During a 2-week period, 90 cesareans were performed and 1056 fetuses examined. Eleven litters contained exencephalic fetuses, one per litter. Thus, the incidence of litters affected (11 of 90) is 12.2 percent, and the incidence of fetuses affected (11 of 1056) is 1.04 percent. No other anomalies were seen.

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References and Notes

1. R. Rugh and E. Grupp, *J. Neuropathol. Exp. Neurol.* **18**, 468 (1959); *Amer. J. Roentgenol. Radium Therapy Nucl. Med.* **81**, 1026 (1959); *J. Exp. Zool.* **141**, 571 (1959); *Amer. J. Roentgenol. Radium Therapy Nucl. Med.* **84**, 125 (1960); *Mil. Med.* **126**, 647 (1961); R. Rugh, *Amer. J. Roentgenol. Radium Therapy Nucl. Med.* **87**, 559 (1962); *ibid.* **89**, 182 (1963).
2. R. Rugh and E. Grupp, *Amer. J. Roentgenol. Radium Therapy Nucl. Med.* **81**, 1026 (1959).
3. R. Rugh, *ibid.* **87**, 559 (1962).
4. G. D. Snell and D. I. Picken, *J. Genet.* **31**, 213 (1935); K. Bonnevie, *Skr. Norske Videnskaps—Akad. Oslo I: Mat.-Naturv. Kl.* **9**, 1 (1936); W. C. Morgan, Jr., *Amer. J. Human Genet.* **7**, 39 (1955); D. Bennett, *J. Hered.* **50**, 265 (1959).
5. R. J. Flynn, *Proc. Animal Care Panel* **4**, 186 (1953).
6. Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.
7. Work performed under the auspices of the U.S. Atomic Energy Commission.

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Caudate Unit Responses to Nigral Stimuli: Evidence for a Possible Nigro-Neostriatal Pathway

Abstract. *Electrical stimulation of the substantia nigra evokes depressant and facilitatory responses from individually recorded caudate nucleus neurons. These effects resemble those elicited from caudate cells by microiontophoretic ejections of dopamine. Since histochemical evidence suggests that dopamine-containing fibers link the substantia nigra with the caudate, this pathway may mediate the changes in caudate spike rates produced by nigral stimuli.*

Andén *et al.* (*1*), using fluorescence histochemical methods, have described a dopaminergic pathway consisting of fine fibers which arise from the substantia nigra and terminate in the neostriatum, especially the caudate nucleus. Numerous clinical and biochemical observations suggest possible involvement of this pathway both in the control of caudate dopamine levels and in the production of "extrapyramidal" motor deficits such as parkinsonism (for a review, see *2*). When dopamine is applied iontophoretically from multibarrel micropipette assemblies (microiontophoresis) near caudate cells, the rate of discharge of 50 to 60 percent of these neurons is depressed, while the spike rate of approximately 10 percent of the cells is facilitated (*3*, *4*). Presumably, then, electrical activation of a dopaminergic neural input into the caudate should also elicit both depression and facilitation of caudate unit firing. Several investigators, however, have reported only facilitatory responses from caudate cells following nigral stimuli (*4*, *5*). The present experiments demonstrate that the effects of nigral stimuli are entirely consonant with local caudate pharmacologic responses. In addition to facilitation, electrical stimulation of the substantia nigra markedly depresses the discharge rates of a relatively large percentage of caudate neurons.

Twenty cats were anesthetized with ether, then electrolytically decerebrated at the midpontine level. After the ether was discontinued, the right cerebral hemisphere was exposed and protected with a pool of warm mineral oil. Bipolar stimulating electrodes were advanced downward through the cortex into the posterior portion (pars compacta) of the right substantia nigra (Fig. 1A). The nigra was stimulated with 10-msec trains at intervals of 1.3 seconds; each stimulus consisted of 4- to 5-volt rectangular pulses of 1 msec duration at a frequency of 400 per second. Multibarrel micropipette electrodes, prepared according to previously published methods (*6*), were placed in the head of the right caudate under stereotaxic control. The boundaries of the area sampled within the caudate were defined by the coordinates (*7*): A 14.5–16.5, L 3.5–6, D (+) 4–7.5 (Fig. 1B). Poststimulus time histograms (*8*) of caudate extracellular spike discharges were obtained with a computer of average transients (CAT 1000).

Most recordings were made from "silent" caudate units made to fire by the continuous ejection with 5 na of current of an excitant amino acid, *dl*-homocysteic acid, at a concentration in the micropipette of 0.5 to 1.0*M* and at *p*H 8.5. The firing patterns of activated units were monitored on an oscilloscope. Only neurons producing spikes uncomplicated by the firing of neighboring cells were studied. In addition, continuous contact with a neuron for a minimum of 10 minutes was required in order to obtain two successive summations of the effects elicited by nigral stimuli on unit discharges. The discharge patterns of 42 of the 100 caudate neurons meeting these criteria were not altered by electrical stimulation of the substantia nigra (Table 1) (Fig. 2D). Nigral stimuli, however, reproducibly depressed the discharge frequencies of 44 caudate cells. The depression periods had a mean poststimulus latency of 18.3 msec and a duration of 58.8 msec; the rates of discharge during the periods of depression were reduced by 75 to 80 percent (Fig. 2A). About half of the units initially depressed by nigral stimuli had periods of later facilitation (Fig. 2B). These delayed facilitations had a mean poststimulus latency of 124 msec and a mean duration of 187 msec. In contrast to the delayed facilitation just described, 14 caudate neurons responded to nigral stimuli with patterns of apparently "pure" facilitation having a much earlier latency of 14.2 msec (Table 1). The number of spikes during

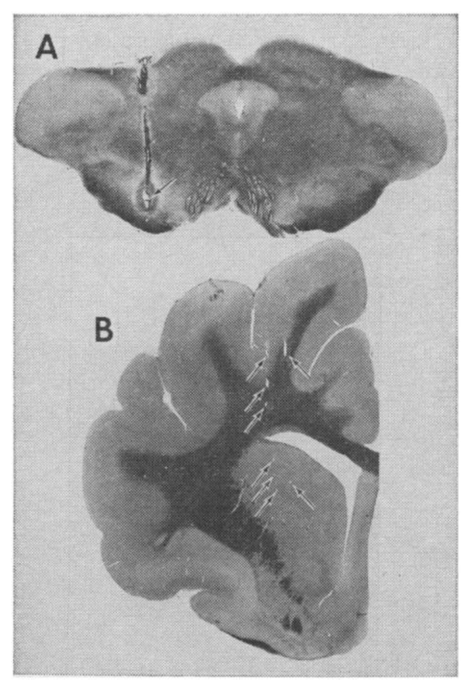

Fig. 1. Transverse sections through the stimulating and recording sites (*11*); Weil stain, 30 μ thickness. (A) Mesencephalon at plane A 3.5–4.0 (*7*). Lesion (arrow) in the substantia nigra was produced postexperimentally by passing electrolytic current through the stimulating electrode. (B) Telencephalon at plane A 15.5–16.0 (*7*). Arrows indicate recording electrode paths of two separate stabs at this level. Units were recorded in the caudate nucleus (lower cluster of arrows).

Table 1. Effects elicited by nigral stimulation on caudate neuronal discharge rates.

Discharge rate responses	Total caudate neurons responding	Distribution among cats	Response latency (mean ± S.E.; msec)	Response duration (mean ± S.E.; msec)
Depressed	44	19	18.3 ± 1.2	58.8 ± 5.7
Facilitated	14	9	14.2 ± 3.7	57.5 ± 11.0
No change	42	20		

these facilitatory periods commonly exceeded the average rate of discharge by a factor of 5 or more (Fig. 2C).

Depressed or facilitated units were rarely encountered near the border of the lateral ventricle. Most of these responses were obtained from neurons in a zone 1.5 to 3.0 mm below the superior margin of the caudate. Cells facilitated or depressed by nigral stimuli were intermingled; both responses were frequently encountered in the same stab. There was no evidence for discrete regional distribution of units yielding similar responses.

The specificity of the site of stimulation was explored in six experiments by using an array of bipolar electrodes. Caudate neurons responsive to nigral stimuli were not affected by subsequent stimulation of several overlying brain structures (*9*). Similarly, in three animals paralyzed with gallamine triethiodide, stimulation of motor tracts in the subjacent cerebral peduncles failed to alter caudate neuronal discharge patterns.

The fluorescent fibers of the proposed nigro-neostriatal pathway resemble peripheral C-fibers in that both are unmyelinated and have diameters of 0.5 to 1.0 μ (*10*); C-fibers have conduction speeds of 0.6 to 2.3 mm/msec. Assuming that the fluorescent fibers also conduct at this rate, and estimating the distance from the point of stimulation to the point of recording as 16 mm, the conduction time should be approximately 10 to 36 msec. The experimental poststimulus latencies of 14.2 and 18.3 msec for facilitated and depressed caudate unit responses fall within this predicted theoretical range.

The results supplement, rather than contradict, the recent observations of other investigators (*4*, *5*). Detection of the depressant effects of nigral stimulation on caudate unit spike patterns was dependent upon several experimental factors. First, ejection of an excitant amino acid near caudate neurons ensured steady neuronal firing rates against which depressant responses could be measured. Furthermore, summation of the effects of a number of nigral stimuli on unit firing patterns introduced a statistical advantage over the technique of examining individual responses elicited by a single stimulus. Finally, preliminary experimentation suggested that depressant responses may be better evoked by short trains of impulses rather than by single shocks of equal or higher voltage.

The changes in caudate unit firing patterns elicited by nigral stimulation are well correlated with the effects of dopamine ejected by microiontophoresis in the vicinity of these neurons. Despite such response similarities, the design of these experiments precludes any precise conclusions about operant receptor mechanisms in the caudate. The data, however, when considered in conjunction with current histochemical and pharmacological observations, suggest that the changes in caudate unit discharges elicited by nigral stimuli may be attributable to activation of a nigro-neostriatal pathway.

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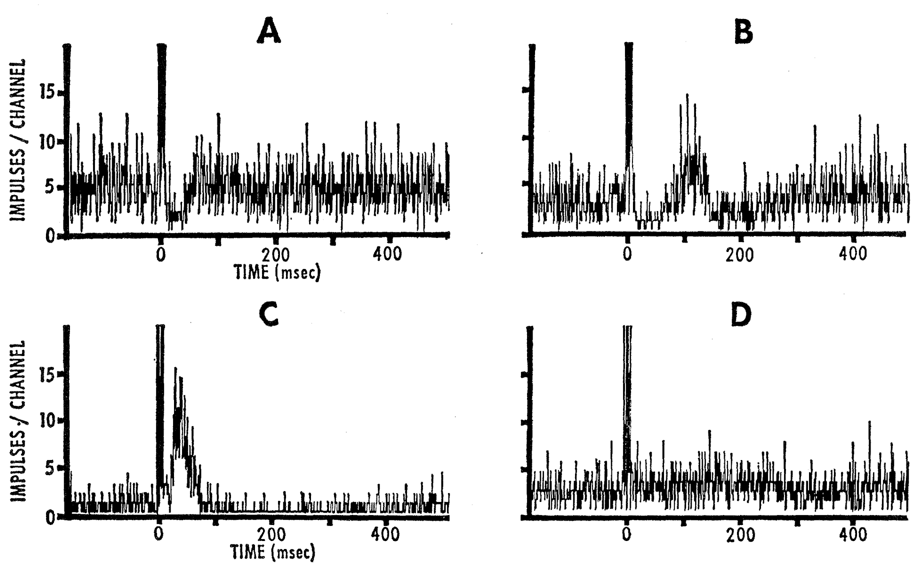

Fig. 2. Effects of substantia nigra stimulation on the occurrence of caudate nucleus unit action potentials recorded extracellularly. Histograms A–D were obtained from different caudate neurons which were excited by continuous application of 5 na of *dl*-homocysteic acid. Abscissa: time after the stimulus artifact (large vertical columns at time zero). Ordinate: number of action potentials deposited in the computer channels (individual channel widths of 1 msec). (A) and (B) are sums of 200 stimuli; (C) and (D), 150 stimuli. (A) Poststimulus firing rate depression. (B) Poststimulus firing rate depression followed by later facilitation. (C) "Pure" poststimulus firing rate facilitation. (D) Histogram from a neuron not responding to nigral stimuli.

References and Notes

1. N.-E. Andén, A. Carlsson, A. Dahlström, K. Fuxe, N.-Å. Hillarp, K. Larsson, *Life Sci.* **3**, 523 (1964); N.-E. Andén, A. Dahlström, K. Fuxe, K. Larsson, *ibid.* **4**, 1275 (1965); ———, *Am. J. Anat.* **116**, 329 (1965).
2. O. Hornykiewicz, *Pharmacol. Rev.* **18**, 925 (1966).
3. F. E. Bloom, E. Costa, G. C. Salmoiraghi, *J. Pharmacol. Exp. Therap.* **150**, 244 (1965).
4. H. McLennan and D. H. York, *J. Physiol. London* **189**, 393 (1967).
5. D. H. York, *Brain Res.* **5**, 263 (1967); T. L. Frigyesi and D. P. Purpura, *ibid.* **6**, 440 (1967).

G. C. Salmoiraghi and F. Weight, *Anesthesiology* **28**, 54 (1967).

7. R. S. Snider and W. T. Niemer, *A Stereotaxic Atlas of the Cat Brain* (Univ. of Chicago Press, Chicago, 1961).
8. G. L. Gerstein, *Science* **131**, 1811 (1960); ——— and N. Y.-S. Kiang, *Biophys. J.* **1**, 15 (1960).
9. Medial lemniscus, mesencephalic reticular formation, red nucleus.
10. A. Dahlström and K. Fuxe, *Acta Physiol. Scand.* **64**, Suppl., 247 (1965); H. D. Patton, in *Neurophysiology*, T. C. Ruch, H. D. Patton, J. W. Woodbury, A. L. Towe, Eds. (Saunders, Philadelphia, 1965), p. 81.
11. Histological identification of electrode tracks was made in collaboration with Dr. T. N. Johnson, George Washington University Medical School, Washington, D.C.
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