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CAUDATE NUCLEUS NEURONES: CORRELATION OF THE EFFECTS OF SUBSTANTIA NIGRA STIMULATION WITH IONTOPHORETIC DOPAMINE

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SUMMARY

1. Extracellular recordings were made from caudate nucleus neurones in decerebrate cats using five-barrel micropipette electrodes.

2. Electrical stimulation of the substantia nigra caused spike rate depressions in 121 of 260 caudate neurones as evidenced by changes in the post-stimulus histograms of their firing patterns. Spike rate facilitation occurred with forty-two caudate units.

3. Neurones depressed by nigral stimuli were consistently depressed by micro-iontophoretic dopamine.

4. Alpha-methyldopamine, administered iontophoretically, antagonized the depressant responses induced by both nigral stimulation and iontophoretic dopamine.

5. The data are compatible with the concept that the caudate neuronal depressant responses produced by nigral stimulation are mediated by a direct dopaminergic nigro-neostriatal pathway. The possibility of mediation via polysynaptic pathways cannot, however, be completely excluded.

INTRODUCTION

Numerous lines of evidence support the hypothesis that a system of dopaminergic fibres links the substantia nigra with the caudate nucleus. An anatomical basis for the proposed pathway was provided by the histofluorescence studies of Andén, Carlsson, Dahlström, Fuxe, Hillarp & Larsson (1964) who described the cytological distribution of dopamine (DA) within the two brain areas. Nigral DA occurs largely within the soma of pars compacta neurones, while caudate DA is concentrated within

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diffuse terminal varicosities distributed in close proximity to non-fluorescent caudate neurones. The course of the interconnecting fibres has been mapped by retrograde degeneration after caudate extirpation (Andén, Dahlström, Fuxe & Larsson, 1965; Andén, Dahlstöm, Fuxe, Larsson, Olson & Ungerstedt, 1966*a*). Electrical stimulation of the nigra evokes changes in both intracellularly and extracellularly recorded responses of caudate neurones (McLennan & York, 1967; Frigyesi & Purpura, 1967; Albe-Fessard, Raieva & Santiago, 1967; Connor, 1968*a*, *b*; Feltz & MacKenzie, 1969). Because the types of responses elicited vary with experimental conditions, the extent to which the fluorescent pathway serves as a mediator of the electrophysiological events is uncertain.

Overt decreases in the nigral neuronal population, either spontaneously occurring (Hornykiewicz, 1966) or experimentally induced (Poirier & Sourkes, 1965), are closely associated with reduced caudate DA levels. Conversely, electrical stimulation of the nigra causes sustained release of homovanillic acid, a DA metabolite, into the ventricular fluid bathing the caudate (Portig & Vogt, 1968). DA, ejected by micro-iontophoresis (Curtis & Eccles, 1958) into the immediate vicinity of caudate neurones, alters unit firing rates (Bloom, Costa & Salmoiraghi, 1965; McLennan & York, 1967; Herz & Zieglgängsberger, 1966). The most frequent DA effect in the caudate is depression of both spontaneous neuronal activity and the firing induced by iontophoretic excitant amino acids.

The foregoing observations, when considered as a group, suggest that dopamine may function as a neurotransmitter in the caudate. A key criterion for transmitter identification is the requirement for 'identical action' (Werman, 1966), i.e. the putative transmitter should evoke the same subsynaptic responses as the 'natural' transmitter. As a corollary, ejection of a suspected transmitter by iontophoresis should elicit the same qualitative changes in a neurone's firing pattern as does release of an endogenous transmitter. In the present investigation the effects produced by iontophoretic DA on caudate neurone firing rates were studied in conjunction with the response of the same neurone to nigral stimulation. The results of this combined approach are offered as a first step toward elucidating the functional significance of caudate DA at the neuronal level.

METHODS

The data were obtained from sixty-four adult cats electrolytically decerebrated at the midpontine level (Batini, Moruzzi, Palestini, Rossi & Zanchetti, 1959; Pl. 1 a) under temporary ether anaesthesia. The pyramidal tracts and ventropontine vasculature remained intact. To minimize pulsations and cerebral oedema a portion of cerebrospinal fluid was removed by cisternal puncture. After the cortex was exposed the brain was covered with a pool of warm paraffin oil. No recordings were attempted until 1·5–2 hr after the ether was discontinued. Respiratory CO_2 was

continuously monitored in all animals and was usually 3·5–4·0 % of expired air. Femoral systolic blood pressures were between 120 and 170 mm Hg. The cats consistently maintained core temperatures of 40–40·5° C (Connor & Crawford, 1969). Six of the decerebrated cats were immobilized with gallamine triethiodide (3 mg/kg I.V., supplemented as needed) and mechanically ventilated with room air.

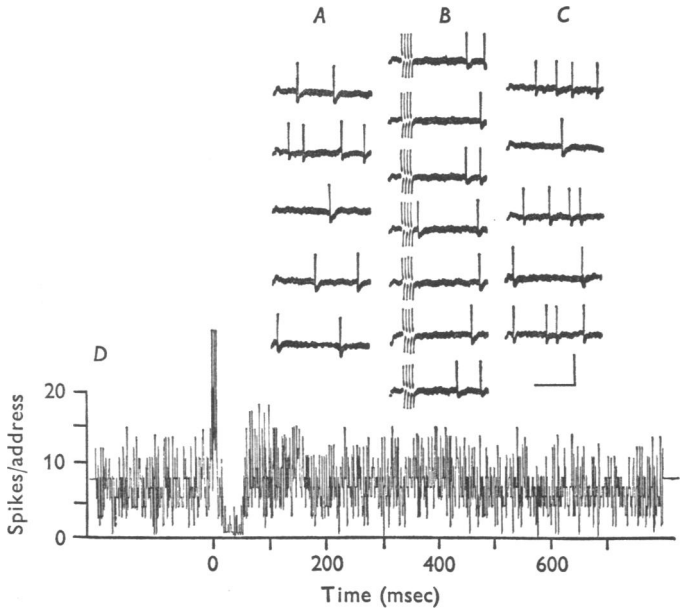
Placement of the stimulating electrodes in the substantia nigra was achieved by utilizing a combination of stereotaxic and electrophysiologic criteria. A silver ball electrode was placed on the lateral surface of the precruciate gyrus (Pl. 1 d), and a large indifferent electrode was inserted into the nuchal musculature. Bipolar stainless-steel stimulating electrodes (oriented parasagittally, DC impedance 30 kΩ in saline, insulated to within 1 mm of their tips, interpolar distance 1 mm) were introduced through the intact cortex at a two-dimensional stereotaxic point above the substantia nigra. While the stimulating electrodes were being advanced ventrally, single square-wave pulses (5 V, 0·1 msec duration, 1/sec) were applied through the electrodes. When the electrodes passed through the area of the medial lemniscus (Pl. 2*B*, c; approximate stereotaxic depth of −2, Snider & Niemer, 1961), a reproducible positive-negative-positive slow wave was monitored from the cortex (Pl. 2*A*, c). Upon further lowering of the electrodes, the evoked response rapidly diminished so that at stereotaxic depths of −4 to −5 the potentials were essentially isolectric (Pl. 2*A*, d). Subsequent histologic examinations of lesioned preparations indicated that at this depth the tips of the electrodes were in the posterior portion of the substantia nigra, stereotaxic co-ordinates A 3·5, L 4·5, D −4 to −5 (Snider & Niemer, 1961; Pl. 1b; Pl. 2*B*, d). If the electrodes were advanced beyond the substantia nigra into the pes pedunculi, a short latency evoked response was elicited (Pl. 2*A* and *B*, e). Normally, the electrodes were not lowered past the depth at which an isoelectric response was obtained.

Nigral stimulation with shock intensities greater than 10 V caused forelimb and neck musculature contractions suggesting considerable current spread. At stimulus voltages which were subthreshold for muscular twitching, single or paired pulses failed to alter caudate neurone firing patterns. Short trains of low voltage stimuli appeared more effective for this purpose. Therefore the nigral stimulation used in this study had the following parameters: 4–6 V, 400 Hz, 10 msec train duration, repetition rate 1·3 sec. A 6-pole stimulating array was used in four experiments to compare the responses elicited by nigral stimulation with the effects produced by stimulation of other mesencephalic areas.

Unit action potentials were recorded extracellularly from caudate neurones in the area delineated by the stereotaxic co-ordinates A 14·5 to 18, L 2 to 5, D +5 to +8 (atlas of Snider & Niemer, 1961; Pl. 1c). Five-barrel glass micropipette electrodes with tip diameters of 3–7 μ were prepared as described previously (Salmoiraghi & Weight, 1967). The centre recording barrel was filled with 4·8 M-NaCl and had a DC impedance in saline of 2–8 MΩ. Three outer barrels contained solutions of ionizable compounds whose effects were to be studied, and the fourth outer barrel contained 2 M-NaCl. The following agents were employed: dopamine HCl (1·0 M), acetylcholine Cl (1·0 M), monosodium-L-glutamate (0·5 M at pH 8), DL-homocysteic acid (0·5 M at pH 8·5), alpha-methyldopamine (courtesy of Dr C. C. Porter, Merck Institute for Therapeutic Research, 0·5 M in 1 % (w/v) aqueous solution ascorbic acid), chlorpromazine HCl (0·05 M in 1 % NaCl), phentolamine HCl (0·01 M), phenoxybenzamine HCl (0·005 M), dichloroisoproterenol HCl (0·5 M), propranolol (0·5 M), D(−)INPEA HCl (courtesy of Dr W. Murmann, Selvi & C., 0·5 M). These compounds were ejected with constant currents of 5–100 nA of appropriate polarity. Retaining currents were employed between drug ejections to retard leakage of active ions. The 2 M-NaCl side barrel was used for current 'neutralization' (Salmoiraghi & Steiner,

1963); it automatically passed currents equal to the sum of the currents flowing in the other barrels but of opposite polarity. In addition, anodal and cathodal currents of 100 nA were routinely passed through this barrel to evaluate the effects of current alone on neuronal responses.

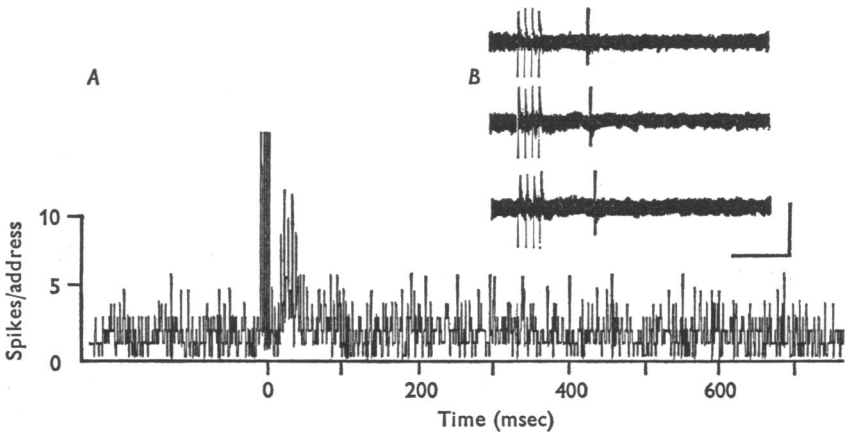
Extracellularly recorded spikes detected by the recording barrel were amplified, displayed on an oscilloscope, and photographed. Action potentials were also voltage gated and converted to pulses of constant amplitude and duration. The pulses were integrated and displayed with a pen recorder as a continuous record of discharge frequency. The current flow in the drug barrels was simultaneously monitored on a polygraph. The gated spike output was also fed to a peak detector and then to a Computer of Average Transients (CAT 1000). On-line post-stimulus time histograms (Gerstein & Kiang, 1960) were obtained by plotting 125–300 summations of unit spike patterns in analog form using a rectilinear pen writing recorder. A change in discharge pattern was considered significant when the spike rate for a period of at least 40 msec was increased or decreased by a factor of 3 or more relative to base line firing.



Text-fig. 1. Depressant changes in firing pattern of a caudate neurone in response to nigral stimulation. *A* to *C*: unit spike responses before (*A*), during (*B*), and after (*C*) stimulation. The photographic records are consecutive. Calibrations: 40 msec and 300 μV. In *B* there is decreased spike tendency during the time period between 10 and 50 msec after the stimulus train artifact. This response pattern corresponds to the period of firing rate depression obtained in analog form (*D*) from the same cell. Histogram *D* is the sum of the effects produced by 300 nigral stimuli. Abscissa: time after the stimulus train artifact at zero. Ordinate: number of spikes deposited in each computer address (1 msec per address).

RESULTS

Few spontaneously active caudate neurones were normally encountered. Most recordings were made from units whose firing was maintained by DL-homocysteic acid or L-glutamic acid, either ejected iontophoretically (5 nA) or allowed to diffuse passively by removing the retaining current. The responses of units producing spikes of at least 150 μV uncomplicated by the firing of other neurones were examined. Maximum spike amplitudes recorded with the multibarrel micropipettes were about 300 μV with base line noise levels of about 60 μV. The amino acids maintained the firing rates of different neurones between 1 and 20/sec.



Text-fig. 2. Facilitation of the firing of a caudate neurone during nigral stimulation. *A*. Histogram obtained by summating 125 sweeps, 1 msec per address. Spike facilitation period has a post-stimulus latency of about 18 sec and a duration of 40 msec. *B*. Sequential oscilloscopic film records from the same neurone. The excitant amino acid was withheld to eliminate random spiking. The driven spike in this small sample occurs about 20 msec after the stimulus train artifact. Calibrations: 20 msec and 200 μV.

Substantia nigra stimulation. The effects of nigral stimuli on the firing patterns of 260 caudate neurones were studied. The post-stimulus time histograms indicated that the firing frequencies of 121 cells were depressed. The periods of firing rate depression occurred approximately 18 msec after the stimulus. Frequently, depressed firing was followed by a period of augmented discharge having an approximate latency of 75–100 msec. An example of the compound response is given in Text-fig. 1. Post-stimulus facilitation of a much earlier latency (12–15 msec) was obtained from forty-two caudate units. A histogram with associated oscilloscope tracings from a facilitated unit are presented in Text-fig. 2. The durations of the

firing pattern changes for depressed cells and facilitated cells were similar (50–70 msec). Responsive units were normally encountered at least 1 mm below the lateral ventricle ependyma in the head of the caudate. Facilitated and depressed neurones were often randomly distributed within the same stab. No generalizations could be made with regard to discrete localization of similarly responding cells. Ninety-seven caudate units in this series did not respond to nigral stimulation.

TABLE 1. Effects of substantia nigra stimulation on unit action potential rates in different telencephalic areas of the cat

Response to nigral stimulation	Caudate neurones	Cortical neurones	Lenticular neurones*
Depression (18 msec latency)	64	0	0
Depression followed by late facilitation	57	0	0
Facilitation (15 msec latency)	42	0	0
Facilitation (125 msec latency)	0	0	6
No change	97	24	16

* Pallidal, putamen and amygdaloid areas.

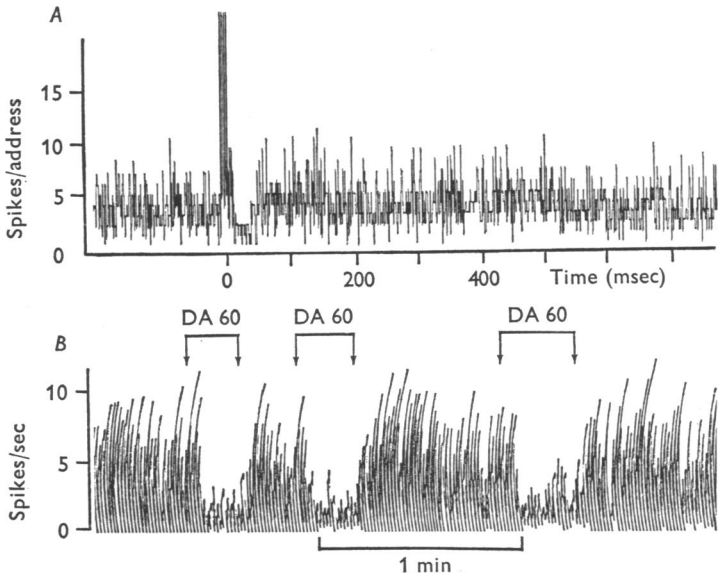
A measure of the specificity of the response can be obtained from Table 1. The firing pattern changes elicited from caudate neurones by nigral stimuli, while suggestive of a mixed population, are completely different from the responses of units recorded in non-caudate sites at the same telencephalic planes. Thus, the caudate neuronal effects evoked by nigral stimuli do not appear to be simply a part of a general phenomenon.

The specificity of the site of stimulation was examined in four experiments by stimulating various central mesencephalic areas through a switch box coupled to an array of electrodes. It was possible to record from five caudate neurones for sufficient time to evaluate the effects of altering the locus of stimulation. Caudate neurones depressed by nigral stimulation were unaffected by trains of equivalent parameters from electrodes whose tips were placed in the medial lemniscus, red nucleus, and mesencephalic reticular formation. When the stimulation was switched back to the nigra, however, the firing patterns of the caudate units were again depressed. Stimulation of the underlying cerebral peduncles of cats immobilized with gallamine triethiodide also failed to change caudate unit discharge patterns.

Effects of iontophoretic DA and acetylcholine. DA was ejected iontophoretically into the extracellular vicinity of 101 caudate neurones whose responses to nigral stimuli were 'identified' on the basis of post-stimulus histograms. Thirty-one units having post-stimulus firing pattern depressions were also depressed by subsequent iontophoretic DA; no instances

TABLE 2. Effects of iontophoretic ejection of DA on spike rates of caudate neurones whose responses to nigral stimuli were previously determined

Response to nigral stimulation	Iontophoretic dopamine effects		
	Depression	Facilitation	No change
Depression	31	0	9
Facilitation	3	7	5
No change	34	6	15



Text-fig. 3. Effects of iontophoretic DA on firing rates of a caudate neurone depressed by nigral stimulation. *A*. Histogram representing the effects of 250 successive stimuli. Note period of post-stimulus depression in firing pattern. *B*. Integrated spike rate from same neurone showing depressant action of repeated iontophoretic applications of DA with 60 nA of current (between arrows).

of DA facilitation were observed with neurones depressed by nigral stimuli. The effects of DA on the firing rates of cells facilitated or non-responsive to nigral stimuli were mixed. These results are summarized in Table 2. The conspicuous lack of DA facilitation among units depressed by nigral stimulation is significantly different ($P = 0{\cdot}03$, Fisher Exact Probability Test; Siegel, 1956) from the DA facilitation observed in the stimulus non-responsive group. The statistical evaluation, however, should be taken with reservations because the stimulus non-responsive population may not be entirely suitable for group comparisons. Text-fig. 3 illustrates

the effects of repeated iontophoretic applications of DA on the spike rate of a caudate neurone depressed by nigral stimulation.

Acetylcholine caused reproducible facilitation of neurone firing rates in twelve of thirty-six trials. It appeared that the excitant amino acids frequently masked the effects of acetylcholine. There was no correlation between DA and acetylcholine responses on the same unit, i.e. cells facilitated by acetylcholine were sometimes depressed by DA, but a few were facilitated by DA, and frequently a neurone responded to only one agent.

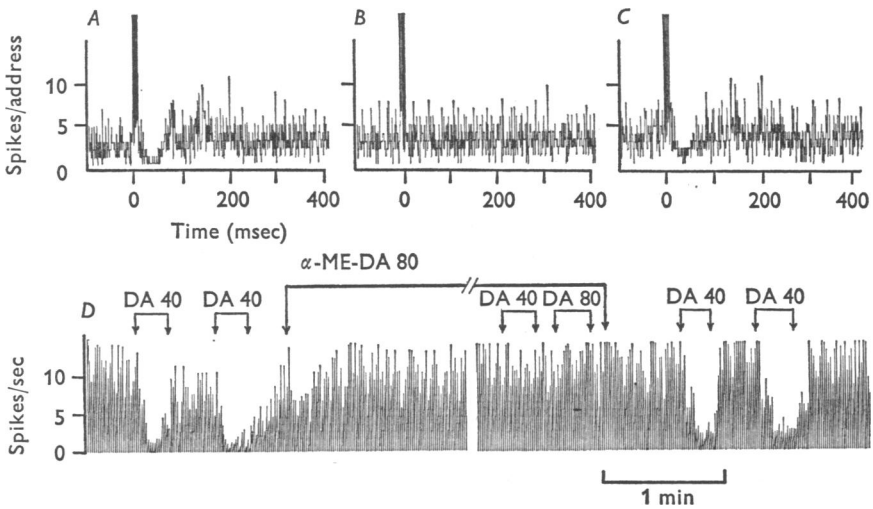


Fig. 4. Pharmacologic antagonism of the depressant effects of nigral stimulation and iontophoretic DA on the same caudate neurone. Histograms (200 summations) *A* and *C* were made before and after iontophoretic ejection of alpha-methyldopamine. Post-stimulus depression was not obtained in histogram *B* begun 6 min after start of alpha-methyldopamine ejection. Integrated spike rate *D* shows the depressant effects of iontophoretic DA (40 nA). During alpha-methyldopamine administration (80 nA), DA (40 and 80 nA) does not elicit a depressant response. After alpha-methyldopamine is turned off DA sensitivity returns. A 1·5 min section of the record was removed as indicated.

Effects of iontophoretic catecholamine antagonists. Several compounds which antagonize catecholamine responses peripherally were ejected from multibarrel micropipettes to study their effects on both iontophoretic DA and nigral stimuli. The use of phentolamine and phenoxybenzamine, adrenergic alpha receptor blockers, was made difficult by the low solubility and poor ionization characteristics of these compounds. In a few cases phentolamine antagonized the effects of iontophoretic DA but no discernible blockade of the response to nigral stimuli was detected. Chlorpromazine and the adrenergic beta-receptor blocking agents, dichloro-

isoproterenol and propranolol, produced relatively strong local anaesthetic effects (a decrease in firing frequency associated with a decrease in spike height) which seriously interfered with their use. INPEA, another beta-receptor blocker in the peripheral nervous system, while apparently devoid of procaine-like action at low current strengths (20–40 nA), failed to antagonize either response, even after continuous applications for up to 10 min.

Iontophoretic administration of alpha-methyldopamine, however, blocked the depressant responses elicited from six caudate neurones by both nigral stimuli and iontophoretic DA. Continuous applications for 4–7 min were required to block the effects of electrical stimulation; antagonism of iontophoretic DA occurred after 2–3 min. These comparatively long lag times are illustrated in the responses of the caudate neurone presented in Text-fig. 4. The compound often caused an initial but transient decrease in caudate unit firing. When the iontophoretic administration of the blocking agent was terminated, there was an abrupt (approximately 1 min) return of the sensitivity to both DA and nigral stimulation. Since the alpha-methyldopamine was dissolved in aqueous ascorbic acid solutions, anionic and cationic currents (100 nA) were passed through a micropipette barrel containing only the vehicle. Ascorbic acid alone did not affect caudate neuronal responses.

DISCUSSION

Biochemical, pharmacological and histochemical studies suggest that there may be a DA-mediated neuronal pathway between the substantia nigra and the caudate nucleus (for a discussion of the clinical significance relative to parkinsonism and motor dysfunctions, cf. review: Hornykiewicz, 1966). If this organizational concept is valid, then electrical stimulation of the nigra and iontophoretic administration of DA should elicit the same effects on caudate neurones. In the present investigation the effects of nigral stimuli on caudate neurone firing patterns were quantitatively similar to the responses elicited by DA (Bloom *et al.* 1965; McLennan & York, 1967). In both experimental situations the spike rates of most responding units were depressed whereas a small percentage were facilitated. Before the electrophysiological responses can be ascribed to activation of a specific pathway, however, several problems of interpretation must be considered. These include the latency and duration of the responses, current spread from the site of stimulation, and the possibility of alternate polysynaptic pathways.

The diameters of the DA fluorescent fibres in the caudate range between 0·1 and 1·0 μ but most are less than 0·4 μ (Fuxe, Hökfelt & Nilsson, 1964). Calculations have been advanced which would suggest that the 15–20 msec

response latencies may be a function of the slow conduction rates usually associated with small calibre fibres (Frigyesi & Purpura, 1967; Connor, 1968*b*). The variations in fibre size within the system could cause a range of conduction rates. Thus, the 50–70 msec response durations may be related to temporal dispersion of fibre depolarizations initiated at the nigra, a conductile distance of 15 mm or more in the adult cat. Spatial dispersion may also contribute to the response durations, as well as to the requirement for stimulus trains, since the fluorescent axons undergo extensive ramification within the caudate. Each fibre is estimated to give rise to 5×10^5 DA terminal varicosities (Andén *et al.* 1966*b*). The post-stimulus time course of caudate depressant responses resembles to some extent the neuronal responses described by Libet & Kobayashi (1969) in sympathetic ganglia. Certain ganglion cells which are hyperpolarized by norepinephrine develop slow onset-long duration inhibitory post-synaptic potentials when stimulated with high frequency trains.

The results obtained in this study with arrays of stimulating electrodes indicate that current spread to extra-nigral loci does not materially contribute to the unit responses obtained in the caudate. However, in addition to the histofluorescent nigro-neostriatal pathway (Andén *et al.* 1964, 1965, 1966*a*, *b*), efferent nigral projections to thalamic, pallidal, and tegmental areas have been described using silver impregnation techniques (Carpenter & McMasters, 1964; Afifi & Kaelber, 1965). It is apparent therefore that even very specific nigral stimuli could affect caudate neurones by one or more polysynaptic pathways as well as by activation of the direct dopaminergic projection. Because the caudate responses observed in this investigation had comparatively long post-stimulus delays, polysynaptic pathways cannot be excluded by electrophysiologic techniques alone. However, a good correlation between DA responses and the effects of nigral stimulation would provide presumptive evidence for the involvement of a direct DA pathway, since histochemical and biochemical studies indicate that the polysynaptic alternatives are not dopaminergic.

A critical comparison of the pharmacologic and electrophysiologic responses of caudate units argues in favour of the DA fluorescent fibres. Neurones depressed by nigral stimuli were consistently depressed by iontophoretic DA. The total correlation for both depression and facilitation among responsive caudate neurones was 38 of 41. Correlation break-down occurred with only three caudate cells which were excited by nigral stimulation but depressed by iontophoretic DA. These neurones may have been activated by polysynaptic routes or by antidromic invasion since the axons of some caudate neurones are known to project to the substantia nigra (Voneida, 1960). In these cases a correlation between the effects of nigral stimulation and iontophoretic DA would not be predicted.

Iontophoretic administration of alpha-methyldopamine blocked the depressant effects produced by DA and nigral stimulation. Although the number of cells in this group is small, the observations reinforce the contention of dopaminergic involvement in the depressant responses. Alpha-methyldopamine apparently functions as a 'false' neurotransmitter at sympathetic neuroeffector sites in the cat (Theonen, Haefely, Gey & Hürlimann, 1967), and it serves as a rather weak agonist at dopaminergic synapses in *Helix* suboesophogeal ganglia (Woodruff & Walker, 1969). There is some basis then for the proposal that alpha-methyldopamine might antagonize catecholamine responses at some neuronal sites. The rather long delay before alpha-methyldopamine blocked the nigra-evoked depressant responses may be explained by factors involving diffusion to active sites and buildup of sufficient concentrations.

Ariëns (1967) has proposed that the pharmacologic responsiveness of DA receptors may be distinctly different from those of the classical adrenergic alpha and beta receptors. This may account for the finding that, aside from the technical difficulties previously mentioned, the prototype adrenergic blockers (phenoxybenzamine, phentolamine, dichloroisoproterenol, propranolol) were not particularly effective in blocking pharmacologic and electrophysiologic responses in the caudate.

It is not possible to rule out completely the existence of a polysynaptic pathway which orginates in the substantia nigra and whose terminals influence the proposed dopaminergic projection by presynaptic modulation of DA release. Resolution of this problem would require intracellular recordings from axon terminals in the caudate, at present a technically forbidding feat. The results of this investigation indicate that nigral stimuli influence caudate neurone firing patterns, and that dopaminergic mechanisms are intimately involved in these responses. These observations provide additional evidence in support of biochemical and histochemical studies which indicate that there is a direct dopaminergic pathway from the substantia nigra to the caudate nucleus.

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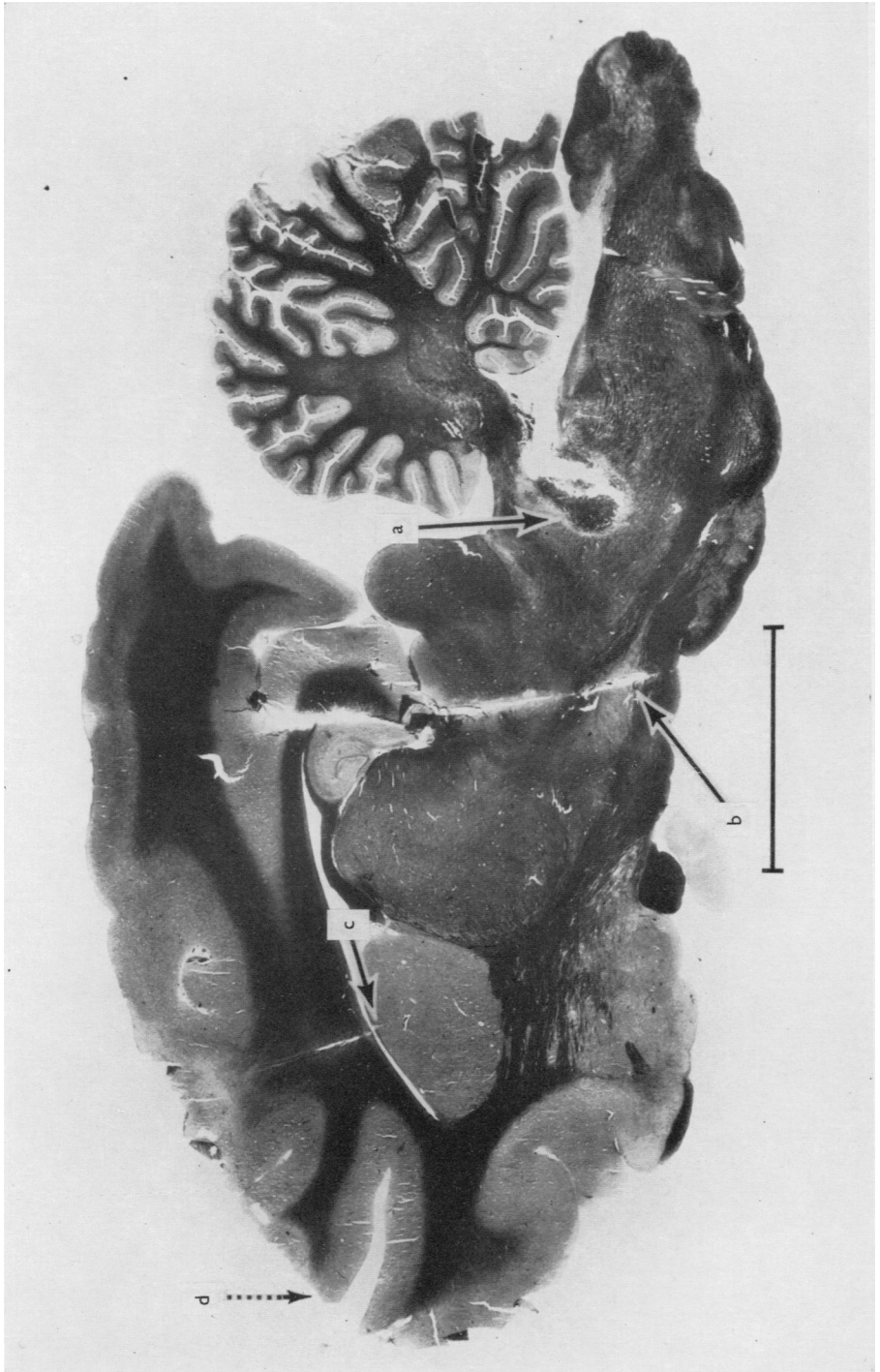

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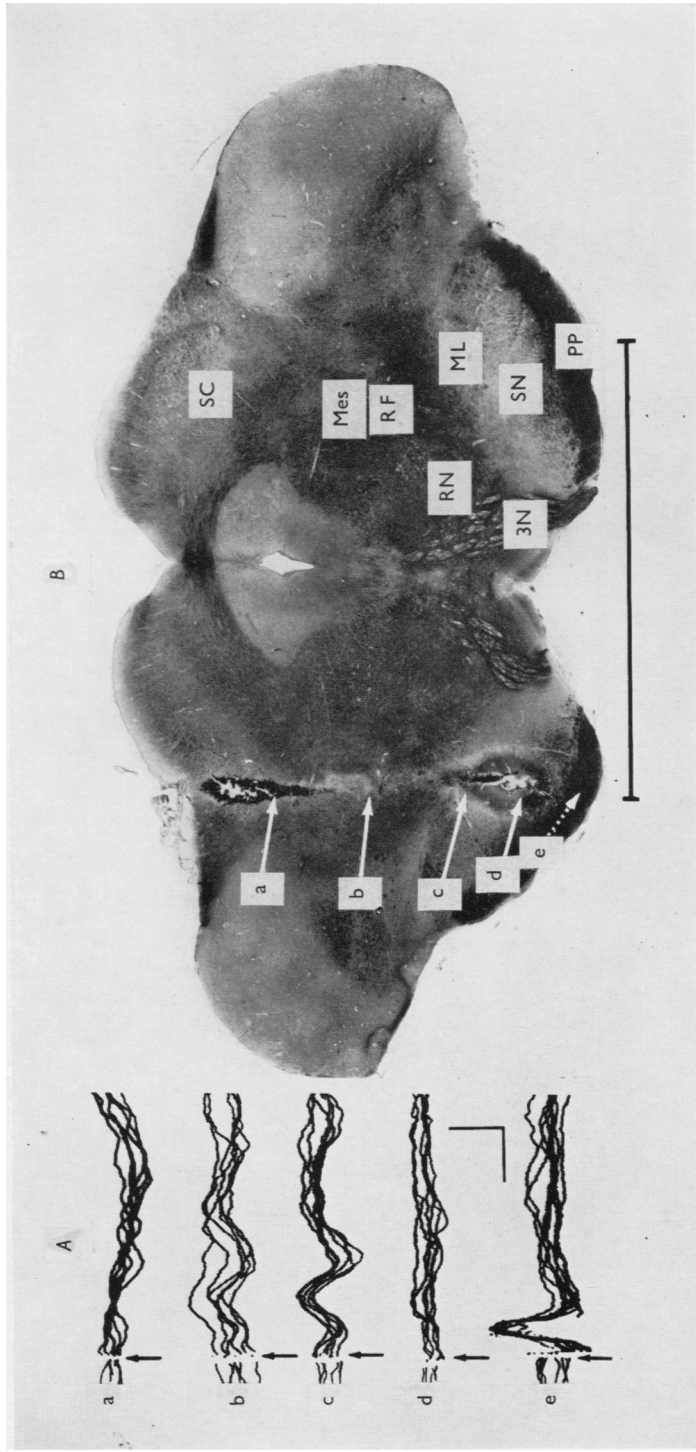
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3N

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EXPLANATION OF PLATES

PLATE 1

Parasagittal section of cat brain showing the essentials of the experimental procedure. a: decerebration lesion; b: stimulating electrode track ending in substantia nigra; c: micropipette electrode track entering the head of the caudate nucleus; d: pericruciate cortex (actual recording site somewhat lateral to the plane of this section). Weil stain. Scale: 1 cm.

PLATE 2

Placement of stimulating electrodes. *A*. Surface monopolar recordings from pericruciate cortex: a–e, potentials evoked while lowering the stimulating electrodes through the ipsilateral mesencephalon (*B*). Lettered points along the electrode track in *B* correspond to the level at which lettered potentials in *A* were obtained. SC, superior colliculus; MesRF, mesencephalic reticular formation; RN, red nucleus, 3N, oculomotor nerve; ML, medial lemniscus; SN, substantia nigra; PP, pes pedunculi. Large lesions at *B*, a and *B*, d were produced post-experimentally by electrolytic current. Time and voltage calibrations for *A*: 20 msec and 0·5 mV, negativity upward. Scale for *B*: 1 cm. Stimulus parameters: single square-wave pulses, 0·1 msec, 5 V at 1 msec intervals.