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Antidromic Activation of Dorsal Raphe Neurons from Neostriatum: Physiological Characterization and Effects of Terminal Autoreceptor Activation

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Three types of neurons, distinguished on the basis of their spontaneous firing rates and patterns, extracellularly recorded waveforms and responses to neostriatal stimulation, were observed in the dorsal raphe nucleus in urethane-anesthetized rats. Type 1 neurons (presumed to be serotonergic) fired spontaneously from 0.1 to 3 spikes/s in a regular pattern, with initial positive-going bi- or triphasic action potentials. Type 1 cells exhibited long-latency antidromic responses to neostriatal stimulation (mean \pm S.E.M. 24.9 \pm 0.3 ms) that sometimes occurred at discrete multiple latencies, and supernormal periods persisting up to 100 ms following spontaneous spikes. Type 2 cells fired spontaneously in an irregular, somewhat bursty pattern from 0 to 2 spikes/s with initial negative-going biphasic spikes, and were antidromically activated from neostriatal stimulation at shorter latencies than Type 1 cells (21.8 \pm 0.9 ms). Type 3 cells were characterized by initial positive-going biphasic waveforms and displayed a higher discharge rate (5-30 spikes/s) than Type 1 or Type 2 cells. Type 3 cells could not be antidromically activated from neostriatal stimulation. The relatively long conduction time to neostriatum of the Type 1 presumed serotonergic neuron is discussed with respect to previous interpretations of the synaptic action of serotonin in the neostriatum. In conjunction with these antidromic activation studies, the neurophysiological consequences of serotonergic terminal autoreceptor activation were examined by measuring changes in the excitability of serotonergic terminal fields in the neostriatum following administration of the serotonin autoreceptor agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT). The excitability of serotonergic terminal fields was decreased by intravenous injection of 40 μ g/kg 5-MeODMT, and by infusion of 10-50 μ M 5-MeODMT directly into the neostriatum. These results are interpreted from the perspective of mechanisms underlying autoreceptor-mediated regulation of serotonin release.

INTRODUCTION

The existence of a projection from the dorsal raphe nucleus (DRN) to the neostriatum (i.e. caudate-putamen) has been well established by a variety of anatomical techniques^{7,19,43,45,56}. This pathway originates predominately in the dorsomedial and ventromedial cell clusters of the DRN^{43,44,51}, giving rise to axons that pass through the ventral tegmentum and occupy the ventro-lateral aspect of the medial forebrain bundle, and eventually turn laterally along their ascending route to innervate the neostriatum^{7,30}. Although the dorsal raphe innervation of the neostriatum is predominately serotonergic, a minor

component arises from neurons that do not exhibit immunocytochemical staining for serotonin⁴³⁻⁴⁵. In addition to this anatomical evidence, it has been shown that stimulation of the DRN alters the firing pattern of neostriatal neurons in rats¹⁵, often with short latencies^{29,36,38,55}. These reports describe orthodromic effects presumed to be mediated by the synaptic action of serotonin onto neostriatal neurons. However, the short latency component of these orthodromic effects^{29,36,38,55} appears to be incompatible with slow conduction velocities of ascending serotonergic fibers in rats as determined from the antidromic activation of DRN serotonergic neurons produced by stimulation of the ventral tegmentum^{37,39,58,59}

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Among the aims of the present report was to provide an electrophysiological characterization of the neurons that participate in the dorsal raphe-neostriatal projection, and to clarify the orthodromic conduction time discrepancy, by antidromically activating DRN neurons with electrical stimulation of the neostriatum.

In conjunction with these antidromic activation studies, experiments were performed to determine the neurophysiological consequences of serotonergic terminal autoreceptor activation. We have previously shown that activation of autoreceptors at the terminal regions of dopaminergic and noradrenergic neurons reduces the excitability of their terminal fields to direct electrical stimulation, an effect that can be reversed by application of a suitable receptor blocking agent^{22,34,35,48,49}. While the inhibitory effects of stimulation of autoreceptors situated on somato-dendritic regions of serotonergic DRN neurons have been well characterized electrophysiologically^{3,16}, the neurophysiological effects associated with autoreceptor activation in serotonergic terminal fields have not been investigated. This issue is of interest in light of numerous reports describing modulation of serotonin release by autoreceptors located on serotonergic terminals^{9,11,20,21,28,32,46}. The results of excitability experiments on serotonergic terminal fields presented in this report are discussed from the perspective of mechanisms underlying autoregulation of neurotransmitter secretion at central serotonin release sites.

MATERIALS AND METHODS

110 male Sprague-Dawley rats, weighing 230–410 g, were used in these experiments. Subjects were anesthetized with 1.3 g urethane/kg body weight, a catheter was positioned in the left femoral vein, and the animal was secured in a stereotaxic instrument (oriented according to König and Klippel²⁶) using blunt earbars. All pressure points and wound margins were coated with 5% xylocaine ointment. Body temperature was maintained at 37 ± 1 °C with a heating pad electronically coupled to a Yellow Springs telethermometer. The electrocardiogram was monitored on an auxiliary oscilloscope. The scalp was removed following subcutaneous injection of 2% xylocaine solution, and the atlanto-occipital membrane

overlying the cisterna magna was punctured and some cerebrospinal fluid allowed to drain in order to reduce brain pulsations. Small burr holes were drilled to allow placement of stimulating electrodes in various regions of one or both neostriata, and for some animals, in the medial forebrain bundle (MFB). Coordinates for the neostriatum were –1.0 to 2.0 mm anterior to bregma, 3.0–4.3 mm lateral to the midline, and 4.2–5.4 mm ventral to the surface of the brain. Coordinates for the MFB were 4.1 mm anterior to lambda, 1.3 mm lateral to the midline, and 7.8 mm ventral to the surface of the brain. Formvar-coated stainless steel bipolar stimulating electrodes with a tip separation of 0.2 mm were either affixed to the skull with cyanoacrylate glue and dental cement or held in position by means of a micromanipulator. A large rectangular hole was drilled to permit access to the DRN, from 1.5 mm posterior to 2.0 mm anterior to lambda, and from 2.0 mm lateral to the midline. The sagittal sinus was ligated, cut and reflected.

Single unit discharges in the DRN were recorded extracellularly with glass microelectrodes filled with 2 or 3 M NaCl with in vitro impedances of 4–10 MΩ at 500 Hz. Neuronal activity was amplified by a WPI (M-707) preamplifier with bandpass filtering set to 0.1–3.0 kHz, displayed on a Tektronix S65 oscilloscope and simultaneously recorded on magnetic tape for off-line analysis.

The recording electrode was lowered into the brain at the midline, about –0.5 to 0.5 mm anterior to lambda. The DRN was usually found at a depth of 5.0–5.5 mm. Identification of the DRN was based on the depth of the microelectrode, the presence of a small (150 μV) field potential evoked by electrical stimulation of the MFB, and the presence of presumed serotonergic neurons. Serotonergic neurons were identified according to previously published electrophysiological criteria^{2,4}, including: (1) a 2–5-ms wide biphasic positive-negative or triphasic positive-negative-small positive waveform; (2) a slow, regular spontaneous discharge rate (0.1–3 spikes/s); and (3) for those cells tested, inhibition of spontaneous unit activity after intravenous injection of the serotonergic autoreceptor agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT)^{31,42}.

Monophasic electrical stimulation of the neostriatum (0.2–2.5 mA, 0.025–0.25 ms duration, 1 Hz) was maintained during the search for antidromically

activated cells. When a well-isolated unit was encountered, stimulating current and/or duration were varied to determine if the neuron could be antidromically driven. Criteria for an antidromic response were an invariant response latency (or in the case of multiple antidromic latencies, invariant discrete latencies) and the collision of spontaneous and stimulation-evoked action potentials¹⁸. For neurons in which collision testing was not possible due to little or no spontaneous activity, the antidromic nature of the response was inferred by its invariant latency and the ability to follow suprathreshold twin pulse electrical stimulation to 200–500 Hz.

In some animals, the neurophysiological consequences of serotonin terminal autoreceptor stimulation were examined by measuring neostriatal stimulating currents necessary to elicit antidromic activation of DRN serotonergic neurons before and after drug administration, using procedures developed in this laboratory and previously described for rat substantia nigra dopaminergic and locus coeruleus noradrenergic neurons^{22,34,48}. Briefly, when a serotonergic neuron was encountered, the stimulating current was adjusted to a setting just sufficient to antidromically activate the cell on 100% of the non-collision trials. This value, termed 'threshold', was used to evaluate changes in terminal excitability. The proportions of antidromic responses to several lower currents were also measured. Between 25 and 50 stimuli (1 Hz) were presented at each current setting, and excitability values were repeated in a counter-balanced manner in order to establish that the excitability measurements were stable. At this point, one group of animals was administered 20 $\mu\text{g}/\text{kg}$ 5-MeODMT intravenously, and terminal excitability measurements were repeated. Approximately 4–8 min later, the animal received a second intravenous injection of 5-MeODMT (cumulative dose of 40 $\mu\text{g}/\text{kg}$), and terminal excitability values were again determined. Only one intravenous drug experiment was performed per animal. Control experiments consisted of two intravenous injections of the vehicle (0.9% saline, 1 ml/kg). In addition, in 2 animals the effect of intravenous injection of 40 $\mu\text{g}/\text{kg}$ 5-MeODMT on the excitability of the preterminal region of serotonergic fibers in the MFB was examined. In the second group of animals, 10 or 50 μM 5-MeODMT was infused directly into the neostriatal

stimulation site. The drug was delivered through a 32-gauge cannula at a rate of 0.06 $\mu\text{l}/\text{min}$ for 5 min (total volume of 0.30 μl). The cannula was situated approximately 50 μm from the exposed tips of the stimulating electrode. Following determination of the post-infusion excitability, a second infusion was performed and excitability measurements were repeated. Only one drug infusion experiment was performed per animal. Control experiments consisted of two infusions of the vehicle (0.9% saline). Control and drug experiments were performed on different neurons.

At the conclusion of the recording session, a lesion was produced at the recording site by passing 30–40 μA anodal current (0.5 Hz, 1 s duration) through the recording microelectrode for 20 min. Animals were perfused with 0.9% saline followed by 10% formalin, and the brains were removed and stored in 10% formalin until processed further. The locations of the recording microelectrode and stimulating electrodes were reconstructed from 100 μm thick coronal sections stained with neutral red.

RESULTS

Antidromic responses of DRN neurons to neostriatal stimulation

Electrical stimulation of the neostriatum evoked antidromic spikes in 343 neurons located in the DRN. Extracellular recordings from antidromically activated neurons were confined mostly to the dorso-medial and ventromedial cell clusters of the DRN. Cells presumed to be serotonergic on the basis of previously discussed criteria comprised the majority of the antidromically excited DRN neurons ($n = 315$), and are termed Type 1 cells. Virtually all of the antidromically excited Type 1 cells exhibited spontaneous activity, typically in the range of 0.5–3.0 spikes/s. Approximately 13% of the Type 1 neurons encountered during the course of these experiments could be antidromically activated by unilateral stimulation of the neostriatum (about 2000 Type 1 DRN cells were examined in this study). As shown in Fig. 1, the antidromic action potential of Type 1 cells consisted of a 2–5-ms wide biphasic positive-negative or triphasic positive-negative-small positive full spike, often with an inflection on the rising phase of the initial positivity, characteristic of an initial seg-

ment-somatodendritic (IS-SD) break (Fig. 1A and B). Some serotonergic waveforms also displayed a

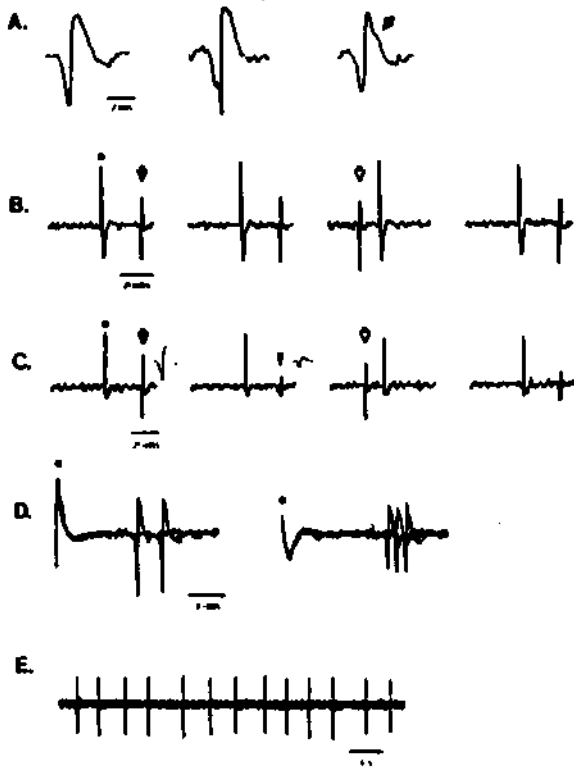


Fig. 1. Antidromic responses recorded from Type 1 (presumed serotonergic) neurons in the DRN upon electrical stimulation of the neostriatum. A: characteristic waveforms recorded from three different antidromically-activated Type 1 neurons. Note the width of the response, the biphasic (positive-negative) or triphasic (positive-negative-small positive) polarity, the presence of an inflection on the rising phase of the initial positivity signifying an initial segment-somatodendritic break (second trace), and a hump riding on the falling phase of the negativity (i.e. a C spike; arrow in third trace). B: electrical stimulation of the neostriatum evokes an antidromic response in a Type 1 neuron (solid arrow), with a latency of 23.5 ms. In the third trace, a spontaneous spike (open arrow) extinguishes the antidromic response. Stimulus artefact is labeled with a solid circle in B, C and D. Traces are presented in serial order in B and C. C: a different antidromically-activated Type 1 neuron that exhibits a mixed population of antidromic full (solid arrow) and initial segment-only (solid arrowhead) spikes. The latency of this response is 28.0 ms. The antidromic response is extinguished by a spontaneous spike (open arrow). Insets: expanded time base showing antidromic full spike and IS-only waveform. D: example of two serotonergic neurons that exhibit multiple discrete antidromic latencies. Left: two discrete, mutually exclusive antidromic latencies (22.0 and 28.5 ms) are evident. The figure is a superimposition of 4 traces, 2 of each latency. Right: 3 discrete antidromic latencies (28.5, 31.0 and 34.0 ms). Superimposition of 6 traces, two of each latency. E: 10 s train of spontaneous activity recorded from a Type 1 cell. Note regularity of firing pattern. In this and subsequent figures, positivity is down.

hump riding on the descending stroke of the negative component, i.e. a C spike³³. The polarity of the Type 1 waveform was not observed to reverse as the microelectrode was advanced from the point at which the action potential was first distinguished from background noise until the point of cell contact and injury. In approximately 10% of the antidromic Type 1 cells, both full spikes and IS-only spikes were observed in response to neostriatal stimulation (Fig. 1C). Spontaneous action potentials in cells having IS-only antidromic responses usually exhibited prominent IS-SD breaks. No spontaneous IS-only spikes were observed. The spontaneous activity of antidromically activated Type 1 neurons was inhibited by intravenous injection of 40 $\mu\text{g}/\text{kg}$ 5-MeODMT, by an average of 65% ($n = 11$).

A second type of DRN neuron, termed the Type 2 cell, could be antidromically activated by stimulation of the neostriatum ($n = 28$), and is shown in Fig. 2. The Type 2 neurons exhibited a 1.5–3.0 ms wide biphasic negative-small positive waveform, with a smaller amplitude spike than the Type 1 neurons. Type 2 IS-SD spike decomposition to an IS-only spike was observed only with high frequency antidromic activation. Initial segment-somatodendritic breaks were evident only when the waveform was electronically differentiated, or at high rates of antidromic stimulation. The shape of the Type 2 waveform was not modified to an appreciable degree as the microelectrode tip was advanced towards the cell, and the initial negativity of the waveform did not reverse polarity. The discharge rate of these cells was low (0–2.0 spikes/s), with 10 out of the 28 antidromically activated Type 2 cells exhibiting either no spontaneous activity or less than 0.1 spikes/s. Furthermore, the spontaneous firing pattern of these cells in the absence of neostriatal stimulation was somewhat bursty, and lacked the regular, 'clock-like' firing pattern typical of serotonergic neurons (Fig. 2E). The spontaneous activity of the Type 2 antidromic neurons was completely inhibited by intravenous administration of 40 $\mu\text{g}/\text{kg}$ 5-MeODMT, with recovery occurring over the course of 10 min ($n = 4$).

For both the Type 1 and the Type 2 antidromic neurons, no cells were encountered that could be antidromically driven from both neostriata.

A third type of neuron (Type 3 cell) was commonly encountered in the DRN. This cell was characterized

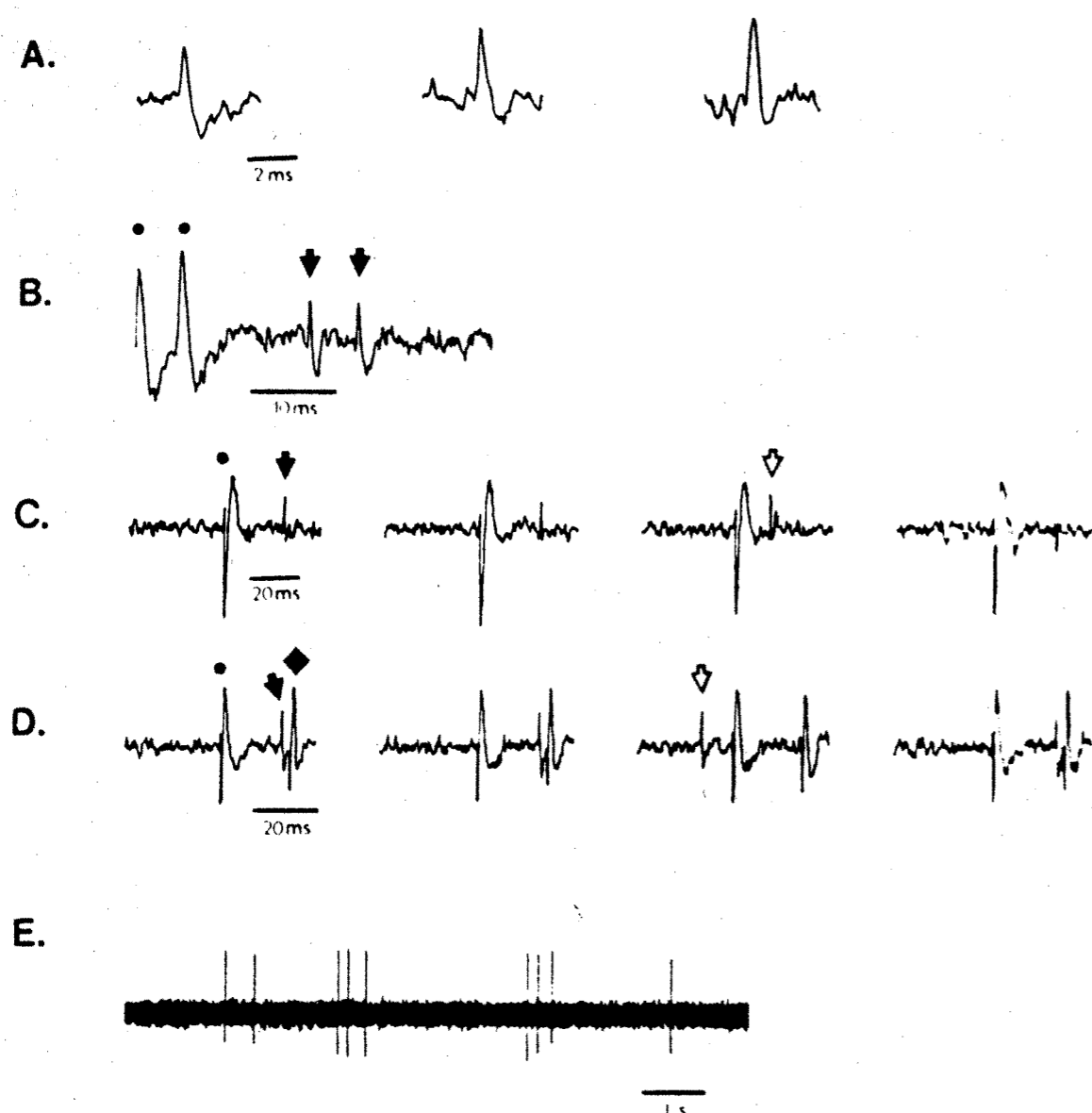


Fig. 2. Antidromic responses recorded from Type 2 (presumably non-serotonergic) neuron in the DRN upon electrical stimulation of the neostriatum. A: characteristic action potential waveforms recorded from three different Type 2 antidromically-activated neurons are distinct from Type 1 neurons (cf. Fig. 1). Note that the Type 2 waveform has a biphasic (negative-small positive) waveform polarity. In addition, a distinct initial segment component is not evident, and the amplitude and width of these responses are not as large as those recorded from Type 1 neurons. B: antidromic Type 2 neuron (solid arrow) that did not exhibit any spontaneous activity follows twin pulse stimulation of the neostriatum. Inter-stimulus interval is 5.0 ms (200 Hz), indicative of antidromic activation. The latency of the response is 19.5 ms. Stimulus artefact is labeled with a solid circle in B, C and D. C: example of an antidromic response from a different Type 2 neuron (solid arrow) with a latency of 22.0 ms. In the third trace, a spontaneous spike (open arrow) extinguishes the antidromic response. Traces are presented in serial order in C and D. D: simultaneous recording from two different antidromically activated DRN neurons. A Type 1 response (diamond) with a latency of 21.0 ms and a Type 2 response (solid arrow) with a latency of 19.0 ms. In the third trace, the Type 2 response is extinguished by a collision with a spontaneous Type 2 action potential (open arrow), but the Type 1 response is unaffected. E: 10 s train of spontaneous activity recorded from a Type 2 cell. Note that in contrast to Type 1 cells, the firing pattern is irregular.

by a 1–3 ms wide, positive-negative waveform and a high rate of spontaneous activity (5–30 spikes/s). Dorsal raphe neurons similar to Type 3 cells have been previously described by other investigators, and have been presumed to be non-serotonergic neurons^{4,37,54}. The Type 3 cell was never observed to be antidromically driven by neostriatal stimulation, but on rare occasions ($n = 2$) it was antidromically activated by stimulation of the MFB, with latencies of 8.0

and 12.7 ms. The effect of 5-MeODMT on the firing rate of Type 3 neurons was not tested.

Neurophysiology of the dorsal raphe-neostriatal projection

The latencies for antidromic responses evoked by stimulation of the neostriatum for Type 1 neurons (24.9 ± 0.3 ms; $n = 315$) and Type 2 neurons (21.8 ± 0.9 ms; $n = 28$) were significantly different ($t = 2.94$; $df = 341$; $P < 0.005$). The antidromic latency distributions for these two classes of neurons are presented in Fig. 3.

The position of the neostriatal stimulating electrode(s) was carefully reconstructed from the histological sections for 205 of the antidromically excited neurons (Fig. 4). There was no significant correlation between the site of neostriatal stimulation and the latency of the response for either the Type 1 ($r = -0.02$; $df = 178$; $P = 0.786$) or the Type 2 ($r = -0.35$; $df = 23$; $P = 0.089$) antidromic responses, despite differences in stimulation sites of as much as 3.8 mm along the rostral-caudal axis. In addition, no prominent differences were observed in the probability of evoking Type 1 or Type 2 antidromic responses for stimulation sites varying along the rostral-caudal dimension of the neostriatum.

Using a straight line estimation of distance between the DRN and the neostriatal stimulation sites, mean conduction velocities for Type 1 neurons ($n = 180$) and Type 2 neurons ($n = 25$) were calculated to be 0.34 ± 0.01 m/s and 0.37 ± 0.01 m/s, respectively.

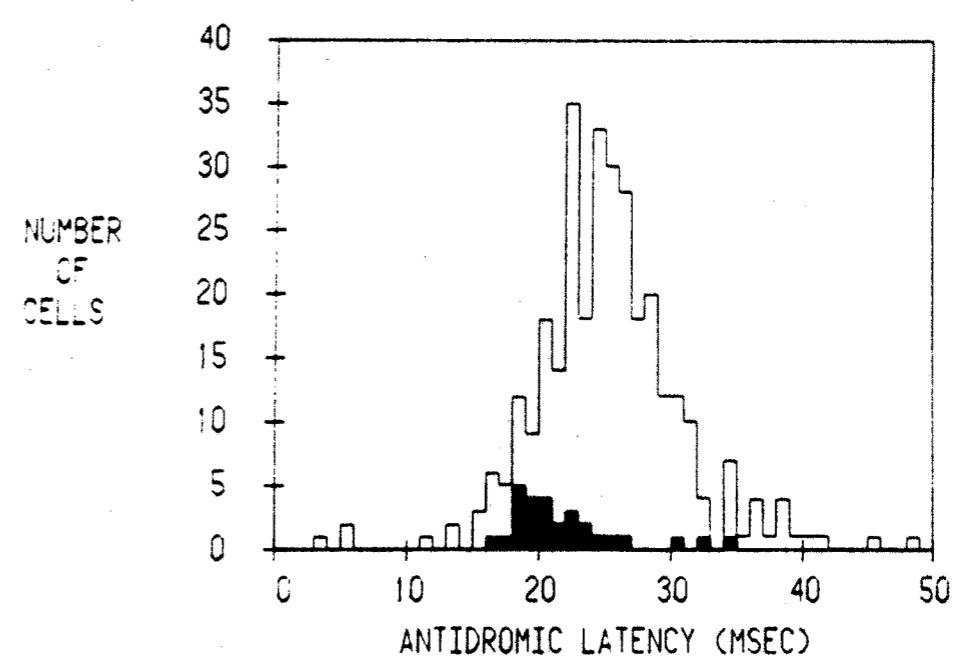


Fig. 3. Frequency distribution of latencies recorded from DRN neurons antidromically-activated by electrical stimulation of the neostriatum. Mean latencies and S.E.M. for Type 1 and Type 2 neurons were 24.9 ± 0.3 and 21.8 ± 0.9 ms, respectively. These values differ significantly ($t = 2.94$; $df = 341$; $P < 0.005$). Open bars = antidromic latencies of Type 1 neurons ($n = 315$); closed bars = antidromic latencies of Type 2 neurons ($n = 28$).

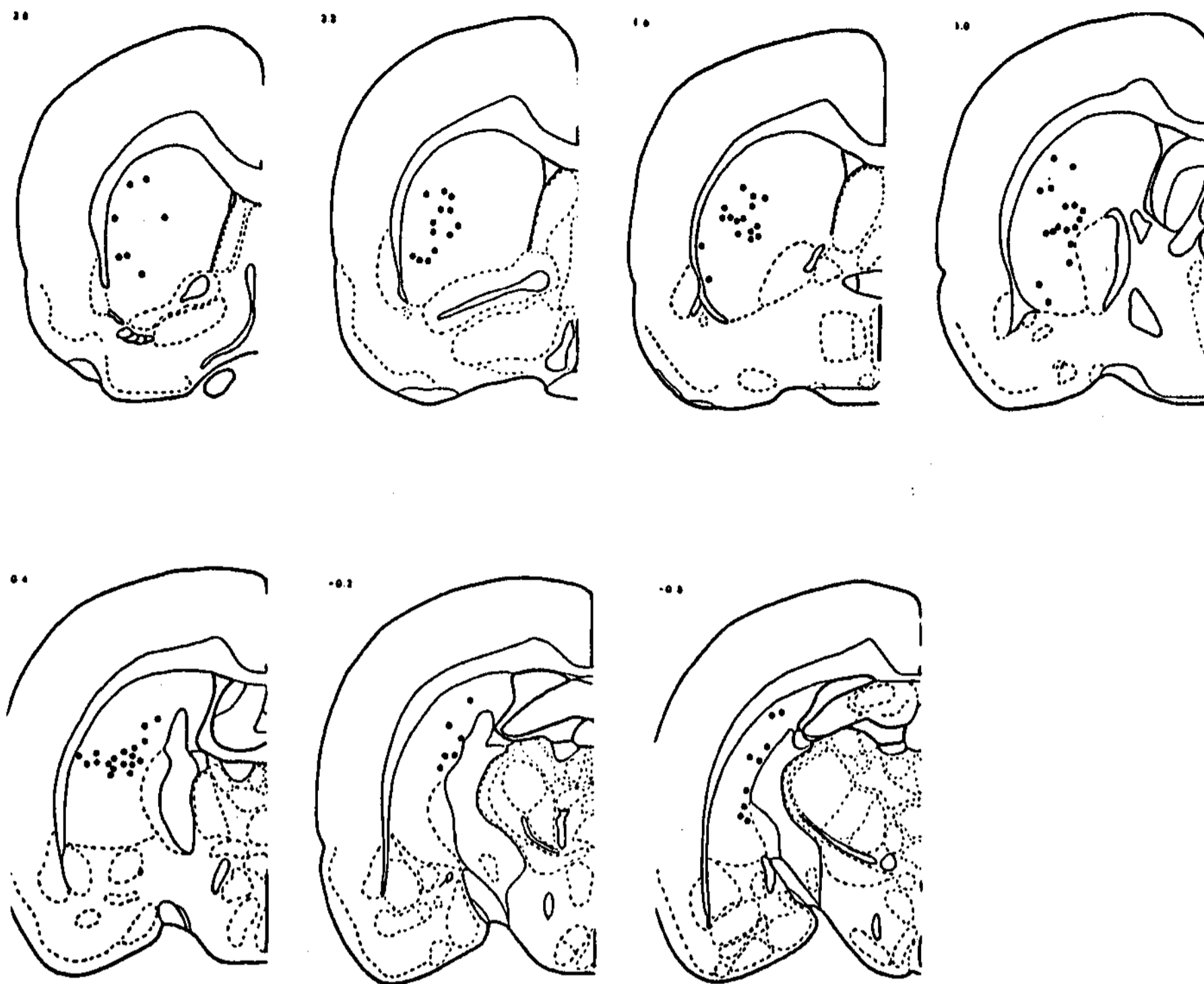


Fig. 4. Schematic serial reconstruction of neostriatal stimulation sites based on neutral red-stained histological sections. All frontal planes refer to atlas of Pellegrino et al.⁴¹.

A similar analysis for MFB stimulation latencies revealed an estimated conduction velocity of 0.61 ± 0.03 m/s ($n = 72$) for Type 1 fibers. A few Type 1 neurons were antidromically stimulated from both the neostriatum and the MFB. Mean conduction velocity between these two sites was calculated to be 0.26 ± 0.04 m/s ($n = 8$).

When an antidromic DRN neuron was encountered, the response was examined at different stimulating current intensities. In 26% of the antidromically responding Type 1 neurons, changing the stimulating current revealed a second or third discrete antidromic latency (Fig. 1D). In these cells, the discrete latency jumps were typically 1–6 ms. Only one antidromic response was observed to a given stimulus, and, almost invariably, a higher stimulating current was required to elicit the shortest latency response. For those cells with multiple antidromic latencies, the shortest latency response was used for calculation

of mean latency and conduction velocity. Type 2 antidromic neurons were observed to have multiple antidromic latencies in 3 cases.

It was often observed that the probability of evoking an antidromic response from Type 1 neurons at a given stimulating current was increased by the occurrence of a spontaneous spike up to 100 ms prior to the collision interval (Fig. 5A). The time course of this impulse-dependent increase in fiber excitability was assessed by determining neostriatal threshold stimulating currents at different time intervals following the spontaneous spike, as shown for a Type 1 neuron in Fig. 5B. Note that following an initial refractory period, there is an interval of increased excitability that persists for 75 ms. Concomitant with increases in fiber excitability, prior impulse activity was also observed to decrease the latency of the antidromic response up to 1 ms (Fig. 5C). These impulse-dependent decreases in conduction time occurred over the

same time interval as the excitability changes. Unlike the multiple discrete antidromic latencies observed in Type 1 neurons (see above), the impulse-dependent antidromic latency changes were not discrete and did not exceed 1 ms. One result of impulse-dependent changes in antidromic latency was 'jitter' in discrete antidromic latencies of about 1 ms.

Following both suprathreshold and subthreshold antidromic stimulating currents, Type 1 neurons displayed a post-stimulus suppression of spontaneous activity for a period of 0.5–1.0 s, in accord with previous reports^{58,59}. Only rarely did a serotonergic neuron show evidence of orthodromic excitation follow-

ing neostriatal stimulation; in such cases, latencies ranged from 10 to 45 ms. In addition, the mean minimum inter-stimulus interval that could evoke an antidromic response to the second stimulus at $1.5 \times$ threshold was 2.5 ± 0.2 ms (MFB stimulation). This value presumably reflects the refractory period of serotonergic neuronal somata.

Effects of autoreceptor stimulation at serotonergic terminals

Intravenous administration of 5-MeODMT (20–40 $\mu\text{g}/\text{kg}$) led to consistent decreases in the excitability (increases in threshold) of presumed seroto-

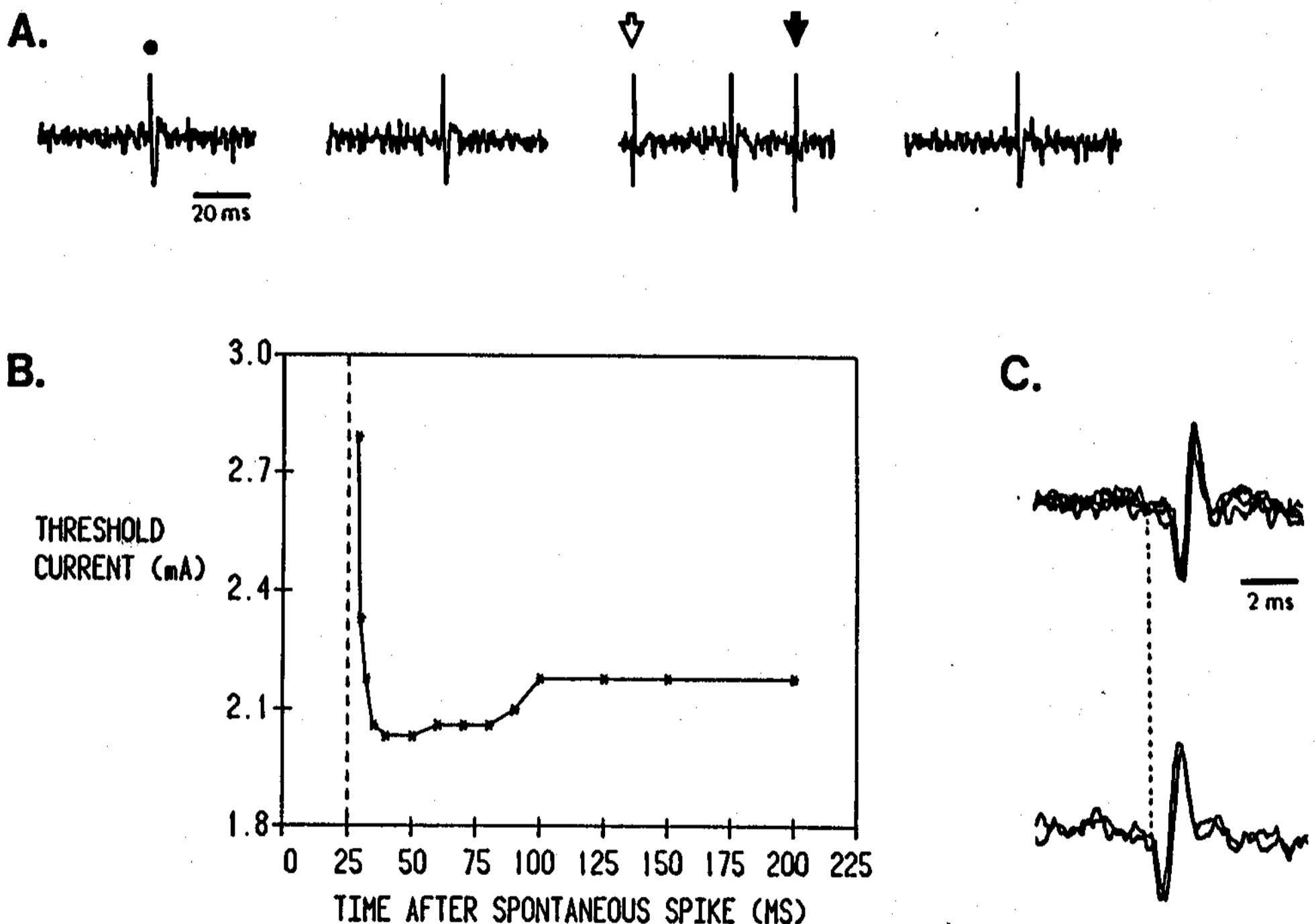


Fig. 5. Impulse-dependent increases in the antidromic excitability and conduction velocity of Type 1 DRN neurons. **A** and **B**: impulse-dependent increases in axon excitability. **A**: subthreshold electrical stimulation of the neostriatum does not elicit an antidromic spike (solid arrow) except when a spontaneous spike (open arrow) occurs within 10–75 ms prior to the collision interval (third trace). Traces are presented in serial order. Stimulus artefact is labeled with a solid circle. **B**: time course of increased excitability following propagation of an orthodromic spike past the site of fiber stimulation in the neostriatum. Stimulating currents just sufficient to evoke 100% antidromic responding on non-collision trials (i.e. threshold) were determined at different time intervals after the occurrence of a spontaneous (orthodromic) spike. Antidromic latency of this Type 1 neuron is 25.0 ms. Immediately after the propagation of the spontaneous orthodromic impulse along the axon there is a short period of reduced excitability, indicative of axon refractoriness. The refractory period was followed by an interval of increased fiber excitability persisting for 75 ms. **C**: impulse-dependent decreases in antidromic latency. Top traces show invariant latency of antidromic spike recorded from a Type 1 neuron in response to stimulation of the neostriatum. Note expanded time scale (stimulus artefact is not shown). Superimposition of four traces. Bottom traces illustrate a decrease in latency of two antidromic spikes by about 1 ms when a spontaneous spike occurs about 50 ms prior to (antidromic) stimulation of the fiber. Different cells in **A**, **B** and **C**.

nergic (Type 1) terminal fields (Table I). A cumulative dose of 40 $\mu\text{g}/\text{kg}$ increased neostriatal threshold stimulating currents in 7 out of 9 neurons when compared to the pre-drug state. One cell exhibited a small decrease in threshold, while the excitability of another cell was unaffected by the serotonin autoreceptor agonist (i.e. a change less than or equal to 5%). The response to intravenous injection of 5-MeODMT is illustrated for a typical Type 1 neuron in Fig. 6. Following an initial injection of 20 $\mu\text{g}/\text{kg}$, the excitability curve is shifted uniformly to the right, demonstrating that increased stimulus currents were necessary to evoke the same proportions of antidromic responding compared to the pre-drug condition. After a second injection of 20 $\mu\text{g}/\text{kg}$ 5-MeODMT, excitability is further reduced, as shown by an additional shift to the right in the excitability curve. Most neurons exhibited a further decrease in terminal excitability following a second injection of 5-MeODMT, but additional injections of 5-MeODMT up to a cumulative dose of 60–100 $\mu\text{g}/\text{kg}$ did not consistently result in a further reduction in terminal excitability. In control experiments, two successive injections of the vehicle (1 ml/kg 0.9% saline) failed to alter terminal field excitability in 6 of 8 cells, with the remaining 2 cases showing small decreases in excitability. Changes in terminal field excitability following intravenous administration of 5-MeODMT differed significantly from saline control experiments (Mann-Whitney $U = 11$; $N_1 = 8$; $N_2 = 9$; $P < 0.02$). Excitability testing was also perform-

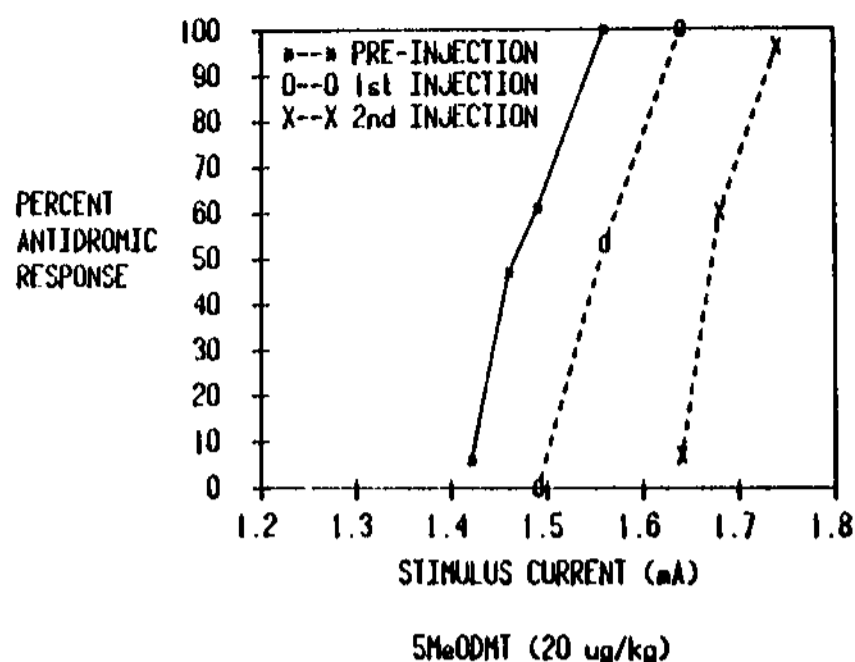


Fig. 6. Intravenous administration of the serotonin autoreceptor agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), reduces the excitability of the terminal field of this presumed serotonergic (Type 1) neuron to neostriatal stimulation. The percentage of stimulus trials on which antidromic spikes are elicited at different currents are expressed on the ordinate, the stimulus current is shown on the abscissa. Solid line represents excitability curve obtained prior to drug injection. The dashed lines represent the excitability curves after intravenous injections of 20 $\mu\text{g}/\text{kg}$ 5-MeODMT. The excitability curve was shifted uniformly to the right, indicating increased stimulus currents are necessary to achieve the pre-drug level of antidromic responding.

ed on preterminal regions of serotonergic fibers in the MFB. In these experiments, intravenous administration of 40 $\mu\text{g}/\text{kg}$ 5-MeODMT did not change the preterminal fiber excitability ($n = 2$).

Following the direct infusion of 10 or 50 μM 5-MeODMT into the neostriatal terminal fields, thresholds were increased in 7 out of 12 cases when compared to pre-infusion threshold values. In 4 ex-

TABLE I

Effects of intravenous administration and infusion of 5-MeODMT and vehicle on antidromic excitability of dorsal raphe-neostriatal serotonergic neurons

Threshold is defined as the stimulating current just sufficient to evoke 100% antidromic responding on non-collision trials. All effects are a comparison of pre- and post-drug excitability values. 'No effect' is considered to be any change less than or equal to 5%. Numbers in each column refer to the number of cases, and the numbers in parentheses refer to the mean change in excitability \pm S.E.M.

Agent	Condition	n	Change in threshold		
			Increase	Decrease	No effect
5-MeODMT (40 $\mu\text{g}/\text{kg}$)	Intravenous, neostriatal stimulation	9*	7 (12.3 \pm 4.0%)	1 (6%)	1
Vehicle (0.9% saline)	Intravenous, neostriatal stimulation	8	2 (6.0 \pm 0.0%)	0	6
5-MeODMT (40 $\mu\text{g}/\text{kg}$)	Intravenous, MFB stimulation	2	0	0	2
5-MeODMT (10 or 50 μM)	Infusion, neostriatal stimulation	12**	7 (14.6 \pm 3.1%)	1 (7%)	4
Vehicle (0.9% saline)	Infusion, neostriatal stimulation	6	0	1 (30%)	5

* Differs significantly from vehicle intravenous group (Mann-Whitney $U = 11$; $N_1 = 8$; $N_2 = 9$; $P < 0.02$).

** Differs significantly from vehicle infusion group (Mann-Whitney $U = 14$; $N_1 = 6$; $N_2 = 12$; $P = 0.05$).

periments, there was no change in threshold, and one cell showed a decrease in threshold. An inverse relationship between the magnitude of the threshold change and the pre-infusion spontaneous firing rate was suggested, but failed to reach statistical significance ($r = -0.542$; $df = 9$; $P = 0.085$). Infusions of 5-MeODMT into the striatum did not produce any consistent effect on the firing rate of DRN serotonergic neurons. Infusions of the vehicle (0.9% saline) had no effect on terminal excitability in 5 out of 6 cases, and increased the excitability of one cell. Changes in terminal excitability following infusion of 5-MeODMT differed significantly from saline control experiments (Mann-Whitney $U = 14$; $N_1 = 6$; $N_2 = 12$; $P = 0.05$).

DISCUSSION

Neurophysiology of the dorsal raphe-neostriatal projection

This report provides an electrophysiological characterization of the dorsal raphe-neostriatal projection by means of antidromic activation techniques. The data presented in this paper are in accord with anatomical studies showing that the dorsal raphe innervation of the neostriatum arises predominately from serotonergic (Type 1) neurons situated in the dorsomedial and ventromedial cell clusters of the DRN^{43-45,51} and is a unilateral projection^{27,52}.

Eight percent of the DRN neurons responding antidromically to neostriatal stimulation were classified into a second group of cells (Type 2 cells) exhibiting electrophysiological properties that were distinct from the serotonergic (Type 1) neurons. Type 2 antidromic neurons had a different waveform (negative-small positive polarity) that was never observed to transform into a serotonergic-like waveform. In addition, Type 2 neurons exhibited an irregular, slow, somewhat bursty firing pattern, and typically shorter antidromic latencies than those observed for presumed serotonergic neurons. The possibility that these responses represent a non-serotonergic projection from the DRN to the neostriatum is supported by a variety of anatomical and physiological studies. Approximately two-thirds of the neurons in the rat DRN are not serotonergic^{12,17}, and anatomical evidence for the existence of a non-serotonergic component in the DRN innervation of the neostriatum has

been reported, based on the combined use of retrogradely transported fluorescent tracers and immunocytochemistry⁴³⁻⁴⁵ (also see ref. 24). Furthermore, the negative-positive polarity and the slow firing rate of Type 2 cells are similar to those of neurons recorded in the DRN that are resistant to the effects of the serotonergic neurotoxic agent, 5,7-dihydroxytryptamine, and were classified as non-serotonergic by Ag-hajanian et al.⁴ However, it is relevant to note that the spontaneous activity of the Type 2 antidromic neuron was inhibited by intravenous injection of a 'presynaptic' dose (40 $\mu\text{g}/\text{kg}$) of the serotonergic autoreceptor agonist 5-MeODMT. If in fact the Type 2 neuron is not serotonergic, then the inhibitory effect of 5-MeODMT on Type 2 discharge rate is probably a reflection of complex synaptic interactions within the DRN involving serotonin. Unlike a previous report⁴ in which presumed non-serotonergic DRN neurons, similar to our Type 2 cells, could not be antidromically activated by stimulation of the ventral tegmentum and were hence postulated to be intrinsic neurons, our results clearly show that at least some of these neurons project to the forebrain.

Recently Park³⁷ has provided electrophysiological evidence in the rat that a class of non-serotonergic dorsal raphe neurons, distinct from the presumed non-serotonergic Type 2 neuron described above, projects out of the nucleus. The cells examined by Park³⁷ could be antidromically activated by stimulation of the ventral tegmentum, and had an intracellularly recorded waveform and a high spontaneous discharge rate that was distinct from serotonergic neurons. The spontaneous activity of this rapidly firing neuron has been shown to be insensitive to the *in vitro* application of 5-MeODMT, further distinguishing it from serotonergic neurons⁵⁴. Although this cell type (i.e. the Type 3 cell) was commonly encountered in our studies, on only two occasions did we observe a Type 3 neuron that was antidromically activated by stimulation of the MFB. No Type 3 cells were antidromically activated by neostriatal stimulation.

A significant proportion of the presumed serotonergic (Type 1) DRN cells displayed multiple discrete antidromic latencies, as described for other central monoaminergic fibers^{13,22,48}. This phenomenon has been interpreted to result from the initiation of the antidromic impulse at different sites along the

axon due to fiber branching and/or fibers that follow a convoluted path. These interpretations seem to apply for the serotonergic dorsal raphe-neostriatal pathway, since, within the confines of the neostriatum, these fibers follow a tortuous path and apparently give off axon collaterals⁵¹. This may also account for the absence of a correlation between the magnitude of antidromic latency and the site of neostriatal stimulation.

Many different types of axons exhibit an increase in conduction velocity and excitability following recent impulse conduction along the axon, an effect termed the supernormal period (for review, see ref. 47). Supernormal periods were observed during antidromic excitation of presumed serotonergic (Type 1) axons, as indicated by increases in excitability of the fiber to electrical stimulation and concomitant decreases in conduction time when an orthodromic action potential passed along the axonal stimulation site within 10–75 ms prior to stimulation. The functional significance of supernormal periods in ascending serotonergic fibers is unclear, since the typical interspike interval of serotonergic DRN neurons greatly exceeds the duration of the supernormal period.

Estimates of conduction velocity for individual serotonergic fibers based on conduction time from neostriatal stimulation ranged from 0.2 to 1.5 m/s, in agreement with previous estimates obtained from stimulation of the ventral tegmentum^{39,58,59}. The slow propagation of action potentials by ascending serotonergic fibers is consistent with the small diameter of these unmyelinated varicose axons^{6,9,19,40}. As noted by others³⁸, none of these estimates takes into account the circuitous nature of the dorsal raphe-neostriatal projection. Hence, these figures represent an underestimate of actual conduction velocities. This argument also applies for the presumed non-serotonergic Type 2 fibers, although less is known about the route of this projection.

It is possible that some of the antidromic responses resulted from stimulation of axons traversing but not innervating the neostriatum. The dorsal raphe-cortical projection is the most likely candidate for such inadvertent activation, since it passes through the most caudal regions of the neostriatum in rats^{7,50}. However, the dorsal raphe-cortical projection is reported to emit collaterals within the neostriatum⁵³, and furthermore, few stimulating electrodes were position-

ed in the most caudal regions of the neostriatum.

Several attempts have been made to elucidate the synaptic action of serotonin in the neostriatum. Davies and Tongroach¹⁵ report an inhibition of rat neostriatal neuronal activity, on rare occasions preceded by excitation, after DRN stimulation with seemingly appropriate latencies of 16–40 ms. However, other studies have reported short latency responses in the neostriatum following DRN stimulation that are not consonant with the slow spike propagation of serotonergic axons observed in the present study. For example, stimulation of the DRN has been reported to result in an 'immediate' inhibitory effect on the extracellularly recorded firing pattern of spontaneously active or glutamate-excited neostriatal neurons in rats^{29,36}. A more direct assessment of serotonergic synaptic actions in spiny neurons of the neostriatum has been attempted by means of intracellular recordings in urethane-anesthetized rats^{38,55}. Electrical stimulation of the DRN was found to elicit a complex monosynaptic excitatory post-synaptic potential (EPSP). The onset of the putative serotonergic component of the EPSP, as determined following pharmacological disruption of serotonin biosynthesis, had a mean latency of 7.2 ms³⁸. However, the antidromic activation latencies for serotonergic DRN neurons reported in this paper are clearly incongruent with a DRN serotonergic mediation of this short latency EPSP. The neurophysiological and anatomical basis for short latency responses in the neostriatum following DRN stimulation remains to be clarified, as does the possibility of a serotonergic mediation of these synaptic actions.

Serotonergic terminal excitability

Terminal excitability experiments were performed in order to examine the neurophysiological effects of presynaptic serotonergic autoreceptor activation under in vivo conditions. We have previously shown that stimulation of autoreceptors on central catecholamine terminals results in decreases in terminal field excitability, an effect that is blocked by application of appropriate autoreceptor antagonists^{22,34,35,48,49}. We report here that intravenous administration of the serotonin autoreceptor agonist, 5-MeODMT, reduced the excitability of serotonergic terminal fields to electrical stimulation. Similar experiments with MFB stimulation did not produce a change in the excitability

ty of preterminal regions of the fiber, suggesting that axonal autoreceptors are localized to the terminal fields of serotonergic axons, similar to previous observations in dopaminergic and noradrenergic neurons^{22,34,48}. Furthermore, decreases in excitability were observed following infusion of the agonist directly into the neostriatum, providing persuasive evidence that activation of presynaptic autoreceptors situated at the terminal regions are crucial for the observed excitability changes.

Decreases in the excitability of terminal fields to direct electrical stimulation have been inferred to result from hyperpolarization of the membrane and/or alterations in membrane conductance^{23,25,34,48,57}. Intracellular recordings from rat serotonergic DRN neurons indicate that activation of somato-dendritic autoreceptors results in a hyperpolarization that is probably mediated by an increase in membrane K⁺ conductance¹. A similar mechanism may be responsible for decreased terminal excitability consequent to 5-MeODMT administration. Alternatively, modification of calcium or chloride conductances could underlie the change in excitability, or the excitability change may be a secondary event, the result of a biochemical process, such as activation of a hyperpolarizing electrogenic ion pump.

Mechanism of autoinhibition of Ca²⁺-dependent serotonin release

It is conceivable that the biophysical membrane events that underlie the observed changes in terminal excitability are also instrumental in the regulation of Ca²⁺-dependent neurotransmitter release by serotonergic terminal autoreceptors^{8,11,20,21,28,32,46}. Under this assumption, the results of the excitability experiments presented in this report permit the following inferences concerning the mechanism of autoreceptor-mediated regulation of secretion.

In general terms, autoinhibition of serotonin release could be mediated by: (1) decreasing stimulus-secretion coupling, or (2) decreasing the recruitment of neurotransmitter release sites, i.e. decreasing the number of release sites that are activated by a given stimulus. The latter process could involve the blockade of action potential propagation along the fiber or at branch points, thus preventing the recruitment of neurotransmitter release sites (presumed to be varicosities) distal to the point of propagation blockade.

Such a mechanism has been proposed to be a primary means of autoinhibition of neurotransmitter secretion from presumably varicose noradrenergic fibers in guinea pig vas deferens^{5,14}. A serotonin autoreceptor-mediated membrane hyperpolarization and/or increase in membrane conductance could be easily incorporated into this proposal, since the production of membrane hyperpolarization or current shunting would be expected to decrease the safety factor for impulse propagation along these varicose fibers. The terminal excitability experiments described in this paper provide a means for testing this hypothesis. Specifically, if blockade of spike propagation within the terminal region of the axon accounts for autoinhibition of serotonin secretion, then one would expect that following exposure to a serotonin autoreceptor agonist it would not be possible to evoke 100% antidromic responding from stimulation of terminal fields. In fact, following infusions and intravenous injections of the autoreceptor agonist, 5-MeODMT, it was possible to obtain 100% antidromic responding (albeit at slightly higher current settings due to decreases in excitability). This finding strongly suggests that conduction blockade is not the primary means of autoinhibition of central serotonin secretion. This interpretation is also consistent with the findings of a study that compared the inhibitory effects of serotonin autoreceptor agonists on electrically-evoked and K⁺-evoked serotonin secretion in a brain slice preparation²⁰. High K⁺ concentrations depolarize the entire length of the terminal fiber. Hence, if autoinhibition of release is mediated by blocking the recruitment of distal secretion sites, then the ability of autoreceptor agonists to inhibit serotonin secretion in high K⁺ concentrations should be attenuated when compared to electrically-evoked release. Gothert²⁰ found that Ca²⁺-dependent serotonin release is inhibited at the same concentrations of exogenously applied serotonin and to the same extent irrespective of whether release is evoked by electrical stimulation or by high K⁺ concentrations, providing biochemical evidence that autoinhibition of central serotonin release does not involve impairment of impulse conduction along the axon. In this respect, the mechanism of serotonergic autoinhibition may differ from that observed in catecholamine neurons^{5,48}.

Taken together, this body of evidence suggests that autoinhibition of serotonin secretion involves a

modification of stimulus-secretion coupling at individual release sites, either by reducing the probability that the propagation of an action potential past a release site will evoke secretion¹⁰ (i.e. recruitment at the level of individual release sites), or by reducing the amount of neurotransmitter that is liberated at individual release sites in response to a given stimulus (i.e. reducing the quantal content of individual release sites). The mechanism of autoreceptor-mediated attenuation of depolarization-secretion coupling at individual release sites is a source of speculation (cf. refs. 23, 34 and 48). Apparently a critical feature in serotonergic autoregulatory processes is the availability of Ca²⁺ for stimulus-secretion coupling²⁰. If the action potential spreads passively across the neurotransmitter release site (varicosity), then autoreceptor-mediated hyperpolarization and/or changes in membrane conductance may alter the electrotonic properties of the release site. Conceivably, such changes in membrane properties could result in an attenuation of the voltage-dependent Ca²⁺

influx or depolarization-secretion coupling that results from the passive invasion of the action potential into the release site. In this way, Ca²⁺ availability for secretion would be decreased. Alternatively, if the neurotransmitter release site is actively invaded by the action potential, autoreceptor-mediated hyperpolarization and/or conductance changes could modify the amplitude or duration of the invading spike, and in this manner modulate secretion, presumably by modifying Ca²⁺ availability.

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