Origins of Postsynaptic Potentials Evoked in Identified Rat Neostriatal Neurons by Stimulation in Substantia Nigra

C.J. Wilson, H.T. Chang, and S.T. Kitai
Department of Anatomy, Michigan State University, A 517 West Fee Hall, East Lansing, MI 48823, USA

SUMMARY

Responses of striatal neurons to stimulation in substantia nigra were recorded intracellularly in intact rats and after acute or chronic unilateral lesions of cerebral cortex or after combined cortical lesions and unilateral thalamic transections. Spiny striatal efferent neurons were identified by intracellular injection of horseradish peroxidase. In intact animals substantia nigra stimulation evoked a complex response with both excitatory and inhibitory phases. Acute unilateral decortication abolished the inhibitory phase of the response and reduced the amplitude of the initial EPSP. Thus, part of the excitatory phase and most or all of the inhibitory phase of the response result from polysynaptic routes to striatum involving cerebral cortex. The remaining EPSP observed in acute decorticate animals exhibited two components distinguished on the basis of their time courses. The latter of these was abolished by thalamic transections. The earlier component was shown to be a monosynaptic EPSP evoked by axon collaterals of cortical efferent neurons projecting to brainstem and was not observed in animals subjected to chronic decortication. After removal of all of these non-nigral response components a small long latency EPSP could be evoked by nigral stimulation. This EPSP is probably due to activation of dopaminergic nigro-striatal axons.

KEY WORDS: Striatal spiny neurons - Nigro-striatal fibers, Striatal PSP's, corpus striatum, Substantia nigra stimulation

INTRODUCTION

Since the discovery of a large dopaminergic axonal projection from the substantia nigra to the neostriatum in mammals, numerous attempts have been made to characterize the postsynaptic action of nigrostriatal axons. In an early study of extracellular unit activity in cats, Frigyesi and Purpura (1967) described long latency (15-20 ms) discharges of neostriatal neurons following substantia nigra stimulation. These responses appeared to be orthodromic in nature and continued to occur despite previous destruction of descending cortical pathways, medial lemniscus and brachium conjunctivum. Subsequent extracellular studies have likewise demonstrated excitation of neostriatal neurons, but have also yielded inhibitory and mixed excitatory and inhibitory responses to nigral stimulation in the cat (e.g. Connor 1970; Feltz and Albe-Fessard 1972; Liles 1974; Zarzecki et al. 1976; Katayama et al. 1980) and rat (e.g. Gonzales-Vegas 1974; Richardson et al. 1977).

Intracellular recording studies of neostriatal neurons have consistently yielded a biphasic sequence of excitation followed by inhibition upon stimulation in substantia nigra (Hull et al. 1970; Hull et al. 1973; Kitai et al. 1975; Kocsis et al. 1977; Kocsis and Kitai 1977; VanderMaelen and Kitai 1981). The initial excitatory response has been shown to be an EPSP, and action potentials triggered from this EPSP occurred at latencies corresponding to those reported from extracellular recordings. The inhibitory phase of the response was less easily studied, its onset being obscured by the falling phase of the EPSP, but corresponded in, duration to extracellularly observed inhibition. It is thus likely that most or all of the various responses observed in extracellular recordings of striatal neurons represent the action of this excitatory-inhibitory response sequence upon neurons varying in excitability and rates of activity (Fuller et al. 1975; VanderMaelen et al. 1979). The origin of neither the excitatory nor the inhibitory portion of this response has been clearly established however, and their relationship if any to nigrostriatal axons is unknown. Numerous non-nigral fibers that could contribute to striatal responses are present in the vicinity of nigral stimulating positions. These include ascending corticopetal fibers in the ventral tegmentum (e.g. from locus coeruleus) and corticofugal fibers of the cerebral peduncle, both of which could elicit polysynaptic responses in neostriatum via cortico-striatal connections. The latter of these might also activate striatal neurons directly via collaterals given off en route through striatum (Donoghue and Kitai 1981). Ascending fibers in the medial lemniscus and nearby cerebellothalamic tract might also gain indirect access to neostriatal neurons via thalamostriatal connections. Monosynaptic effects on neostriatal neurons might also be exerted by raphe-striatal axons present in and around substantia nigra or from recurrent collaterals of striatonigral neurons activated antidromically by substantia nigra stimulation.

Consistent with the possibility that responses evoked from substantia nigra stimulation are not exclusively nigral in origin is the observation that striatal PSP's evoked in this way may be complex in nature. In both cats (Kocsis and Kitai 1977) and rats (VanderMaelen and Kitai 1981) the initial EPSP evoked by nigral stimulation has been shown to consist of at least two components distinguished on the basis of latency and resistance to inhibition. In both species the earliest component arrived at latencies too short to correspond to conduction time along dopaminergic nigro-striatal axons (Preston et al. 1981; Guyenet and Aghajanian 1978; Deniau et al. 1978). This earliest EPSP component was interpreted as monosynaptic

on the basis of its latency invariance over changes in stimulus intensity and frequency. The later and larger EPSP component and the subsequent inhibitory response were both within the latency range of dopaminergic nigro-striatal fibers. The onsets of these responses were obscured by earlier components however, and so were less accessible for tests of monosynapticity. The possibility that some component or components of neostriatal neuronal responses to substantia nigra stimulation may result from inadvertent stimulation of non-nigral fibers was tested in the experiments reported here. These studies indicate a direct involvement of collaterals of descending cortical efferent fibers activated by substantia nigra stimulation, and an indirect involvement of both cortico-striatal and thalamostriatal fibers activated polysynaptically from stimulation in that area.

METHODS

Subjects were 44 male Long-Evans rats weighing from 236 to 546 g. Of these, 14 rats were subjected to chronic unilateral lesions of the cerebral cortex, eight received acute cortical lesions and in three rats both acute decortication and unilateral thalamic transections were performed. The remaining 19 animals were not subjected to any lesion.

Chronic Lesions:

Unilateral aspiration lesions of the cerebral cortex were performed from 2 to 4 days prior to intracellular recording. These animals were anesthetized with sodium pentobarbital (Nembutal, 55 mg/kg i.p.) and placed in a stereotaxic frame. A large flap of skull overlying the cerebral cortex on one side was removed and the dura was cut and retracted. Suction applied to a small curved pipette was used under visual control to remove frontal and parietal cortex, including the entire frontal pole rostral to striatum, the dorsal medial surface to the midline and the dorsal lateral surface as closely as possible to the rhinal sulcus. The exposed surface was covered with oxidized cellulose (Oxycel), the wound closed and an antibiotic administered (Depo Penicillin, 30,000 units i.m.) to prevent infection.

Acute Lesions:

In 11 subjects, acute unilateral decortication was performed by aspiration in the same manner described above, but under urethane anesthesia during preparation for intracellular recording. In three of these cases a knife cut was also made through the thalamus on the decorticate side. These lesions were performed using a thin plastic knife 3 mm wide and shaped to conform to the ventral contour of the thalamus. It was oriented mediolaterally and lowered stereotaxically into the thalamus at the level of the rostral border of the parafascicular complex (5.0-5.5 mm anterior to the interaural line). Penetration of the knife was sufficient to cut the entire thalamus, including the medial lemniscus ventrally and penetrating slightly into zona incerta.

Surgery, Stimulation and Recording:

All animals were anesthetized with urethane (1.0-1.5 g/kg i.p.) supplemented as needed with intramuscular injections of ketamine (Vetalar, 30 mg/kg) and placed in a stereotaxic frame. Dexamethasone (0.5 mg/kg i.m.) was given to reduce brain edema. The skull was exposed and holes drilled for placement of stimulating and recording electrodes. Cisternal drainage was performed by puncture of the posterior atlanto-occipital membrane.

Stimulating electrodes were pairs of stainless steel insect pins (No. 00) insulated with epoxylite except for 0.2-0.5 mm at the tip and separated by 0.5-1.0 mm. They were oriented mediolaterally and placed in the ventral pons at the level of the pontine nuclei (AP 0.0; ML 0.5, 1.2; DV -4.5) and in the substantia nigra (AP 3.5; ML 1.5, 2.5; DV -2.8) (all coordinates are referred to a point on the midline and 5 mm above the interaural line). In some cases stimulating electrodes were also placed in intralaminar nuclei of the thalamus and in sensorimotor cortex of either side. Stimulating electrodes were secured to the skull with dental acrylic. Stimuli were rectangular pulses of 0.1-0.5 ms duration and were presented at 1.2 s intervals.

Recording micropipettes were broken under microscopic control to tip diameters of from 0.2 to 0.5 um and filled with 4% horseradish peroxidase (HRP, Sigma Type VI or Worthington HPOFF) in 0.05M Tris buffer (pH 7.6) and 0.5M potassium chloride, potassium acetate or potassium methylsulfate. After placement of the microelectrode in striatum the exposed surface of the brain was covered with soft paraffin wax to reduce brain pulsation. Recordings were made using a conventional active bridge amplifier. Neurons with stable membrane potentials of 45 mV or greater and action potentials of at least this amplitude were selected for study. Responses to stimulation of substantia nigra and ventral pons were recorded photographically from the oscilloscope face and also fed to a Nicolet 1074 signal averager (sample period 100 us) for storage on digital magnetic tape. Averages were computed for 4-8 consecutive sweeps and at least two averages of each response were taken as a check for reproducibility of significant features in the averaged record.

Neurons continuing to exhibit stable membrane potentials at the completion of the recording protocol were labelled with HRP by application of 300 ms positive ejection pulses (1-5 nA, 50% duty cycle) for 2-10 min through the bridge amplifier. Membrane potential and often evoked responses were monitored throughout the period of the injection. Intracellular current injection was terminated immediately upon any sign of deterioration of recording conditions.

Fixation and Tissue Preparation

At the end of the experiment a lethal dose of urethane was administered and animals were perfused intracardially with isotonic buffered saline followed by 2% formaldehyde, 2% glutaraldehyde in 0.15M phosphate buffer (pH 7.4). The brains were removed and stored in fixative overnight. Fifty micrometer Vibratome sections were cut in the sagittal plane and reacted for peroxidase activity as previously described (Chang et al. 1981; Wilson and Groves 1979). Sections were examined in buffer and those containing intracellularly labelled neurons were postfixed in osmium tetroxide and section embedded (Wilson and Groves 1979). The remainder were mounted on slides and stained with cresyl violet for reconstruction of electrode placements.

RESULTS

Identification of Spiny Neurons:

Intracellularly labelled striatal spiny neurons were identified by their somato-dendritic morphology according to established criteria derived from Golgi stained (Fox et al. 1971/72; Kemp and Powell 1971; DiFiglia et al. 1976; Somogyi and Smith 1979) and HRP labelled (Kocsis et al. 1977; Preston et al. 1980; Wilson and Groves 1980) material. They were characterized by their spine-free somata and dendrites heavily laden with spines. The dendrites of these cells radiated in an approximately 0.5 mm diameter spherical pattern except where distorted by the presence of fascicles of internal capsule fibers or the border of the nucleus. Their axons emitted several initial collaterals from which arose elaborate terminal arborizations mostly within the dendritic fields of the cells of origin. In several cases the main axons of these neurons were followed into globus pallidus where they were observed to make synaptic contacts, or were activated antidromically from substantia nigra. These observations have been documented elsewhere (Chang et al. 1981).

Included in the sample of unidentified neurons were several neurons which were intracellularly labelled but could not be identified because of their extreme state of degeneration and several other neurons which were well preserved but were not of the spiny cell type. The small size of the sample of aspiny neurons prevented an analysis of these cells according to cell type, but there were no noticeable differences between them and identified spiny neurons in their responses to substantia nigra stimulation.

Responses in Intact Animals:

Responses to stimulation in the substantia nigra were studied in 33 unidentified neurons and 28 identified spiny cells in the striatum of 19 intact rats. All of these neurons responded to SN stimulation with an initial EPSP followed

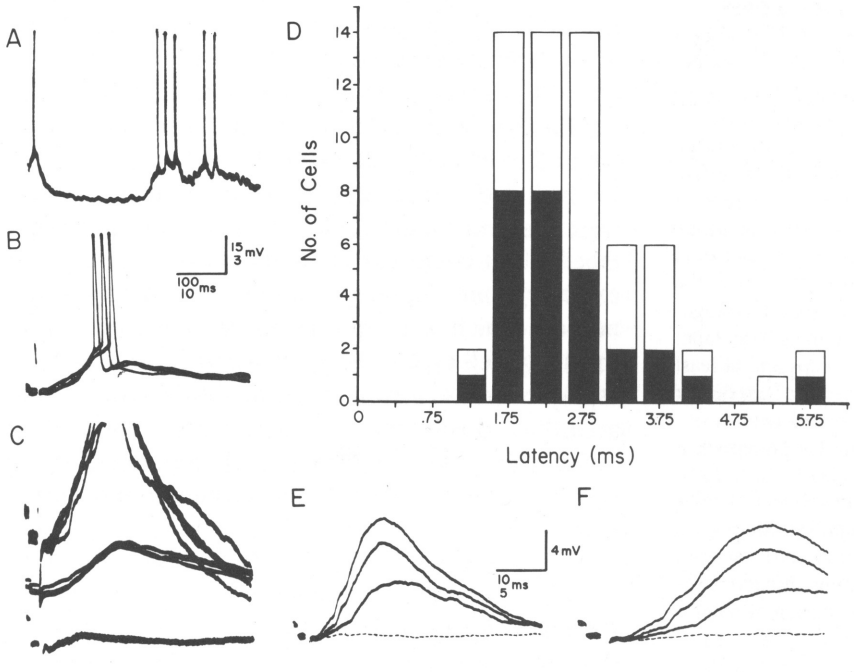

Fig. 1. Responses of a striatal spiny neuron to stimulation of substantia nigra in an intact rat (A, B, C, E, F). A Low gain DC record at slow sweep speed showing the initial EPSP, prolonged hyperpolarization and subsequent excitation following a single stimulus in substantia nigra. B Three superimposed traces at a faster sweep speed showing action potentials in response to maximal stimulation in substantia nigra. C High and low gain superimposed responses to the same maximal stimulus shown in B. Action potentials were prevented by application of 0.5 nA hyperpolarizing current through the microelectrode. Extracellular control is shown at high gain below. Calibrations for A and C are shown in B. D Distribution of EPSP onset latencies for spiny neurons (filled bars) and unidentified neurons (open bars). E and F Averaged EPSP's from the same spiny neuron at varying stimulus intensities. Extracellular control is shown as a dotted line. Calibration pulses at beginning of traces in this and all subsequent figures are 2 mV in amplitude and 1 ms in duration

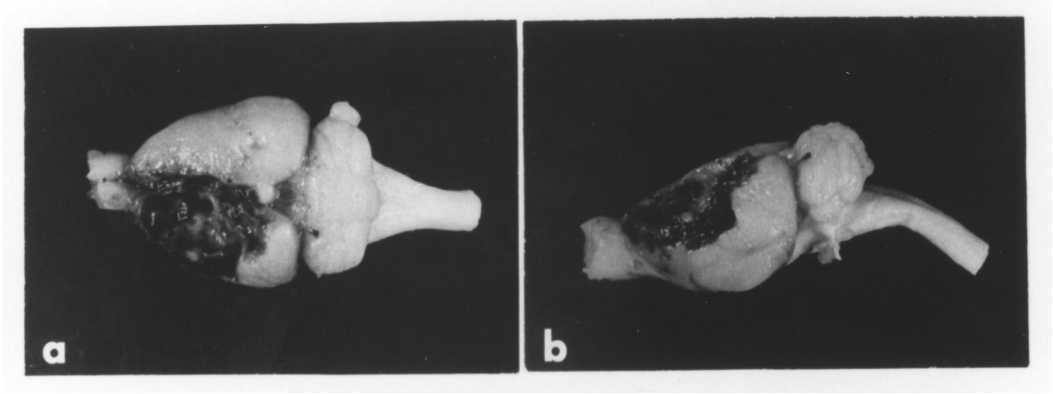

Fig. 2. Dorsal (a) and lateral (b) views of a rat brain showing the extent of unilateral cortical lesions

by a prolonged period of membrane hyperpolarization and often a subsequent variable excitation accompanied by action potentials. Examples of this response from a representative spiny neuron are shown in Fig. 1. The latency of the initial EPSP varied among neurons from 1.5 to 6.0 ms (mean = 2.84, SD = 1.01). No difference in mean latency could be detected between the sample of spiny neurons and unidentified cells. The latency to EPSP onset was constant for individual neurons and could not be made to vary by alteration of stimulus intensity or frequency (Fig. 1). Peak EPSP amplitude occurred at latencies varying from 8-20 ms (mean = 14.1, SD = 4.3, N = 61). Maximal EPSP amplitudes were generally obtained with stimuli from 2 to 5 times the threshold intensity and varied from 4 to 20 mV (mean = 9.3, SD = 4.3, N = 59). Only one or two action potentials could generally be evoked from the maximal response (Fig. 1A, B). Examination of averaged EPSPs consistently revealed the presence of one or two inflections on the rising phase of the initial excitation, and a more variable one was often seen on the falling phase of the response (Fig. 1E, F). Although these inflections suggested the presence of more than one EPSP component, they were not sufficiently distinct to allow a systematic analysis of their latencies or relative contribution to response amplitude.

The initial excitatory phase of the response was terminated by the onset of the hyperpolarization at latencies ranging from 25-50 ms (mean = 35.1, SD = 8.6, N = 60). This later inhibitory response to SN stimulation varied in duration from 150-300 ms and attained amplitudes as great as 15 mV below resting potential (Fig. 1A). It was usually followed abruptly by a variable period of noisy depolarization, and this very long latency excitation was often very effective in eliciting action potentials. In many cases firing at long latencies was observed using stimuli below firing threshold for the short latency initial excitation.

Responses After Acute Decortication:

The possibility that some component or components of the response to substantia nigra stimulation might be mediated via polysynaptic pathways involving intracortical synaptic connections was tested in 8 animals subjected to

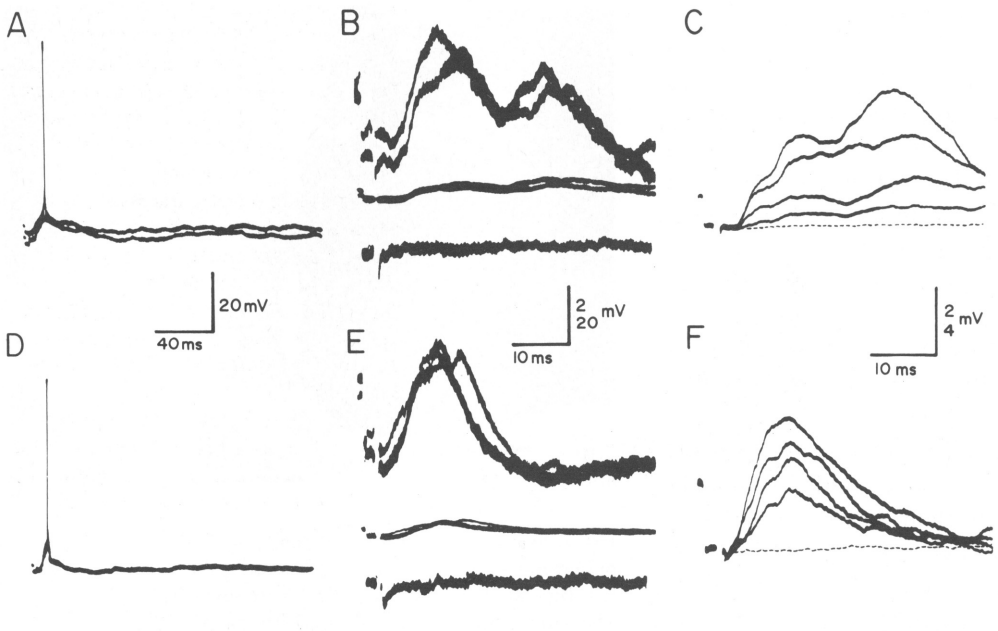

Fig. 3. Responses of spiny neurons to substantia nigra stimulation in an animal with an acute cortical lesion (A, B, C) and an animal subjected to both acute decortication and thalamic transection (D, E, F). Note absence of inhibitory responses with cortical lesions and loss of late excitatory component after thalamic transection. Averages traces in C and F show responses at various stimulus intensities. Gain in F is twice that used in C. Extracellular controls are shown below B and E and as dotted lines in C and F

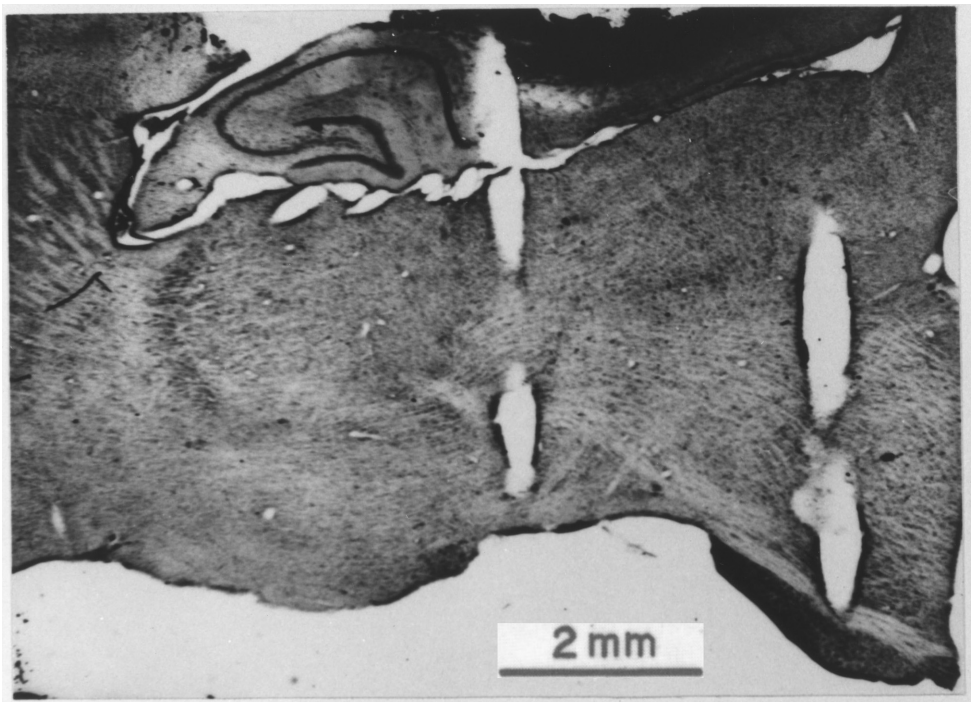

Fig. 4. Sagittal section from an animal with stimulating electrode placements in substantia nigra and ventral pons. Rostral is to the left. Bipolar stimulating electrodes were separated mediolaterally. The more medial nigral electrode track and the more lateral pontine electrode track are visible

unilateral decortication immediately prior to preparation for intracellular recording. In this preparation synaptic activation or inhibition of ipsilateral cortico-striatal neurons is eliminated without destruction of cortical axons either in the brainstem or in the striatum. Presumably because of the wide-spread distribution of cortico-striatal neurons (e.g. Jones et al. 1977, Hedreen 1977), large cortical lesions were required to obtain consistent results in this preparation. Photographs of the brain of one of these animals are shown in Fig. 2. These lesions generally spared the subcortical white matter, and in some experiments the integrity of the crossed cortico-striatal pathway was tested by stimulation of cerebral cortex on the intact side. In two cases there was inadvertent damage to the corpus callosum and crossed cortico-striatal responses could not be elicited. Responses to ipsilateral substantia nigra stimulation in these animals were not noticeably different from those with comparable ipsilateral destruction but sparing of callosal fibers.

Responses to substantia nigra stimulation in 13 unidentified neurons and 16 spiny neurons in acute decorticate rats differed markedly from those obtained in intact animals. Responses from an identified cell are shown in Fig. 3. EPSP amplitudes were reduced after cortical aspiration, ranging from 2.5 to 9.0 mV when maximal (mean = 4.6, SD = 1.9, N = 29). The difference between normal and decorticate preparations in mean amplitude was statistically significant ($t = 5.70$, $df = 86$, $p < 0.01$), and apparently resulted from the loss of a large excitatory component with onset latency in the 6-12 ms range and a peak amplitude corresponding approximately to that observed for the EPSP in intact animals. EPSP's recorded

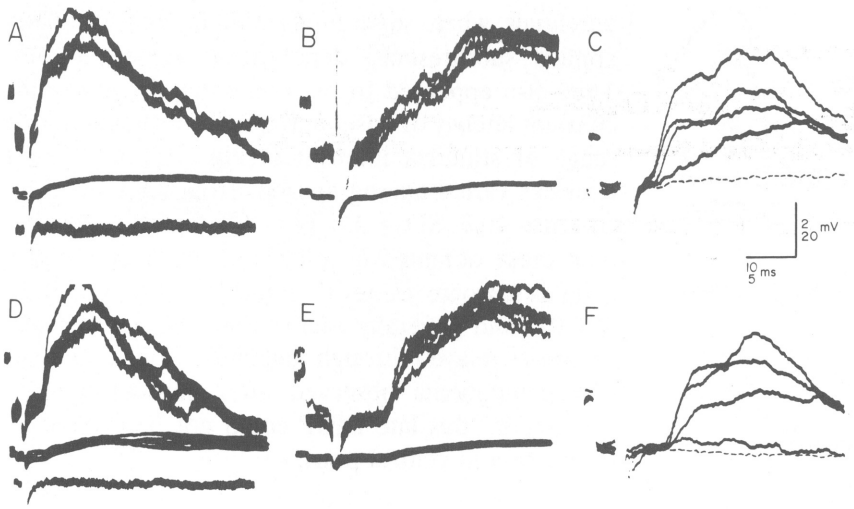

Fig. 5. Responses of a spiny neuron to substantia nigra (A, B, C) and ventral pontine (D, E, F) stimulation. Note latency difference in the responses. Extracellular controls are shown below A, B, D, and E and as dotted lines in the averaged traces (C and F)

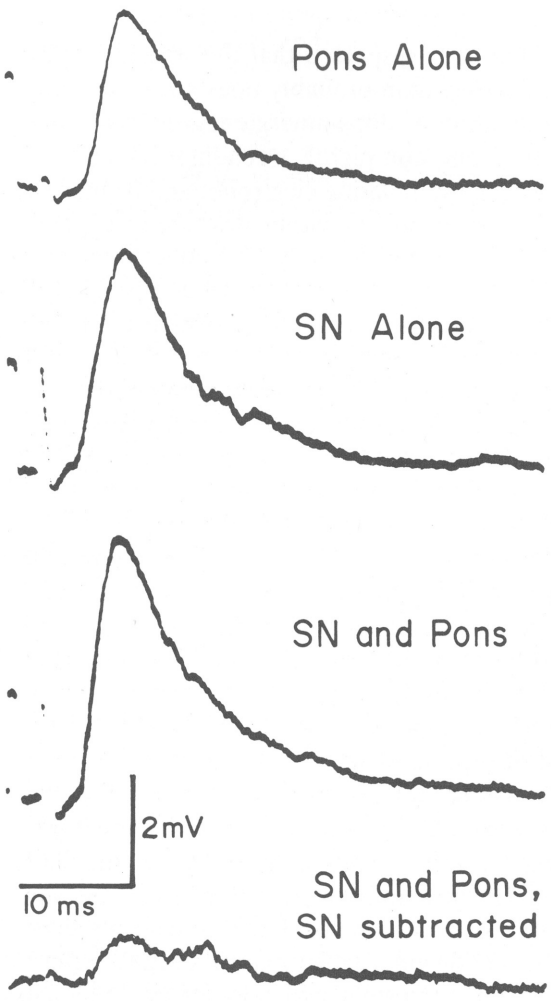

Fig. 6. Collision of the early EPSP component upon simultaneous stimulation in substantia nigra and ventral pons. Acute decorticate preparation with thalamic transection. Top two traces show averaged EPSP to substantia nigra and pontine stimulation presented singly. The response to simultaneous stimulation is shown in the third trace. The failure of these responses to summate is demonstrated in the bottom trace, which shows the result of subtracting the response to substantia nigra stimulation from the response obtained by simultaneous stimulation at both sites

from both spiny and unidentified striatal neurons in decorticate preparations exhibited two clear amplitude peaks (Fig. 3B, C). Onset of the earliest remaining excitatory component did not differ from that seen in intact animals, ranging from 2.0 to 6.5 ms (mean 3.2, SD = 1.1, N = 29). The second major excitatory component was more variable in onset, ranging from 17 to 48 ms, but corresponded approximately with the inflection seen on the falling phase of the EPSP observed in intact animals. These two components of the initial EPSP were well separated in time for most neurons. In many instances the second component showed small but systematic decreases in latency with increasing stimulus suggesting that it may be polysynaptic in nature (Fig. 3).

The normally prominent hyperpolarizing component of the response to substantia nigra stimulation could not be demonstrated in the acutely decorticated preparation (Fig. 3). Concurrent passage of constant depolarizing current sufficient to produce high frequency repetitive firing or hyperpolarizing current up to 2 nA was attempted as a test for the possibility that this response might somehow be masked by changes in resting membrane potential or reversal potential for this response. These tests were likewise negative. Also absent was the long latency excitatory response (compare Figs. 3A with 1A). Consistent with the loss of the inhibitory phase of the response was a small but statistically significant increase in the mean duration of the initial EPSP. Duration of the EPSP ranged from 33 to 65 ms in the acute decorticate animals (mean = 47.9, SD = 11.4, N = 29) in contrast with the 20 to 50 ms range obtained for intact animals (mean = 35.1, SD = 8.6, N = 59) ($t = 5.93$, $df = 86$, $p < 0.01$).

The variability of the second of the two EPSP components observed in acute decorticate animals suggested the possibility that it may result from polysynaptic pathways not destroyed by cortical removal. The possibility of thalamic participation in striatal neuronal responses to SN stimulation was examined in three rats subjected to both acute decortication and a unilateral knife cut through thalamus at the level of the rostral border of the parafascicular nucleus. The knife cut extended ventrally 200-400 um into zona incerta ventral to thalamus, laterally to the lateral edge of thalamus, and medially to within 0.3 mm of the midline. Thalamic nuclei rostral to the knife cut were isolated from ascending fibers of the medial lemniscus, cerebello-thalamic pathway and retrothalamic bundle, which are most likely to be stimulated from nigral electrode positions, while more caudal thalamic nuclei were separated from their ascending axons.

Responses of an identified spiny striatal neuron in this preparation are shown in Fig. D-F. All striatal neurons in these animals exhibited highly abbreviated, relatively simple monosynaptic EPSP's in response to substantia nigra stimulation. The latencies and peak amplitudes of these responses were identical to those of the early EPSP component seen in the acute decorticate preparation (Fig. 3B, E). In three spiny neurons and six unidentified neurons, this EPSP had maximal amplitude ranging from 2.0 to 6.0 mV (mean = 4.0, SD = 1.2, N = 9). Latencies varied from 1.5 to 4.0 ms (mean = 2.6, SD = 0.9, N = 9) and peak amplitudes occurred at latencies ranging from 5.0 to 13.0 (mean = 9.3, SD = 2.8, N = 9). Mean duration of the EPSP was 27.3 ms (SD = 4.5, N = 8), and the reduction of EPSP duration from that seen in acute decorticate animals was statistically significant despite the small sample of neurons in the combined lesion group ($t = 5.00$, $df = 35$, $p < 0.01$).

Origin of the Short Latency EPSP:

The resistance of the short latency component of substantia nigra-evoked striatal EPSP's to destruction of polysynaptic pathways through thalamus and cerebral cortex, as well as its constant latency and time course over changes in stimulus intensity, suggests that this component of the response results from stimulation of fibers making monosynaptic connections on striatal neurons. The short latency of this EPSP is not consistent with the known conduction times of dopaminergic nigrostriatal neurons in substantia nigra, pars compacta. It could be reconciled with the much higher conduction velocity of a smaller population of presumably nondopaminergic nigro-striatal projection neurons in substantia nigra pars reticulata (e.g. Deniau et al. 1978; Guyenet and Aghajanian 1978). Another alternative is the antidromic activation of descending cortical efferent fibers passing near the stimulation position in substantia nigra and contributing collaterals to striatum (Donoghue and Kitai 1981). As a test for this last possibility stimulating electrodes were placed in ventral pons in the region of descending cortical efferent fibers of the cortico-pontine, cortico-bulbar and cortico-spinal pathways. This stimulating position, as well as the distance between pontine and substantia nigra stimulating sites, is shown in Fig. 4. Responses to substantia nigra and ventral pons were compared in both intact and acute decorticate animals, and in animals with acute cortical and thalamic lesions. All components of substantia nigra evoked responses described above could be reproduced by stimulation at the pontine site, including late components of the EPSP in decorticate and intact animals and the prolonged hyperpolarizing response seen in intact animals. Examples of responses from an identified spiny neuron in an acute decorticate animal are shown in Fig. 5. Responses to pontine stimulation consistently occurred at latencies from 0.2 to 1.5 ms longer than those of nigra-evoked responses in the same neurons. Assuming that these responses result from activation of the same fibers, but at points separated by the 3.0-3.5 mm distance between stimulation sites, this latency difference suggests a 2-10 m/s conduction velocity for fibers responsible for the earliest component of the EPSP in striatal neurons. Evidence for a single set of fibers mediating substantia nigra and pontine evoked responses was obtained from collision experiments in which both sites were stimulated either simultaneously or at very short interstimulus intervals. If pontine and substantia nigra evoked responses were independently generated some degree of summation should occur with simultaneous stimulation. The result of this test is shown for a spiny neuron in an animal with combined acute cortical and thalamic lesion in Fig. 6. As shown in that example, little or no summation of substantia nigra and pons evoked EPSP's occurred with simultaneous stimulation of both structures. Results of this kind were obtained consistently in both intact and acute decorticate preparations.

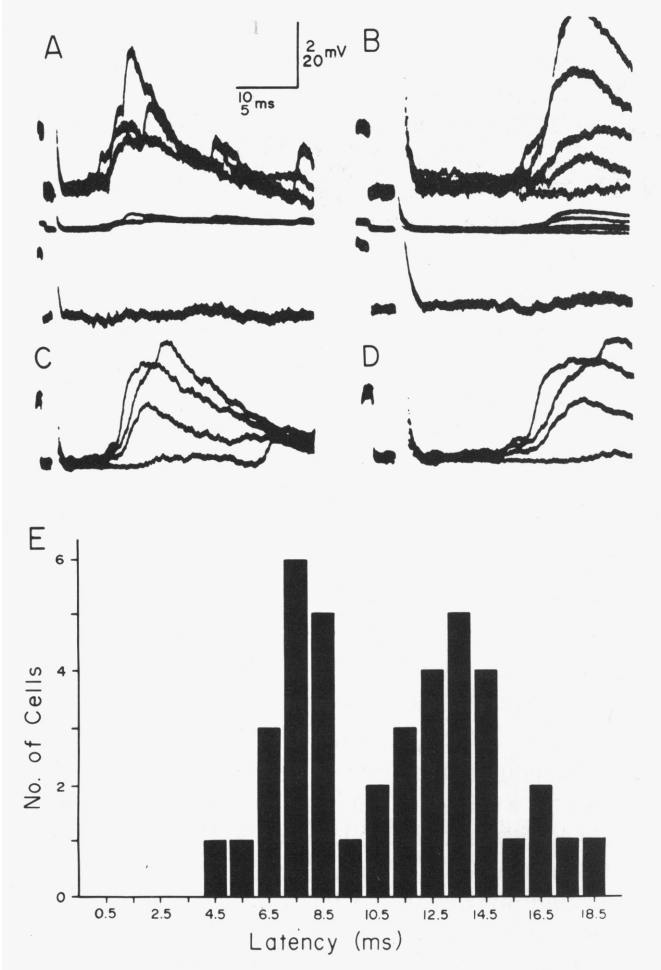

Fig. 7. Responses of a spiny neuron to substantia nigra stimulation after degeneration of cortical efferent axons (A-D). A Superimposed traces showing time course of long latency EPSP. B Superimposed traces at higher sweep speed and with varying stimulus intensity. C and D Averaged responses of the same neuron with varying stimulus intensity. Extracellular controls are shown below A and B. E Distnbution of EPSP latencies for 40 striatal neurons responding to substantia nigra stimulation 2-4 days after decortication

Responses After Chronic Decortication:

The most likely candidate for the high conduction velocity fibers activated from both substantia nigra and ventral pontine stimulation sites and responsible for the short latency EPSP in striatal neurons is cortical efferent fibers descending through the mid-brain. In acute decorticate preparations the descending axons of cortical efferent neurons are likely to remain functional, and any collaterals which they might give off in striatum could remain capable of eliciting EPSP's in striatal neurons. A direct test of this possibility was afforded by examination of striatal neuronal responses in animals subject to the same lesion but surviving for a sufficient period of time to allow degeneration of cortical axons in striatum. Intracel-

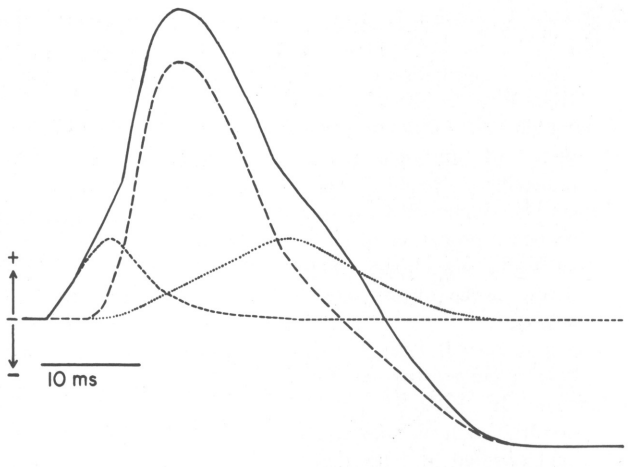

Fig. 8. Reconstruction of PSP components contributing to the response to substantia nigra or ventral pons stimulation in the intact animal. Time course of the early EPSP component (short dashes) is based on maximal responses obtained in animals with acute cortical lesions and thalamic transections. Time course of the late thalamic component (dotted line) was obtained by comparison of responses in acute decorticate animals with and without thalamic transections. Time course of the large polysynaptic cortical component (long dashes) was estimated by comparison of the average response in intact (solid line) and acute decorticate rats. Onsets of polysynaptic components are approximate

lular recordings of striatal neurons were obtained in 14 rats surviving 2, 3, or 4 days after large unilateral cortical aspiration lesions. At all of these survival periods early EPSPs from substantia nigra or pontine stimulation were absent in most (47 of 55 striatal neurons. Eight striatal neurons exhibiting early EPSP's were located primarily in ventral portions of striatum and probably resulted from failure to remove areas of cortex projecting to this region. The remaining 47 neurons lacking short latency EPSP's in response to substantia nigra or ventral pontine stimulation likewise failed to exhibit any of the other response components normally evoked from these sites at stimulus intensities within the normally effective range (0-1.0 mA, 0.1 ms duration).

With longer substantia nigra stimulus durations 0.2-0.5 ms) or increased stimulating currents (1.0-2.0 mA), a long latency EPSP could be observed in 40 neurons, including 16 identified spiny cells. Examples of these responses from one spiny neuron are shown in Fig. 7. Although usually not attaining amplitudes sufficient to trigger action potentials, these long latency EPSPs could fire action potentials when superimposed upon intracellularly applied subthreshold depolarizing current pulses. They also appeared to be monosynaptic, exhibiting constant latency of onset and time course over a wide range of stimulus intensities (Fig. 7C, D). Onset latencies varied among neurons from 4.8 to 18.5 ms (mean = 10.4, SD = 3.6, N = 40), significantly later than those obtained in normal or acute decorticate animals (chronic lesions vs. intact, $t = 7.8$, $df = 97$, $p < 0.01$) and generally earlier than the polysynaptic responses relayed through thalamus. Unlike all other PSP components observed after substantia nigra stimulation, this late EPSP could not be evoked by stimulation in ventral pons.

DISCUSSION

It has long been suspected that the striatal response to nigral stimulation probably does not result simply from activation of dopaminergic nigro-striatal fibers but also from non-nigral ascending fibers in the vicinity of the stimulating electrodes and from activation of intrinsic circuits within striatum (e.g. Hull et al. 1973; Kocsis and Kitai 1977; VanderMaelen and Kitai 1981). The relative contributions of these various components to the total response has however remained controversial. The results of the present experiments indicate that under the usual circumstances of stimulation in substantia nigra neither nigro-striatal fibers nor intrastriatal circuitry plays a major role in generating the responses of striatal neurons. Although both of these are no doubt present to some degree, the major components of the intracellularly recorded neuronal response can be accounted for by the monosynaptic and polysynaptic activation of cortico-striatal and thalamo-striatal pathways. Analysis of intra-striatal connections and nigro-striatal responses will depend upon the experimental disruption of pathways responsible for these large response components which mask the other more subtle effects of substantia nigra stimulation.

The shortest latency component of the EPSP evoked by substantia nigra stimulation in intact animals is a monosynaptic EPSP apparently generated by antidromic activation of cortical efferent fibers in the brainstem and invasion of their axon collaterals within the striatum. Evidence supporting this view includes the observations that: (1) it is monosynaptic, (2) it is resistant to acute decortication and interruption of thalamo-striatal connections, (3) it is elicited from stimulating positions along the course of cortico-pontine, cortico-bulbar and corticospinal fibers in the brainstem, and (4) it is abolished after degeneration of cortical efferent fibers in striatum. The existence of intrastriatal axon collaterals of cortical efferent fibers in the internal capsule has been suggested from studies using the Golgi methods (Cajal 1911; Webster 1961; Kemp and Powell 1971) and from experiments employing antidromic activation of cortical neurons (Endo et al. 1973; Oka and Jinnai 1978; Miller 1976; Jinnai and Matsuda 1979). Recently these collaterals have been unambiguously demonstrated by Donoghue and Kitai (1981) using intracellular HRP labelling of identified brainstem projecting neurons in the sensorimotor cortex of the rat. The latency differences obtained for substantia nigra and pontine-evoked EPSPs in the present study suggest conduction velocities in the 2-10 m/s range, in good agreement with the 3-8 m/s figure obtained by Donoghue

and Kitai for antidromic activation of cortico-striatal neurons also projecting to brainstem. The total EPSP latency from either substantia nigra or pontine stimulation suggests a somewhat lower mean conduction velocity which may be accounted for by the small diameter and long trajectory of intrastriatal collaterals described by those authors.

A large component of the initial EPSP evoked by both substantia nigra and ventral pontine stimulation requires the integrity of intra-cortical connections as indicated by its disappearance after acute decortication. The approximate time course and maximal amplitude of this portion of the response can be estimated from a comparison of EPSPs evoked in intact and acute decorticate preparations. The result of such a comparison is shown in Fig. 8. The large maximal amplitude of this component suggests that it may not result simply from synaptic excitation of the same cortico-striatal neurons responsible for the early EPSP component. The existence of two distinct classes of cortico-striatal neurons, one branching to brainstem and one projecting exclusively to striatum has been suggested from the results of experiments using striatal-evoked antidromic activation of cortical neurons (Jinnai and Matsuda 1979). It is possible that fibers activated by substantia nigra and ventral pontine stimulation are effective in recruiting a larger population of unbranched cortico-striatal neurons which give rise to the large polysynaptic EPSP component. Synaptically activated cortical neurons might also be capable of recruiting thalamo-striatal neurons (Cherubini et al. 1979).

The finding that prolonged inhibitory responses to substantia nigra stimulation are abolished after decortication suggests that these responses are also polysynaptic and depend upon intracortical synaptic connections. A long duration inhibitory component has been observed in striatal responses to all known afferents to that structure (e.g., Hull et al. 1973; Purpura and Malliani 1967; Kocsis and Kitai 1977; VanderMaelen and Kitai 1981; VanderMaelen et al. 1979), and has usually been interpreted as the result of inhibitory interactions between striatal neurons. Such long duration inhibitory responses have been reported to be absent in striatal slices maintained in vitro however (Misgeld et al. 1979; Lighthall et al. 1981). Many cortical neurons exhibit EPSP-IPSP sequences in response to stimulation in cerebral peduncle or thalamus (for review see Phillips and Porter 1977). If such responses occur in cortico-striatal neurons, they could account for polysynaptic EPSP's in striatal neurons, followed by a 200-300 ms period of disfacilitation. The inhibitory component of striatal responses may thus represent primarily a temporary inhibition of a tonic cortico-striatal excitatory synaptic discharge.

A small and slowly rising polysynaptic EPSP contributing to substantia nigra and pontine-evoked striatal responses continues to occur despite acute decortication. This response component, however, requires intact intrathalamic connections for its expression as indicated by its loss after acute thalamic knife cuts. A number of routes are known through which stimulation in substantia nigra or ventral pons might elicit excitation of thalamo-striatal neurons. The loss of this response component in animals surviving decortication for 2-4 days but not after acute decortication suggests that it may be somehow dependent upon intact cortical efferent fibers. Anti-dromic activation of pyramidal tract neurons from thalamic nuclei has been reported by Araki and Endo (1976). Although anatomical evidence for such collateralization is lacking (Jones 1976), a cortical axon reflex exciting thalamo-striatal neurons would help to explain the present findings.

At 2-4 days after extensive unilateral decortication a long latency apparently monosynaptic EPSP was evoked from substantia nigra but not pontine stimulation sites. The time course of this EPSP did not match that of any of the major components contributing to the response observed in intact animals, and its appearance usually required the use of stimulus intensities higher than those normally used in the other preparations. Probably because of its low amplitude and high threshold in comparison with non-nigral response components, this EPSP was not detected in normal or acute decorticate animals. Possible non-nigral sources for this response include activation of raphe striatal fibers coursing near nigral stimulative electrodes and recurrent excitation from antidromically activated striato-nigral fibers. Excitatory striato-nigral responses were described by Frigyesi and Purpura (1967) and have been reported by subsequent investigators (e.g., Feger and Ohye 1975; Dray et al. 1976; Kanazawa and Yoshida 1980). Neither the neurons responsible for these responses nor any intrastriatal axon collaterals they may possess have yet been identified, and no direct evidence for recurrent excitation in striatum has yet been reported. The latency of this response is in agreement with published antidromic conduction times for nigro-striatal neurons, however, and no other candidate response components for nigro-striatal fibers could be identified. Inhibitory postsynaptic potentials have not been observed in response to substantia nigra stimulation after chronic decortication. It is thus likely that the monosynaptic long latency EPSP observed in these animals represents the postsynaptic action of dopaminergic nigro-striatal fibers.

Acknowledgements. We thank Bill Bitzinger and Julie King for their technical assistance and Carol Meister for typing the manuscript. A preliminary report of this work was presented at the 10th Annual Meeting of the Society for Neuroscience, November, 1980. Supported by USPHS grant NS17294 (to C. J. Wilson), USPHS grant NS 14866 (to S. T. Kitai), and NIH BRSG RR 0572 to the College of Osteopathic Medicine, Michigan State University

REFERENCES

Araki T, Endo K (1976) Short latency EPSPs of pyramidal tract cells evoked by stimulation of the centrum medianum-parafascicular complex and the nucleus ventralis anterior of the thalamus. Brain Res 113: 405-410.

Chang HT, Wilson CJ, Kitai ST (1981) Single neostriatal efferent axons in the globus pallidus. A light and electron microscopic study. Science (in press)

Cherubini E, Novack G, Hull CD, Buchwald NA, Levine MA (1979) Caudate neuronal responses evoked by cortical stimulation. Contribution of an indirect corticothalamic pathway. Brain Res 173: 331-336

Connor JD (1970) Caudate nucleus neurones: Correlation of the effects of substantia nigra stimulation with iontophoretic dopamine. J Physiol (Lond) 208: 691-703

Deniau JM, Hammond C, Riszak A, Feger J (1978) Electrophysiological properties of identified output neurons of the rat substantia nigra (pars compacta and pars reticulata). Evidences for the existence of branched axons. Exp Brain Res 32: 409-422

DiFiglia M, Pasik P, Pasik T (1976) A Golgi study of neuronal cell types in the neostriatum of monkeys. Brain Res 114: 245-256

Donoghue JP, Kitai ST (1981) A collateral pathway to the neostriatum from neurons in somatic sensory-motor cortex demonstrated by intracellular labeling with HRP. J Comp Neurol (in press)

Dray A, Gonye TJ, Oakley NR (1976) Caudate stimulation and substantia nigra activity in the rat. J Physiol (Lond) 259: 825-849

Endo K, Araki T, Yagi N (1973) The distribution and patterns of axon branching of pyramidal tract cells. Brain Res 57: 484-491

Feger J, Ohye C (1975) The unitary activity of the substantia nigra following stimulation of the striatum in the awake monkey. Brain Res. 89: 155-159

Feltz P, Albe-Fessard D (1972) A study of an ascending nigro-caudate pathway. Electroencephalogr Clin Neurophysiol 33: 179-193

Fox CA, Andrade AN, Hillman DE, Schwyn RC (1971/72) The spiny neurons in the primate striatum. A Golgi and electron microscopic study. J Hirnforsch 13: 181-201

Frigyesi TL, Purpura DP (1967) Electrophysiological analysis of reciprocal caudato-nigral relations. Brain Res 6: 440-456

Fuller DRG, Hull CD, Buchwald NA (1975) Intracellular responses of caudate output neurons to orthodromic stimulation. Brain Res 96: 337-341

Gonzalez-Vegas JA (1974) Antagonism of dopamine-mediated inhibition in the nigro-striatal pathway: A mode of action of some catatonia-inducing drugs. Brain Res 80: 219-228

Guyenet PG, Aghajanian GK (1978) Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra. Brain Res 150: 69-84

Hedreen JC (1977) Cortico-striatal cells identified by the peroxidase method. Neurosci Lett 4: 1-7

Hull CD, Bernardi G, Buchwald NA (1970) Intracellular responses of caudate neurons to brain stem stimulation. Brain Res 22: 163-179

Hull CD, Bernardi G, Price DD, Buchwald NA (1973) Intracellular responses of caudate neurons to temporally and spatially combined stimuli. Exp Neurol 38: 324-336

Jinnai K, Matsuda Y (1979) Neurons of the motor cortex projecting commonly on the caudate nucleus and the lower brainstem in the cat. Neurosci Lett 13: 121-126

Jones EG (1976) Areal differences in the laminar distribution of thalamic afferents in cortical fields of the insular, parietal and temporal regions of primates. J Comp Neurol 168: 197-247

Jones EG, Coulter JD, Burton H, Porter R (1977) Cells of origin and terminal distribution of corticostriatal fibers arising in the sensory-motor cortex of monkeys. J Comp Neurol 173: 53-80

Kanazawa L, Yoshida M (1980) Electrophysiological evidence for the existence of excitatory fibers in the caudato-nigral pathway in the cat. Neurosci Lett 20: 301-306

Katayama Y, Tsubokawa T, Moriyasu N (1980) Slow rhythmic activity of caudate neurons in the cat. Statistical analysis of caudate neuronal spike trains. Exp Neurol 68: 310-321

Kemp JM, Powell TPS (1971) The structure of the caudate nucleus of the cat: Light and electron microscopy. Philos Trans R Soc Lond [Biol] 262: 383-401

Kitai ST, Wagner A, Precht W, Ohno T (1975) Nigro-caudate and caudato-nigral relationship. An electrophysiological study. Brain Res 85: 44-48

Kocsis JD, Kitai ST (1977) Dual excitatory inputs to caudate spiny neurons from substantia nigra stimulation. Brain Res 138: 271-283

Kocsis JD, Sugimori M, Kitai ST (1977) Convergence of excitatory inputs to caudate spiny neurons. Brain Res 124: 403-413

Lighthall JW, Park MR, Kitai ST (1981) Inhibition in slices of rat striatum. Brain Res 212: 182-187

Liles SL (1974) Single-unit responses of caudate neurons to stimulation of frontal cortex, substantia nigra and entopeduncular nucleus in cats. J Neurophysiol 37: 254-265

Miller R (1976) Distribution and properties of commissural and other neurons in cat sensorimotor cortex. J Comp Neurol 164: 361-374

Misgeld U, Okada Y, Hassler R (1979) Locally evoked potentials in slices of rat neostriatum: A tool for the investigation of intrinsic excitatory processes. Exp Brain Res 34: 575-590

Oka H, Jinnai K (1978) Common projection of the motor cortex to the caudate nucleus and the cerebellum. Exp Brain Res 31: 31-42

Phillips CG, Porter R (1977) Corticospinal neurones. Academic Press, New York

Preston RJ, Bishop GA, Kitai ST (1980) Medium spiny projection from the rat striatum: An intracellular horseradish peroxidase study. Brain Res 183: 253-263

Preston RJ, McCrea RA, Chang HT, Kitai ST (1981) Anatomy and physiology of substantia nigra and retrorubral neurons studied by extra- and intracellular recording and by horseradish peroxidase labelling. Neuroscience (in press)

Purpura DP, Milliani A (1967) Intracellular studies of the corpus striatum. I. Synaptic potentials and discharge

characteristics of caudate neurons activated by thalamic stimulation. Brain Res 6: 325-340
Ramon y Cajal S (1911) Histologie du systeme nerveux de l'homme et des vertebres, vol 2. Maloine, Paris
Richardson TL, Miller JJ, McLennan H (1977) Mechanisms of excitation and inhibition in the nigrostriatal system. Brain Res 127: 219-234
Somogyi P, Smith AD (1979) Projection of neostriatal spiny neurons to the substantia nigra. Application of a combined Golgi-staining and horseradish peroxidase transport procedure at both light and electron microscopic levels. Brain Res 178: 3-15
VanderMaelen CP, Bonduki AC, Kitai ST (1979) Excitation of caudate-putamen neurons following stimulation of the dorsal raphe nucleus in the rat. Brain Res 175: 356-361
VanderMaelen CP, Kitai ST (1981) Intracellular analysis of synaptic potentials in rat neostriatum following stimulation of the cerebral cortex, thalamus and substantia nigra. Brain Res Bull 5: 725-733
Webster KE (1961) Cortico-striate interrelations in the albino rat. J Anat 95: 532-544
Wilson CJ, Groves PM (1979) A simple and rapid section embedding technique for sequential light and electron microscopic examination of individually-stained central neurons. J Neurosci Meth 1: 383-391
Wilson CJ, Groves PM (1980) Fine structure and synaptic connections of the common spiny neuron of the rat neostriatum. A study employing intracellular injection of horseradish peroxidase. J Comp Neurol 194: 599-615
Zarzecki P, Blake DJ, Somjen GG (1976) Interactions of nigro-striate synaptic transmission, iontophoretic o-methylated phenethylamines, dopamine, apomorphine and acetylcholine. Brain Res 115: 357-372