to our results, a group of cells located in the intermediate and deep layers of the SC were inhibited by SN stimulation with latencies ranging from 1-4 ms. As the antidromic activation of SN cells by SC stimulation has a latency of 0.5-3 ms (4), the above mentioned short latency inhibitory responses can be accepted as the direct effect of nigral projection on collicular neurons. Similar results have been obtained by others (3). On the contrary, YORKS and FABER (15) have proposed that the nigrotectal pathway is excitatory, since SN stimulation induced short latency (3-4 ms) activation of collicular cells. In experiments reported here, SN stimulation only produced long latency (10-16 ms) excitatory effects on SC units, but as indicated by DENIAU *et al.* (3) electrical stimulation of cerebral peduncle fibers activates collicular cells with latencies ranging from 2 to 13 ms and the evoked responses were similar to the excitatory effects induced by SN stimulation. It is then possible that the short latency activation of collicular cells reported by YORKS and FABER (15) could be induced by unwanted activation of cerebral peduncle fibers and not by the activation of the nigrotectal pathway. In this regard, it is worth noting that, in results reported here, stimulation sites from which it was possible to induce short latency inhibition of SC neurons were located within SN boundaries. When the stimulating electrode was placed outside the limits of SN short latency inhibitory effects were not longer obtained (3).

The fact that SC cells inhibited with short latency by SN stimulation were located in the intermediate and deep layers of the nucleus is in agreement with previous findings according to which nigrotectal fibers terminate in those layers (7, 8).

The other effects of SN stimulation obtained in present experiments, namely, long latency activation and inhibition could be attributed to current spread to neighbouring structures, to activation of fibers travelling through the nigra or, finally, to polysynaptic effects induced in the intrinsic SC circuitry. In fact and according to present results, short latency inhibition is the only direct effect of SN stimulation on collicular neurons.

Resumen

La inyección de peroxidasa de rábano en el colículo superior de la rata marca neuronas en la pars reticulata de la sustancia negra. Las neuronas nigrotectales se organizan en una banda que se extiende desde la zona ventromedial de la pars reticulata rostral hasta la porción laterodorsal de la pars reticulata caudal. La estimulación eléctrica de la sustancia negra disminuye la actividad espontánea e inducida farmacológicamente de neuronas coliculares con latencias cortas (1-4 ms). Las neuronas inhibidas se localizan en las capas profundas del colículo superior. La estimulación de la sustancia negra también indujo activaciones e inhibiciones de latencia larga. Se concluye que la vía nigrotectal en la rata es de carácter inhibidor.

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