EXPERIMENTAL NEUROLOGY 78, 685–702 (1982)

Excitability Changes Along Pyramidal Tract Axons after Sensory Stimulation

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Received April 6, 1982; revision received July 14, 1982

Recordings were made extracellularly of the activity of single pyramidal tract (PT) neurons in the forepaw focus of the cat's postcruciate cerebral cortex. Activity was evoked antidromically by near-threshold stimulation of the contralateral dorsolateral funiculus of the C6 spinal cord (corticospinal tract), the ipsilateral medullary pyramid, the ipsilateral cerebral peduncle, and the ipsilateral internal capsule. Orthodromic activity was initiated by shocks to each of the four paws. The excitability of PT axons at the spinal cord, medullary pyramid, cerebral peduncle, and internal capsule was tested by measuring the threshold to antidromic activation in conditioning–testing interactions. The PT axons with small contralateral receptive fields (small-field cells) showed no change in excitability at any of the four test sites after stimulation of any of the four paws. In contrast, most PT axons with large bilateral receptive fields (wide-field cells) showed increases in excitability after stimulation of the contralateral forepaw at one or more of the test sites, in many cases, at all four sites. Although not tested in all neurons, increases in excitability of wide-field axons were also seen after stimulation of the ipsilateral forepaw and the contralateral and ipsilateral hind paws. The increased excitability was detectable 20 to 25 ms after the contralateral forepaw stimulus, was maximum at 25 to 30 ms, and then returned to baseline by 75 to 100 ms. Maximum changes in excitability varied from 5% to more than 60% in different axons. The time course of the change in excitability was nearly the same at each site, but the magnitude of the change was most often greater at the pyramid and cerebral peduncle than at the internal capsule. In some cells, a period of decreased excitability, in most cases apparent inexcitability, followed the period of increased excitability. This could also be observed at all four test sites. The

Abbreviation: PT—pyramidal tract.

[1] This work was supported by National Science Foundation research grant BNS 76-14743. The authors thank Douglas Martin for technical assistance and Dr. C. F. Tyner for reading and commenting upon initial drafts of the manuscript. Please send correspondence to Dr. Michael D. Mann, Department of Physiology and Biophysics, University of Nebraska Medical Center, 42nd St. and Dewey Ave., Omaha, NE 68105.

0014-4886/82/120685-18$02.00/0

period of increased excitability most likely resulted from depolarization of terminals of branches of the PT axon near the testing site, whereas the period of decreased excitability may have resulted from strong depolarization or hyperpolarization of such terminals or from hyperpolarization of one or more nodes of Ranvier, the site situated nearer to the soma than the test site.

INTRODUCTION

Most axons of the pyramidal tract (PT) undergo a transient increase in excitability at the level of the medullary pyramids after electrical stimulation of the central footpad of any paw in cats (17). In particular, it is the wide-field or *m* subset of PT axons that experiences this change in excitability. The *m* subset consists of those neurons that respond to electrical stimulation of all four paws and is distinguished from the small-field or *s*a subset consisting of PT neurons that respond only to stimulation of the contralateral forepaw (when the somata are in the forepaw focus of motor–sensory cortex). The *s*a subset undergoes no such change in excitability.

In the conditioning–testing paradigm for measuring excitability, the increased excitability of PT *m* axons begins shortly after the conditioning stimulus to the paw and is manifested as a decreased threshold for initiating antidromic propagation along the axon from the medullary pyramids to the cortex. In some but not all PT *m* axons, this period of increased excitability is followed by a period of decreased excitability in which maximum stimulator output (30-mA, 0.05-ms constant current pulses) is frequently insufficient to evoke the antidromic spike (17).

Similar changes in excitability of corticospinal axons after stimulation of either peripheral nerves or the cerebral cortex were reported for the corticospinal subset of PT axons by Gugino *et al.* (9). These changes in excitability of corticospinal axons at the cervical enlargement followed either cutaneous or muscle nerve stimulation at just above threshold strength. Similar results were also reported for corticofugal axons entering the trigeminal nuclei and the thalamic nucleus ventralis posteromedialis after stimulation of the infraorbital nerve. The time course of this increase in excitability was roughly correlated with that of a reduction in transmission from the cortex to cells of the main sensory trigeminal nucleus (5, 24).

These increases in excitability of corticofugal axons have all been interpreted as the result of depolarization of terminals on short branches of the axons near the site of excitability testing (5, 9, 17, 24). This interpretation was reached primarily because of the long time course of the excitability increase, similar to that observed on primary afferent neurons and thought to reflect presynaptic inhibition (23). Consistent with this interpretation, collaterals of PT axons are known to exist in the reticular nuclei and the inferior olive (14, 15, 19, 22), all near the testing site in the medullary

pyramids, in the trigeminal nuclei (5, 24), and in the spinal cord (26). We know of no report of axoaxonic synapses (the presumed substrate of this effect) in the reticular nuclei or the inferior olive, although Walberg (34) presented several electron micrographs which appear to contain axoaxonic synapses. Axoaxonic synapses are present within the trigeminal nuclei (8). Also consistent with an interpretation in terms of presynaptic inhibition on PT axon terminals is the observation that cortically induced responses, both field potentials and single-unit responses, from the reticular formation are blocked for as long as 150 ms by a preceding stimulus to a peripheral nerve (10).

Increased excitabilities of similar time course were also observed in axons of the visual callosal system (27), but these changes were interpreted as the result of a supernormal period of excitability such as follows the axon spike in peripheral nerve and is associated with a period of afterdepolarization (7, 27). This interpretation was suggested by the lack of reports of axoaxonic synapses on visual callosal axon terminals. Such depolarizing afterpotentials are usually small or absent in recordings from the somata of PT neurons (20, 29), but the relationship between the properties of axon and soma spikes in PT neurons is unknown. It is therefore possible that the increase in excitability of PT axons is also the result of afterdepolarization.

If the increase in excitability of PT axons were due to afterdepolarization, then the increase should be measurable anywhere along the axon, i.e., it should be distributed continuously along the length of the axon. On the other hand, the pyramidal tract gives off collaterals to a number of sites within the brain stem and spinal cord (6, 26), but presumably the distribution of such collaterals is not uniform along the axons' lengths. One would, therefore, expect to find some regions of the axons which are more than two space constants away from a depolarized terminal and which experience no detectable change in excitability due to presynaptic depolarization. The existence of branches of PT axons at the level of the red nucleus, thalamus, and spinal cord (6, 26, 32) suggests that some axons may experience increases in excitability by presynaptic depolarization at the cerebral peduncle, internal capsule, or the C6 spinal cord, but not every axon need experience such changes at each site. The present experiments were to distinguish these two interpretations of pyramidal excitability changes by testing the excitability of the same PT axon at these different points along its course though the brain stem.

METHODS

Domestic cats weighing 2.5 to 4.0 kg were anesthetized with α-chloralose (50 to 60 mg/kg, i.p.), placed on artificial respiration, and paralyzed with

either decamethonium bromide or gallamine triethiodide as required throughout the experiments. The right femoral vein was cannulated, the medullary pyramids were exposed through the standard ventral approach, the right anterior cerebral hemisphere was exposed, a craniectomy was made over the right cerebral peduncle and internal capsule, and a bilateral pneumothorax was produced. Upon reflection of the dura mater, the pericruciate cerebral cortex was covered with a protective polyethylene sheet and other exposed areas of cortex were covered with Ringer's agar. Further reduction in pulsations was achieved by suspending the animal from one upper thoracic and lower lumbar spinous vertebral process. Respiration was adjusted to maintain expired CO_2 at about 4.0%. Rectal temperature was maintained at 38.5°C by a servocontrolled DC heating pad placed under the animal. In several animals, a laminectomy was made over the C6 spinal cord, the dura mater was reflected, and the spinal cord covered with mineral oil that was maintained at 38.5°C.

A small hole was made in the polyethylene sheet to allow placement of a glass micropipet, filled with 2.5 M NaCl or 1.25 M Na citrate, onto the postcruciate recording site in the forepaw focus of field 4γ (31). Signals were led through a Grass P15B preamplifier, with filters at 30 Hz and 10 kHz half-amplitude settings, to a Tektronix 565 oscilloscope for photographic recording using a Grass C4 kymograph camera.

A pair of silver ball electrodes (with 1.75-mm tip separation) was placed along the long axes of both the medullary pyramid at the midolivary level ipsilateral to the cerebral recording site and the contralateral C6 dorsolateral funiculus 0.5 to 1.0 mm lateral to the dorsal root entry zone. Concentric bipolar electrodes, with 0.5-mm tip exposures and 1.0-mm tip separations, were lowered stereotaxically into the cerebral peduncle (A 4, L 4.5, H −6) and internal capsule (A 13.5, L 5.5, H 1.5). While the electrodes were being inserted, recordings were made from the center wire. The final position of the tip of the electrode in the horizontal plane was the position of the largest response to stimulation of the postcruciate recording site and the medullary pyramid. Proper positioning of the electrodes was confirmed histologically after the experiment. Bipolar needle electrodes were inserted into the central footpad of each limb for cutaneous stimulation. Electrical stimuli were generated using a Tektronix 2600 pulse generator and delivered using a 2620 photon-coupled stimulus isolator. Cutaneous stimuli consisted of 0.1-ms pulses of 25 mA constant current; stimuli in the central nervous system consisted of 0.05-ms pulses of various strengths, depending on the neuron and the experimental maneuver.

Neurons were isolated using two per second stimulation of the medullary pyramid at an intensity supramaximal for the a wave recorded from the postcruciate cortex and sufficient to evoke the d wave (12). The PT neurons were identified on the basis of short, invariant latency of a response to

pyramid stimulation, ability to follow such stimulation at frequencies in excess of 100 per second and in many cases, by collision of a suspected antidromic spike with a known orthodromic spike. A total of 259 PT neurons was examined. Neurons were further classified as *s*a, *s*b, *s*c, or *m* neurons if they responded to stimulation of the contralateral forepaw, both forepaws, both contralateral paws, or all four paws. Recordings were made of responses of 11 *s*a, 15 *s*b, 185 *m*, and 4 *s*c neurons. In addition, one cell responded only to stimulation of the contralateral hind paw, whereas 59 cells did not respond to stimulation of any paw (PT mute neurons).

Excitabilities of PT axons were tested with the conditioning–testing paradigm as used by Mann *et al.* (17). Thresholds were determined by increasing the stimulus strength from a minimal value until the axon discharged in response to 50% of the test stimulus trials. The stimulus strength was raised until the axon responded to each stimulus and then lowered again to a strength which yielded responses to 50% of the test stimuli. Threshold was taken as the midpoint between the "ascending" strength which yielded 50% responses and the "descending" strength which yielded 50% responses.

RESULTS

Increased Axonal Excitability. The PT *m* neuron responds to stimulation of the medullary pyramid with a characteristic antidromic latency that does not vary greatly as stimulus strength is lowered toward threshold. If the antidromic stimulus strength is set just below threshold, the neuron, of course, fails to respond, but if this ineffective stimulus was preceded at 25 to 100 ms by a supramaximal stimulus to the contralateral forepaw, the neuron responds antidromically. This was the basic observation of Mann *et al.* (17) and is shown in Fig. 1, column A. A response to a just-suprathreshold pyramid stimulus is shown in A1. In A2, the neuron failed to respond to a just-subthreshold stimulus; however, in A3, it responded to the same stimulus when it was preceded at about 75 ms by a stimulus to the contralateral forepaw.

Similar increases in excitability of PT *m* axons could be detected at the level of the cerebral peduncle and the internal capsule. This is illustrated for the same axon in Fig. 1B (peduncle) and C (capsule). Antidromic responses to suprathreshold stimuli are shown in B1 and C1, no responses to just subthreshold stimuli are shown in B2 and C2, and responses to conditioned, subthreshold stimuli are shown in B3 and C3 at the same condition–test interval as in A.

A similar increase in excitability of PT *m* axons that were also corticospinal axons could be detected in the same manner at the C6 spinal cord. Figure 2A shows an increase in excitability at the C6 level with a response to a suprathreshold stimulus to the contralateral dorsolateral funiculus in A1, a failure to respond to a subthreshold stimulus in A2, and a response

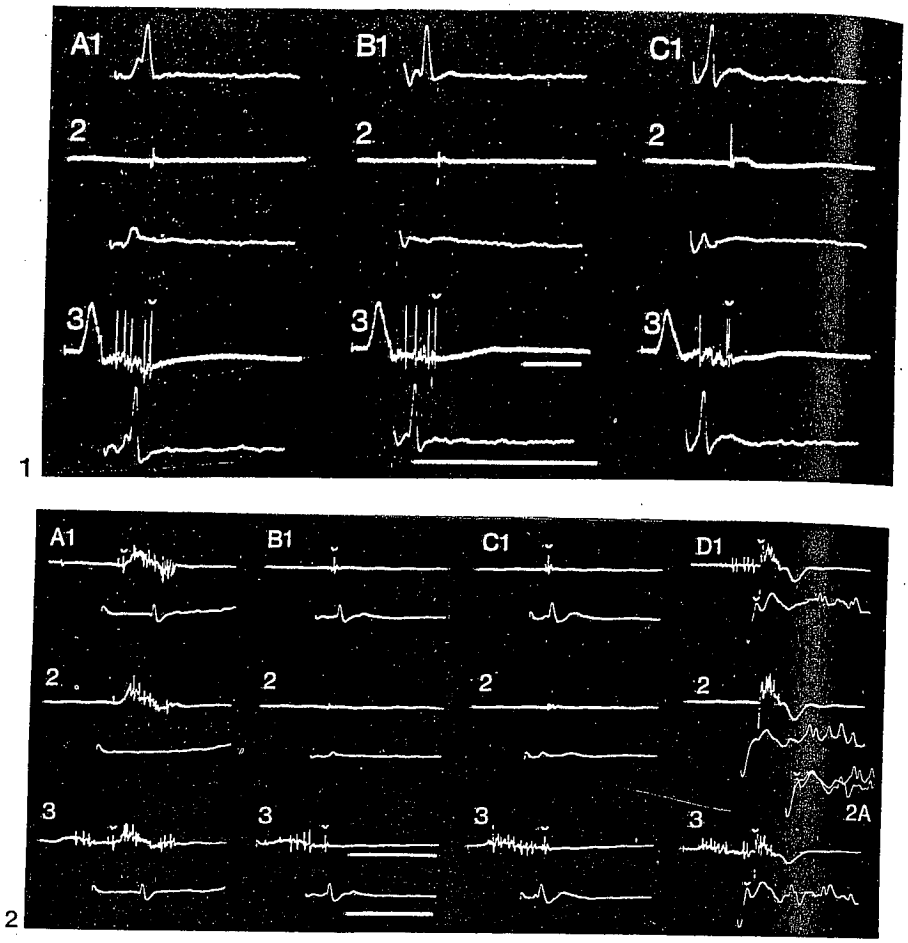


FIG. 1. Condition–test measurements of the excitability of a PT *m* axon. Row 1—antidromic response to suprathreshold stimulation of the medullary pyramid (A), cerebral peduncle (B), and internal capsule (C). Row 2—just-subthreshold stimulation as above fails to evoke responses. Row 3—effect of conditioning with supramaximal shocks to the contralateral forepaw on responsiveness of the axon to the stimulation as in row 2. Responses are shown in fast sweeps only in row 1 and in both fast and slow sweeps in rows 2 and 3. The position of the antidromic spike is indicated by the caret above the slow sweeps. All trials at one per 2 seconds. Time in B3: 50 ms, upper traces; 5 ms, lower.

FIG. 2. Condition–test measurements of the excitability of a PT *m* axon. Row 1—antidromic response to suprathreshold stimulation of the C6 spinal cord (A), medullary pyramid (B), cerebral peduncle (C), and internal capsule (D) at two sweep speeds. Row 2—just-subthreshold stimulation as above fails to evoke responses. Row 3—effect of supramaximal shocks to the contralateral forepaw on responsiveness of the axon to the same stimulation as in row 2. The position of the antidromic spike is indicated by the caret above the record for all slow sweeps and for internal capsule records only in fast sweeps. The antidromic spike was identified by its all-or-none character. The inset, D2A, shows fast traces with and without the antidromic spike superimposed. All trials repeated at 2-s intervals. Time in B3: 50 ms, upper traces; 5 ms, lower.

a conditioned, subthreshold stimulus in A3. In Fig. 2, the conditioning–
ting interval is approximately 30 ms. Changes in excitability of this cor-
ospinal axon are illustrated at the pyramids in B, at the cerebral peduncle
C, and at the internal capsule in D.

Distibution of Increased Excitability. As shown in Figs. 1 and 2, a single
on could experience increased excitability at several sites along its course.
ıt, not every PT axon experienced such changes at every site tested. Tests
axonal excitability were carried out at at least one site along the axon
208 PT neurons, the results of which are summarized in Table 1. We
ıve never seen any change in excitability of a PT *sa* axon at the medullary
ramid (17) or at the C6 spinal cord, cerebral peduncle, or internal capsule.
hus, PT *sa* axons apparently did not undergo changes in excitability any-
here along their course, at least to the lower cervical spinal cord.

In the present study, 147 PT *m* axons were tested. Six of these showed
o change in excitability at the pyramids; most of these were observed in
single experiment in which no change occurred in any neuron tested. The
maining 141 showed an increased excitability at one or more sites after
nsory stimulation. Each row in Table 1 consists of a combination of effects
r lack of effects of contralateral forepaw stimulation upon axon excitability.

TABLE 1

Distribution of Increased Excitability Along Pyramidal Tract (PT) Axons of Different Subsets

Position[a]	Neuron subset				
	sa	*sb*	*sc*	*m*	mute
PT + CP + IC + CST	0	2	0	11	1
PT + ~CP + ~IC + ~CST	0	0	0	1	0
PT + CP + IC	0	2	2	33	0
PT + CP + ~IC	0	0	0	5	0
PT + ~CP + ~IC	0	1	0	1	0
PT + CP	0	2	0	25	1
PT + ~CP	0	0	0	3	0
PT + IC	0	0	0	18	1
PT + ~IC	0	0	0	3	1
PT + CST	0	0	0	3	0
PT + ~CST	0	0	0	2	0
PT + ~CP + ~CST	0	0	0	1	0
PT	0	1	0	35	4
No effect at any site tested	8	2	0	6	33
Total	8	10	2	147	41

[a] PT—medullary pyramid, CP—cerebral peduncle, IC—internal capsule, CST—cortico-spinal tract, ~CP—not CP, ~IC—not IC, ~CST—not CST.

TABLE 2

Proportion of Axons Tested at a Level that Showed Excitability Increase at that Level

Level	Neuron set				
	sa[a]	*sb*[a]	*sc*	*m*	mute[a]
PT	0/7 (0)[b]	8/10 (80)	2/2 (100)	141/147 (96)	8/41 (20)
CP	0/5 (0)	6/8 (75)	2/2 (100)	73/79 (92)	2/14 (14)
IC	0/3 (0)	4/6 (67)	2/2 (100)	62/72 (86)	2/10 (20)
CST	0/1 (0)	2/2 (100)	—	14/18 (78)	1/1 (100)

[a] Proportions cannot be calculated from Table 1 because some "No effect" axons were tested at several levels.

[b] Percentage in parentheses.

Thus, an entry in the first row indicates a cell that showed increased excitability at the pyramid, cerebral peduncle, internal capsule, and C6 spinal cord, whereas an entry in the second row indicates a cell that experienced such changes at the pyramid but not at the other three sites. The absence of a position indicator in a row indicates that excitability was not tested at that site. Not every possible combination of effects was found.

Presented in this form, the data are difficult to interpret because such a large proportion of the axons were not tested at every level. This difficulty results from three factors: the long time required to complete tests, the fact that not all axons were driven antidromically from every site, and, particularly for the internal capsule, the frequent obscuring of antidromic spikes by a large stimulus artifact. Attempts to reduce the artifact electronically usually also made the antidromic spike indistinct. Among PT *m* axons tested at all four sites, however, only 1 in 12 failed to show an increase in excitability at each site. Of PT *m* axons tested at two or more sites, 95 of 106 showed a change in excitability at two or more sites.

Another way of looking at these data is to calculate the proportion of axons tested at a site that showed an increase in excitability at that site. The result of this calculation is shown in Table 2. For PT *m* axons, 78% or more tested at a site showed a change in excitability at that site. Only 6 of 147 PT *m* axons failed to show such a change at the pyramids and only 6 of 79 failed to show a change at the cerebral peduncle. Ten of 72 axons tested at the internal capsule and 4 of 18 tested at the C6 spinal cord failed to show such a change in excitability. Overall, two-thirds or more of PT *sb*, *sc*, and *m* axons tested at each site showed an increase in excitability at that site. Thus, there appears to be a wide distribution of this effect along PT axons but the distribution is not continuous.

Time Course and Magnitude. When tested at the medullary pyramid, the

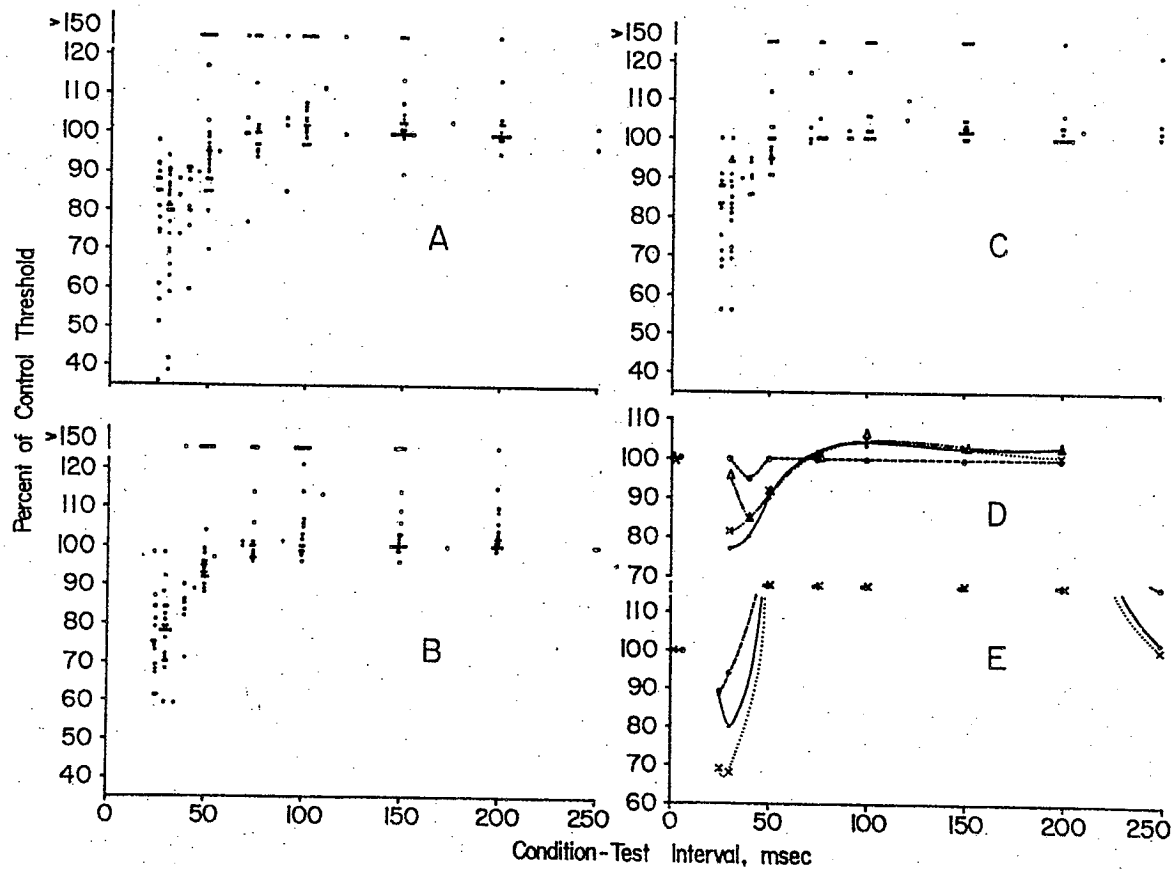
reased excitability of PT axons was maximum at about 25 to 30 ms after onditioning stimulus to the contralateral forepaw. It ended at 75 to 100 , and in some cells the period of increased excitability was immediately lowed by a period of decreased excitability, with a maximum duration 200 ms, during which time the axons often became completely inexcitable en at maximum stimulator output (30 mA, 0.05 ms, one per two seconds). iis time course is illustrated in Fig. 3A where thresholds relative to control reshold are plotted as a function of time after a conditioning stimulus to e contralateral forepaw for 26 PT *m* axons.

Similar time courses of changes in excitability could be detected at both e cerebral peduncle and the internal capsule. As shown in Fig. 3 for 22 T *m* neurons at the cerebral peduncle (B) and 18 PT *m* neurons at the ternal capsule (C), maximum excitability occurred 25 to 30 ms after the nditioning stimulus and excitability had returned to control values by 75 is. As at the pyramid, some axons showed a period of decreased excitability eginning immediately thereafter and lasting to 300 ms.

Not enough excitabilities were tested at the C6 spinal cord level to plot n excitability curve like those in Fig. 3A–C. Figure 3D shows excitability urves for a single PT *m* axon, the excitability of which was tested at all our levels. In this cell, as in the others similarly examined, the excitability urve for the C6 level (triangles) did not differ greatly from that at other evels (e.g., at the pyramids, filled circles). For this axon, the maximum ncrease in excitability occurred earlier at the C6 level and at the pyramid han it did at the peduncle or internal capsule, but this was not uniformly found. Figure 3E shows records from a PT *m* axon, tested at three levels, in which the maximum occurred earlier at the internal capsule than at the peduncle or pyramid.

In Fig. 3, it appears that the magnitude of the change in excitability was greater at the pyramid than at the internal capsule or the peduncle. The mean decrease in threshold at the pyramids was $29.5 \pm 12.5\%$ (mean $\pm$ SD), at the peduncles was $25.3 \pm 10.2\%$, and at the internal capsule was $18.9 \pm 11.7\%$. Only the pyramid–internal capsule difference was statistically significant ($P < 0.05$, Student's $t = 2.3236$, df = 36; $P < 0.02$, Mann–Whitney $U = 94$, $n_1/n_2 = 19/19$). When the effects on single cells were examined, however, the magnitude of the change was sometimes greater at the peduncle or internal capsule than at the pyramid. Figure 4 is a plot of the maximum change in threshold at the pyramids against both the maximum at the internal capsule (open circles) and the maximum at the peduncle (filled circles). More cells experienced a greater change at the pyramids (points below the equal magnitude line) but many experienced a greater change at the peduncle or internal capsule (points above the line).

The linear regression lines calculated for these data,


Percent of Control Threshold
>150
120
110
100
90
80
70
60
50
40
A
B
C
D
E
0
50
100
150
200
250
Condition-Test Interval, msec

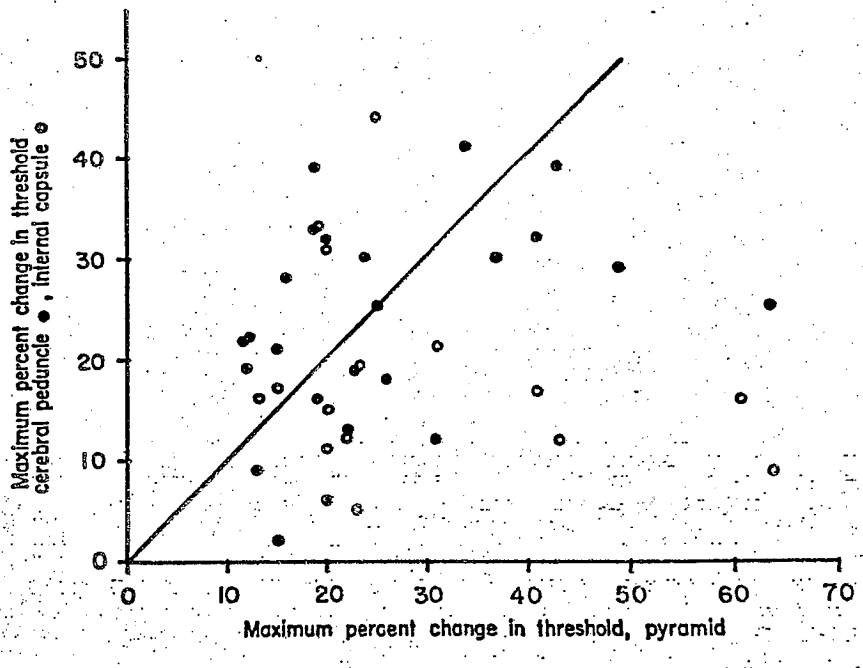


FIG. 4. Maximum percent decrease in threshold for PT *m* axons at the medullary pyramid plotted on the abscissa against the maximum percent decrease in threshold at the cerebral peduncle (●) or the internal capsule (○) for the same axon plotted on the ordinate.

$$\text{cerebral peduncle \% change} = 19.58 + 0.22 \times \text{pyramid \% change}$$

and

$$\text{internal capsule \% change} = 18.70 + 0.006 \times \text{pyramid \% change},$$

are not descriptive. This is reflected in the Pearson's correlation r values of 0.27 and 0.008 and the Spearman's correlation ρ values of 0.11 and −0.11, for the pyramid–cerebral peduncle and pyramid–internal capsule comparisons, respectively.

FIG. 3. Excitability curves for PT *m* axons. Threshold shock strength for the antidromic spike is plotted on the ordinates as percentage of the resting threshold for each axon against the time after a supramaximal shock to the contralateral forepaw on the abscissa. In A–C, each point at a given interval is for a different axon. Excitability for 26 axons as tested at the medullary pyramid (A), for 22 axons as tested at the cerebral peduncle (B), and for 18 axons as tested at the internal capsule (C). D and E—excitability curves for two axons tested at the internal capsule (○), cerebral peduncle (×), medullary pyramid (●), and, in D only, the C6 spinal cord (Δ). Note the broken ordinate in A–C and E indicating periods of decreased excitability.

Conditioning Threshold. When the question of whether or not the observed increase in excitability might be caused by a depolarizing spike afterpotential arose, a number of cells were examined with conditioning stimuli at threshold intensity. At this strength, the PT cell discharged orthodromically on some trials, not on others. If the increased excitability still occurred on trials when the conditioning stimulus did not evoke an orthodromic discharge, then it was not likely a result of afterpotentials. In most cells, the threshold for the change in excitability of the axon and the threshold for the orthodromic response to contralateral forepaw stimulation were the same, i.e., when there was no orthodromic response, the antidromic spike did not occur in conditioning trials. This does not necessarily mean that the change in excitability is tied to the occurrence of the spike as an afterpotential is; it simply means the thresholds are the same. In 10 cells,

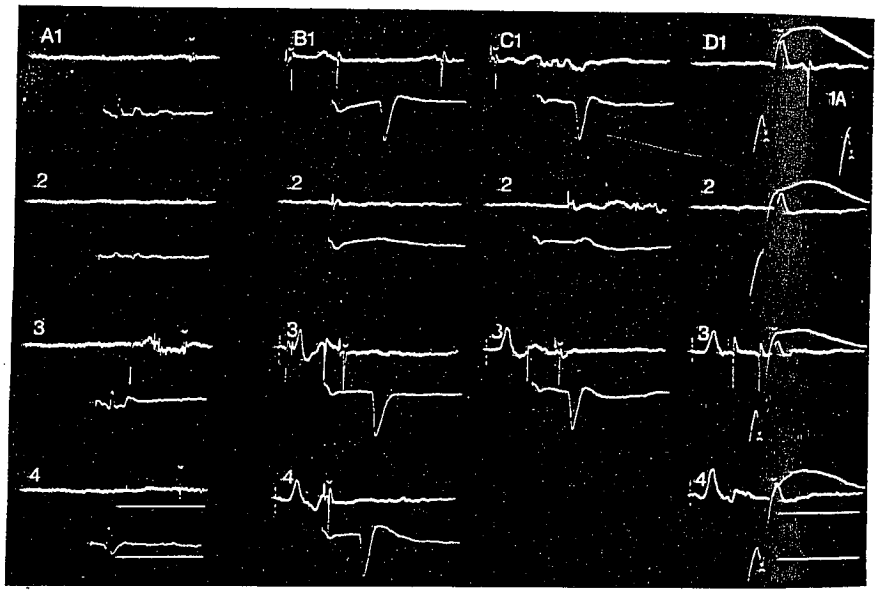


FIG. 5. Condition–test measurements of excitability of two PT *m* axons. Responses of one neuron are shown in A, the other in B–D. Row 1—antidromic response to suprathreshold stimulation of the medullary pyramid (A–B), cerebral peduncle (C), and internal capsule (D) at two sweep speeds. Row 2—just-subthreshold stimulation as above failed to evoke responses. Row 3—effect of maximal (A) and threshold (B–D) shocks to the contralateral forepaw on responsiveness of the axon to the same stimulation as in row 2. Row 4—effect of threshold shocks (A–D) but traces in which the conditioning stimulus itself failed to evoke a response. The position of the antidromic spike is indicated by the caret above the record for all slow sweeps and below the trace for internal capsule records only in fast sweeps. The inset, D1A, shows fast traces with and without the antidromic spike superimposed. The antidromic spike was identified by its all-or-none character. Record B4 was taken from a different series of trials than B3; the condition–test interval was changed, but other parameters were the same. Time in A4 and D4: 50 ms, upper traces; 5 ms, lower.

TABLE 3

Latencies for Antidromic Activation of PT *m* Neurons

Parameter	Stimulation site			
	C6 Spinal cord	Pyramid	Peduncle	Internal capsule
N	21	123	99	84
Mean, ms	2.61	1.69	1.15	0.82
Standard deviation	0.65	1.07	0.66	0.44
Range, ms	1.3–5.5	0.7–6.5	0.45–4.0	0.3–2.8

the threshold for the change in excitability of the axon was lower than the threshold for the orthodromic response in the soma. Discharges of two of these cells are shown in Fig. 5. In A1, the antidromic response of one cell to suprathreshold stimulation of the medullary pyramid is shown. The failure of the antidromic response is shown for just subthreshold stimulation in A2, and the response to the same stimulation, conditioned by a previous supramaximal forepaw stimulus, is shown in A3. In A4, the forepaw stimulus strength was reduced to threshold, but the axon still responded to the antidromic stimulus even when the forepaw stimulus failed to evoke a response.

Figure 5B–D shows the same sort of change in excitability in the absence of a response to conditioning stimulus for another PT *m* axon. Column B shows records for excitability testing at the pyramid, column C at the cerebral peduncle, and column D at the internal capsule. Row 4 shows the excitability change without a preceding discharge from the axon; row 3 shows the change with a preceding discharge. For this axon, the threshold for the effect at the cerebral peduncle was the same as that for the response to contralateral forepaw stimulation. Therefore, there is no trace B4. Unfortunately, records were not kept of the total number of axons tested in this way, so it is not possible to give percentage figures. It is clear that some axons, however, did not have to discharge in the 100 ms preceding the test stimulus to undergo a change in excitability.

Antidromic Latency. Antidromic latencies were measured with 0.1-ms accuracy from film records for 123 PT *m* neurons. The results are shown in Table 3. Conduction distances were estimated as the straight line distances between stereotaxic coordinates of the stimulation sites, taking P 8.5, H 11, L 1, and A 27, H 10, L 8 as the coordinates of the pyramid and cortex, respectively. The distance from the pyramid to the peduncle was estimated at 13.9 mm, from the peduncle to the internal capsule at 12.1 mm, and from the internal capsule to the cortex at 16.2 mm. Using the mean latencies of Table 3, velocities of 25.8, 36.8, and 19.7 m/s were calculated for the three axon segments.

The increase in apparent conduction velocity in the axon segment between the cerebral peduncle and the internal capsule was not simply a sampling error created by different-size samples at each site. Reducing the sample of Table 3 to only those 64 axons in which latencies were obtained for all three sites changed the average latencies to 1.58 ± 0.92, 1.15 ± 0.67, and 0.75 ± 0.36 ms, but it did not eliminate the increase in velocity, yielding 32.4 (pyramid to cerebral peduncle), 30.4 (cerebral peduncle to internal capsule), and 21.5 m/s (internal capsule to cortex). Correcting for a utilization time of 0.02 ms changed the internal capsule to cortex velocity to 22.2 m/s but did not change the sense of the data.

Using the same conduction distance estimates, conduction velocities were computed for each of the 64 axons in which latencies were obtained for all three sites. In 50 of the 64 axons, there was an increase in velocity from the segment between the internal capsule and the cortex to that between the peduncle and the internal capsule. On the other hand, only 34 of the 64 axons showed an increase in velocity in the segment between the pyramid and the peduncle over that in the segment between the peduncle and the internal capsule.

DISCUSSION

Nature of the Changes in Excitability. In their original description of the rapid decrease in threshold of PT axons, Mann *et al.* (17) concluded that the phenomenon was best explained in terms of depolarization of terminals of the axons near the site of testing. Other workers accepted the same explanation for similar changes in other corticofugal systems (5, 9, 24). The present research can be taken to indicate that PT *m* axons have branches at several sites along their course to the pyramids (and, in the case of corticospinal axons, to the spinal cord) and they experience both increased and decreased excitability at nearby terminals of these braches at many sites. Or perhaps there is some other explanation for the phenomenon, some process that occurs all along the axon.

The only known process that fits this second situation is that resulting in afterpotentials. Swadlow and coworkers (27, 28) demonstrated, in visual callosal axons, increases in excitability that have a time course similar to those shown in the present study for PT axons. They ascribe these increases to effects of depolarizing afterpotentials because there are no known synaptic junctions on terminals of visual callosal axons. They found decreases in latency of as much as 2.8 ms for the second of two closely spaced antidromically conducted spikes. Axons with conduction velocities in excess of 7 m/s, however, had maximum latency shifts of less than 0.1 ms under these conditions. We found that, in PT *m* axons in the presence of a conditioning stimulus to the contralateral forepaw, there was always a slight decrease in latency of the antidromic response initiated by a just-supra-

threshold test stimulus. This occurred at all test sites at which there was an increase in excitability. The magnitude of this decrease in latency never exceeded 0.2 ms and usually did not exceed 0.1 ms. Similarly decreased latency was also seen for the second of two closely spaced antidromically conducted spikes in PT *m* axons, almost all of which had conduction velocities in excess of 7 m/s.

If depolarizing afterpotentials explain the increase in excitability of PT *m* axons following sensory stimulation, then one is forced to the conclusion that PT *sa* axons do not have such afterpotentials or they are too small to be detected using the techniques of these experiments. Swadlow and coworkers (27, 28), however, showed that the magnitudes of changes in excitability and latency of visual callosal axons were inversely related to conduction velocity. The *sa* subset of PT neurons includes both fast and slow PT cells (29), and some might reasonably be expected to show major changes in excitability. We have never seen any change in excitability of a PT *sa* axon.

It is doubtful that the increased excitability of all PT *m* axons is due to depolarizing afterpotentials. Such afterpotentials might reasonably be expected to occur at all points along the axon. (It would be interesting if they did not!) Yet some axons that experienced a change at the pyramids (Table 1) did not experience a change in excitability at either the internal capsule or the cerebral peduncle or both. Furthermore, at least some PT axons do not have to respond to the conditioning stimulus with a spike for the increase in their excitability to occur (Fig. 5). We have also seen PT axons in which a conditioning stimulus to some site within the central nervous system (e.g., the head of the caudate nucleus) failed to evoke a discharge but still increased the axon's excitability along a time course much like that described here (unpublished observations). Without a previous spike, there cannot have been an afterpotential. For these axons, and perhaps all PT axons, the increased excitability induced by the conditioning stimulus is not the result of afterpotentials.

It is also difficult to see how an hyperpolarizing afterpotential could account for the observed decrease in excitability in some axons. Such afterpotentials can elevate the threshold for excitation, but, because of their small size, they would not be expected to render the axon inexcitable. It appears therefore, that an explanation in terms of presynaptic depolarization is still best for the increased excitability of PT *m* axons. The PT *sa* axons may not experience this increased excitability either because they do not enter the nuclei where the effects occur or because they do not receive presynaptic influences there, or both. The decreased excitability is probably still best explained in terms of strong hyperpolarization on terminals of a short branch or on some nodes of Ranvier or strong depolarization on terminals of a short branch, driving the axon into sodium inactivation (17).

Sites of Action. If the increased excitability of PT *m* axons is to be explained in terms of depolarization on terminals of short branches, then such branches must exist near the testing sites. Branches of PT axons are known to occur in the basal ganglia, ventralis lateralis, ventralis posterolateralis, and centromedianus (2, 6, 13, 25), all sites close to the internal capsule testing site. Similarly, branches of PT axons are known to enter both the red nucleus and the pontine nuclei (1, 6, 11, 15, 19, 22), both sites near the cerebral peduncle. There are also collaterals of PT axons to multiple levels of the spinal cord including the C6 level (26). Clearly, the branches required by explanation in terms of terminal depolarizations exist.

Conduction Velocity and Fiber Size. When it is known that axons branch along their course, it is usually assumed that the axons' diameters and therefore conduction velocities are decreasing along that course. Certainly, this is the case for the primary afferent neurons in the dorsal columns (3, 16). Our estimates indicate that the conduction velocity and presumably also axon diameter increases along the pyramidal tract in spite of the branching. Clough *et al.* (4) reported a similar increase in conduction velocity of the peripheral portion of motor axons in the forelimb of baboons, although these probably do not branch over the measured course. Similar results have also been reported for human peripheral nerve (18). Whether this is a common characteristic of neurons with long axons and with somata in the central nervous system is unknown.

It is also possible that fibers in the pyramidal tract follow a tortuous rather than straight course, leading to an underestimate of the actual conduction distance. Valverde (33) has shown that pyramidal tract axons in rodents follow a very tortuous course, but they seem more likely to do this near termination sites than near the soma. One might reasonably expect the estimated distances to be more inaccurate at the level of the pyramids than at the internal capsule. It is possible to calculate what the conduction distances should be if the conduction velocity were constant along the axon. On the assumption that the average conduction velocity is a constant 32.4 m/s (the average from pyramids to peduncle), the conduction distances were calculated at 13.0 mm from peduncles to internal capsule and 23.6 mm from internal capsule to cortex. When these values are compared with our estimates of 12.1 mm and 16.2 mm, it is clear that our estimate for the internal capsule to cortex distance would have to be insufficient by nearly 50% to explain the apparent difference in velocity in terms of this sort of error. At the same time, the error for the peduncle to internal capsule distance would be less than 7%.

The segment of axon from the internal capsule to the cortex contains a myelinated portion and an unmyelinated portion near the soma. If this unmyelinated portion were inordinately long, then an underestimate in conduction velocity of the myelinated portion would occur. The length of

such a segment of unmyelinated axon can be estimated by assuming that the axis cylinder is of constant diameter in both myelinated and unmyelinated segments. Using the relation $gd = s/t$, where d is the diameter of the axis cylinder, s is the conduction distance, t is the time, and g is a constant of proportionality, we can calculate the diameter of the average axis cylinder at the pyramids. At this site, $t = 0.43$ ms, $s = 13.9$ mm, and, as estimated by Towe and Harding (30), $g = 4.72$ mm/ms/μm. The calculated mean axis cylinder diameter is $d = 6.85$ μm. A constant of proportionality for unmyelinated fibers in peripheral nerve has been estimated at 1.73 mm/ms/μm (21). Using this value and the previous equation, a conduction velocity of 11.85 m/s was calculated for the unmyelinated segment.

The conduction time from the internal capsule to the cortex at 32.4 m/s should be 0.5 ms, leaving 0.73 − 0.50 or 0.23 ms of unaccounted for time. At 11.85 m/s, the spike could traverse 2.7 mm of unmyelinated axon, clearly beyond any initial segment length reported. Therefore, it seems likely that the differences in conduction velocity reported here are real.

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