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Effect of Strychnine on the Primary Evoked Response and on the Corticofugal Reflex Discharge

A. L. TOWE AND M. D. MANN [1]

Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195

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Primary evoked responses and the concomitant corticofugal reflex discharges were recorded from pericruciate cortex and the medullary pyramid in chloralose-anesthetized cats. Strychnine applied directly to postcruciate arm cortex was found to enhance the primary response markedly and to increase the corticofugal reflex discharge evoked by stimulation of the contralateral forepaw, but it had no effect on the responses evoked by stimulation of the other paws. In precruciate arm focus, the strychnine effect followed stimulation of either forepaw, but not either hindpaw. Ablation of the opposite pericruciate cortex abolished the responses to ipsilateral paw stimulation without affecting those to contralateral paw stimulation. The pattern of effects is uniquely associated with the activity of *s* neurons, but not with *m* neurons. The effects on the corticofugal reflex could be accounted for by enhancement of the facilitatory action of *s* neurons onto the *m* neurons of the pyramidal tract. Possible reasons for the specificity of the strychnine action are discussed.

INTRODUCTION

It has long been known that strychnine has a profound influence on the excitability of the mammalian cerebral cortex, the first indications of its action having been published in 1909 by Baglioni and Magnini (2) in connection with a study of the central action of alkaloids in general. At about the same time, Sherrington (22) and Owen and Sherrington (15) examined the action of strychnine on spinal reflexes, and showed that intravenously administered strychnine depresses or even reverses reflex inhibi-

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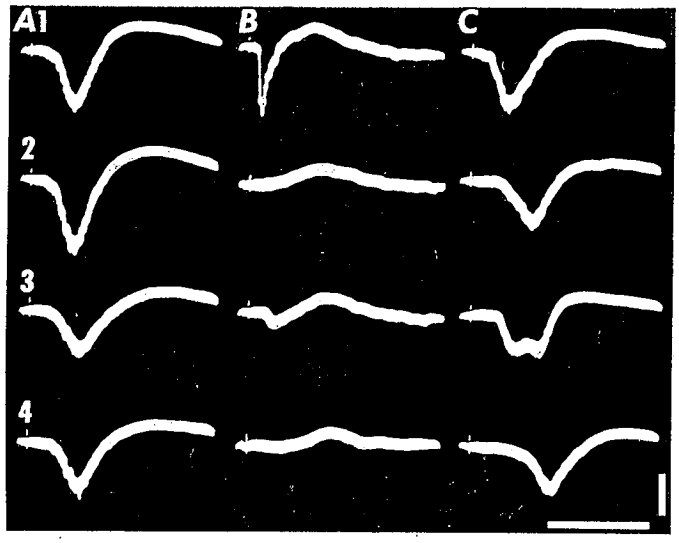

FIG. 1. Responses recorded from brain stem at mid-olivary level after stimulation of CFP (1), IFP (2), CHP (3), and IHP (4) at supramaximal strength. Electrode 6 mm deep to ventral surface records medial reticular responses (A); when withdrawn to 2 mm from ventral surface, medial lemniscus responses (B) are maximal. Just 0.2 mm below the ventral surface, responses in medullary pyramid (C) are seen. Penetration in sagittal plane, 1.5 mm from midline. Time: 50 msec; voltage: 0.5 mv.

tion. However, a full description of the action of this alkaloid on cerebral excitability had to await the development of convenient electrical amplifying and recording equipment. In 1933, Bartley (4) reported that topical application of strychnine enhances the primary evoked response to light and induces the appearance of "spontaneous strychnine spikes" in the treated tissue. It soon became evident that as the action of strychnine develops and then slowly subsides, not only the size but also the configuration of the primary evoked response and of the spontaneous strychnine spike gradually changes. Bartley (4) and Dusser de Barenne, Marshall, Nims, and Stone (9) thought of this effect as an increase in the synchrony of discharge among the cerebral neurons, and Chang (7) ascribed this increased synchrony to an acceleration of the spike initiation process. However, it is now known that strychnine markedly enhances the excitatory postsynaptic potentials (EPSP) of at least some cerebral neurons, causing them to discharge with a high-frequency burst of spikes [2] (13, 21). What

[2] This enhancement has the indirect effect of slightly decreasing spike latency and thereby slightly decreasing the dispersion of spike activity among cerebral neurons. Thus, Bartley (4), Dusser de Barenne *et al.* (9), and Chang (7) were basically correct in their thinking.

happens to postsynaptic hyperpolarizing potentials is less clear: intracellular recordings show that hyperpolarizing responses are depressed or even reversed in the presence of strychnine (17, 21, 23), whereas indirect assessment of excitability often reveals no change (5, 8, 12). The increase in membrane conductance associated with hyperpolarizing responses is normal in some cells but is abnormally large in others in the presence of strychnine (18); the same is presumably true during the depolarizing responses. It thus seems likely that the changes in size and shape of both spontaneous and evoked cerebral potentials may be ascribed to the growth and diminution of postsynaptic membrane responses as a consequence of the alterations in membrane conductance on some cells.

It has long been believed that the primary evoked response represents the sum of the postsynaptic membrane responses on neurons in the neighborhood of the recording electrode. In particular, it is the neurons of layers II and III that contribute in a major way to the primary evoked response (1, 24). These neurons have small excitatory receptive fields, and behave in the same manner under different anesthetic states and in the intact, waking, drowsy or sleeping cat (3). When strychnine is applied to the surface of the cerebral cortex, causing the primary evoked response to increase rapidly in size, the effect may be due to alteration of the excitability of these superficial neurons, or of the apical dendritic arborizations of the deeper neurons, or both. It was the intent of the present study to examine this issue in a region of cerebral cortex where several different functional neuronal types could be identified. Of the several ways of approaching

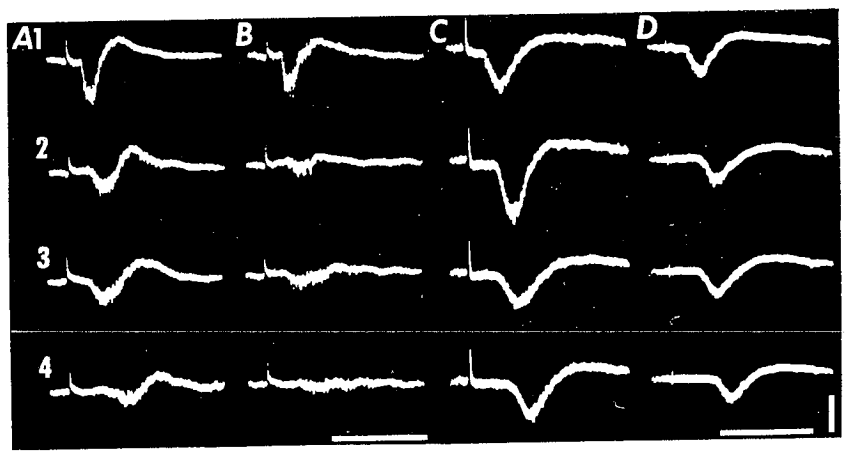

FIG. 2. Responses recorded from medullary pyramid in two animals before (A, C) and after (B, D) exposure of the anterior cerebral cortex ipsilateral to recording site. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength. Time: 50 msec; voltage: 0.5 mv.

this issue, this paper presents the results of one in which the primary evoked response is used as an indicator of postsynaptic membrane responses and the corticofugal reflex (16) is used as an indicator of the discharge of pyramidal tract neurons.

METHODS

Twenty cats, anesthetized with alpha chloralose (50–60 mg/kg, ip) and paralyzed with decamethonium bromide (1 ml/40 min, iv), were used in this study. They were respirated artificially at 17–19 strokes/min, 25–38 ml/stroke, and were heated through an abdominal d-c heating pad under servocontrol via a rectal thermocouple. The medullary pyramids were exposed through a ventral approach, removing basioccipital bone and reflecting the dura mater. One or both anterior cerebral hemispheres were exposed, and immediately covered with polyethylene sheeting or protected with a cap of bone wax, formed to replace the bony covering.

Bipolar needle electrodes were inserted into the central footpad of each limb; square stimulus pulses of 0.1-msec duration and variable amplitude

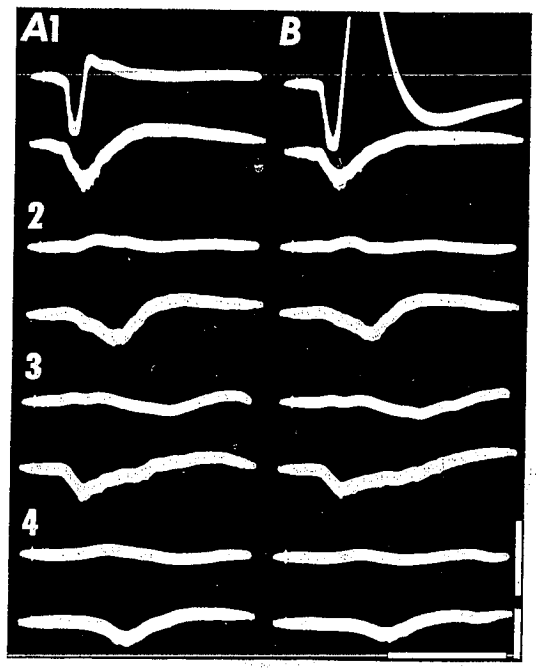

FIG. 3. Responses from postcruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A) and 1 min after (B) topical strychnine treatment for 30 sec. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength. Time: 50 msec; voltages: 2 mv (upper) and 0.5 mv (lower).

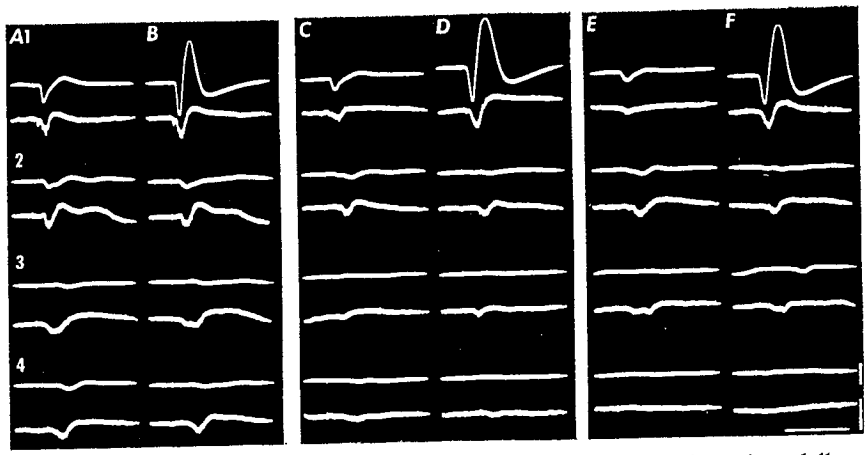

FIG. 4. Responses from postcruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A, C, E) and 1–3 min after (B, D, F) topical strychnine treatment for 30 sec. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength (A, B), median *sa* neuron threshold strength (C, D) and median *m* neuron threshold strength (E, F). Note small medial lemniscal component in records from pyramid after CFP stimulation (row 1, records A through D). Time: 100 msec; voltages: 2 mv (upper) and 0.4 mv (lower).

were applied via these leads. A blunt silver wire electrode was used for recording the primary evoked response from the surface of the cerebral cortex. In order to remove any vestige of strychnine, the electrode was thoroughly washed whenever the recording site was changed. A sharp tungsten wire electrode, insulated except for an 0.3-mm length of the tip, was inserted into the medullary pyramid ipsilateral to the site of cerebral recording. This produced a good killed-end recording of the corticofugal reflex discharge.

A 2% strychnine sulfate solution was used throughout this study. In a few experiments, a strychnine-soaked cotton pellet was touched to the exposed tip of the silver wire recording electrode, and the electrode was then placed on the cerebral surface. In most experiments, a drop of the strychnine solution was placed onto a glass surface, and a 1.5 × 1.5-mm square of paper towel was set in the drop. When thoroughly soaked, the square was placed on the cerebral surface for 30–40 sec. It was then removed, the recording electrode was replaced on the treated area and recording was immediately resumed. All times were referenced to the moment of contact of the strychnine-soaked square to the cerebral surface.

In several experiments, the cortex of the opposite anterior cerebral hemisphere was ablated by suction. The ablation included both sigmoid gyri, into the depths of the cruciate sulcus and along the mesial surface. The extent of the lesion was evaluated by visual inspection during the experi-

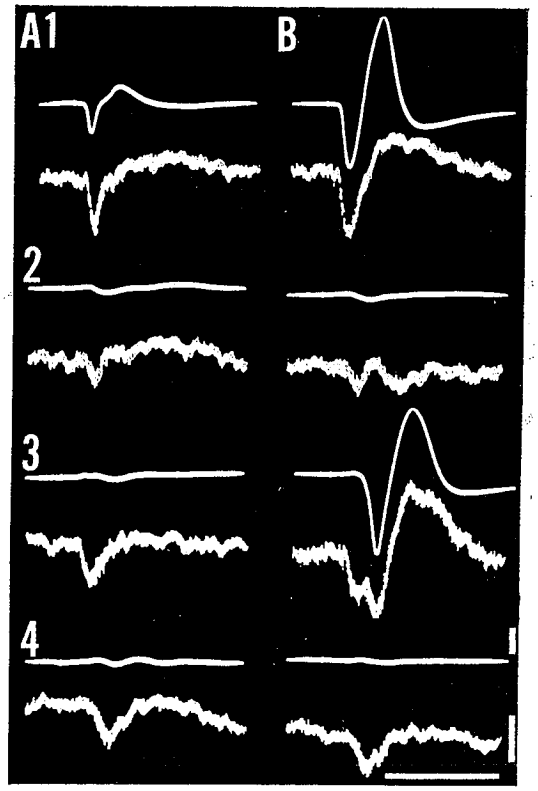

FIG. 5. Responses from postcruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A) and 1 min after (B) topical strychnine treatment for 30 sec. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal strength. Time: 50 msec; voltages: 2 mv (upper) and 0.2 mv (lower).

ment; histological reconstruction was not considered essential for the interpretation of the results.

Recording was done via two Grass P5 amplifiers, each set at 1.5-Hz and 30-kHz half-amplitude frequency response. The output of the P5 amplifiers was led into the d-c amplifiers of a Tektronix 502A dual-beam oscilloscope for photographic recording.

RESULTS

A primary measure in this study was the output of pyramidal tract neurons, as reflected in killed-end recording from the medullary pyramid in response to cutaneous stimulation. This is the well-known corticofugal reflex discharge (16), which has two not-so-well-known properties that

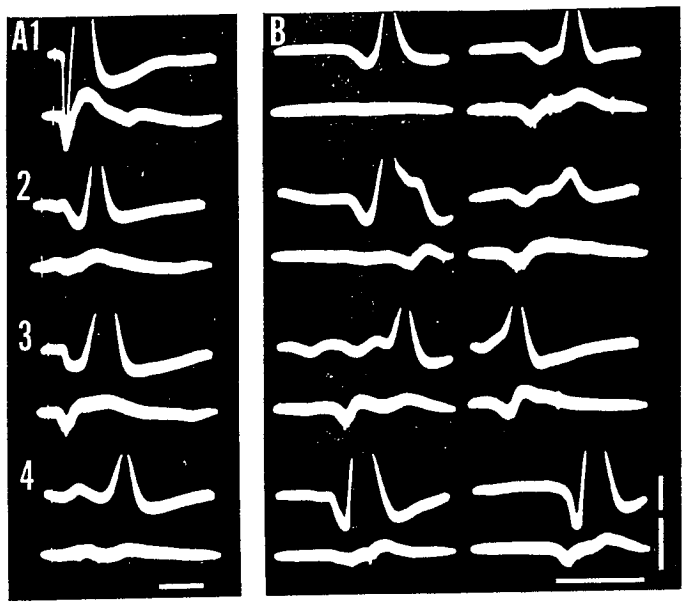

FIG. 6. Records from postcruciate arm cortex (upper traces) and medullary pyramid (lower traces) after copious strychnine application to cerebral surface. Evoked responses (A) after CFP (1), IFP (2), CHP (3), IHP (4) stimulation 1/sec at supramaximal strength, showing late "off focus" strychnine spikes. Samples of spontaneous strychnine spike (B), showing variations in configuration and also variations in relation to corticofugal discharge. Time: 50 msec; voltages: 2 mv (upper) and 0.5 mv (lower).

are of importance to this and many other studies. These are its marked similarity to concomitant events in the brain stem and its marked fragility.

Pyramidal Tract Recording Site. Because the corticofugal reflex discharge can be confused with a related response in the ventromedial reticular region of the medulla, special attention must be taken to verify the site of recording. When a gross electrode is inserted several millimeters into the medulla via the ventral surface and is then slowly withdrawn, the sequence of responses shown in Fig. 1 may be obtained. Deep (dorsal) to the medial lemniscus, the responses of the ventromedial reticular region may be seen (Fig. 1, column A). They look very much like the responses of the pyramidal tract (Fig. 1, column C), recorded just superficial (ventral) to the medial lemniscus, making confusion of one with the other possible. The characteristic patterns of response latencies in the two regions when all four limbs are stimulated reveal the site of recording. However, the unique response pattern of the medial lemniscus—the short-latency, short-duration volley evoked by contralateral forepaw stimulation, contrasted with the near lack of response to stimulation of the other limbs

(Fig. 1, column B)—forms a distinctive boundary between the pyramidal tract and reticular responses, and constitutes a reliable indicator in verifying the site of recording.

Fragility of Corticofugal Reflex. The mere act of exposing the sigmoid gyri for recording often results in a marked decrease in size of the corticofugal reflex discharge. The decrease in reflex size occurs rapidly as the cranium is opened and the dura mater is resected; however, the reflex size remains fairly stable throughout an experiment, and occasionally even regains some amplitude. As illustrated in Fig. 2A and B, the effect is usually much less marked when the "on focus" skin site—contralateral forepaw, in this case—is stimulated than when "off focus" sites are stimu-

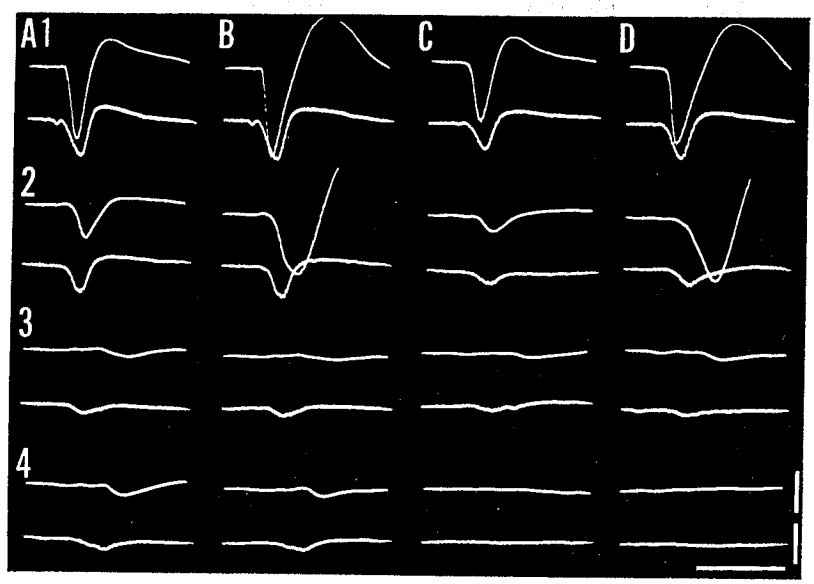

FIG. 7. Responses from precruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A, C) and 1–2 min after (B, D) topical strychnine treatment for 40 sec. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength (A, B) and median s_a neuron threshold strength (C, D). Time: 50 msec; voltages: 2 mv (upper) and 0.4 mv (lower).

lated. Occasionally, the effect was slight and more uniformly distributed, as shown in Fig. 2C and D; animals showing this latter condition could be effectively used in this experiment.

The abruptness of the change in reflex size disqualifies both heat loss and dehydration as major factors; a more likely factor is the abrupt circulatory change that occurs as cerebrospinal fluid pressure is reduced and fluid drains through the basioccipital exposure, allowing the brain to

settle to the floor of the brain case. Consistent with this idea is the observation that the decrease in reflex size occurs in *both* pyramidal tracts when only one side of the cerebrum is exposed. The effect may be minimized by rotating the preparation such that the brain comes to rest against the side of the brain case.

Postcruciate Cortex. The forepaw focus of the cat extends as a band of tissue all the way from the coronal sulcus rostromedially to the lateral third of the cruciate sulcus and anterior to that sulcus (29). Single-neuron studies reveal that evoked neuron activity is equally intense along this band, though the timing of the neuronal activity varies (29, 30). In order to compare the activity of a known set of cerebral neurons—the pyramidal

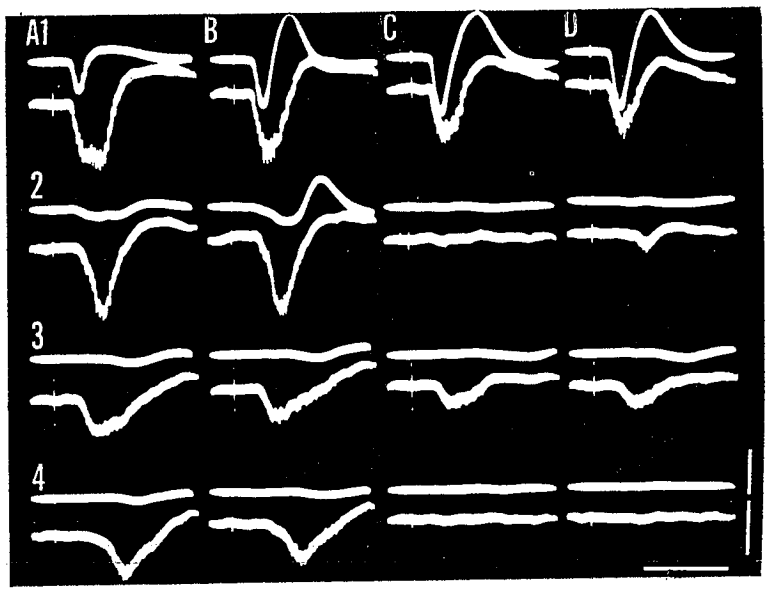

FIG. 8. Responses from precruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A) and 3 min after (B) strychnine treatment for 40 sec, immediately after ablation of the opposite pericruciate cortex (C) and 15 min after the ablation (D). Four paws (CFP : 1, IFP : 2, CHP : 3, IHP : 4) stimulated 1/sec at supramaximal stimulus strength. Time: 50 msec; voltages: 2 mv (upper) and 0.5 mv (lower).

tract (PT) neurons—with the surface primary evoked responses, recordings were confined to area 4γ (10), on either side of the lateral third of the cruciate sulcus. Figure 3A shows the responses commonly seen caudal to the sulcus (upper trace of each pair), along with the associated corticofugal reflex discharges (lower trace of each pair). Within 90 sec after the start of a 30-sec topical strychnine treatment, the effect shown in Fig. 3B

develops. Aside from the large strychnine spike evoked by contralateral forepaw (CFP) stimulation, no change is apparent. This circumstance continues throughout the strychnine effect—as the CFP-evoked strychnine spike grows, changes configuration, and finally disappears about 1 hr after the strychnine treatment. Other than the exceptions outlined below, this result was obtained in all animals tested.

Occasionally, in response to supramaximal stimulation, the corticofugal reflex discharge to CFP stimulation increased slightly, with no change in the reflex discharges to off-focus stimulation (Fig. 4A and B). The application of strychnine to a limited portion of the tissue from which the corticofugal reflex discharge originates, coupled with supramaximal stimulation, may have made detection of an effect on PT neurons difficult. For this reason, tests were carried out at near-threshold stimulus strengths.[3] As shown in Fig. 4C through F, the corticofugal reflex evoked by weak CFP stimulation was enhanced by strychnine. However, at no stimulus strength could any effect be detected when off-focus paws were stimulated.

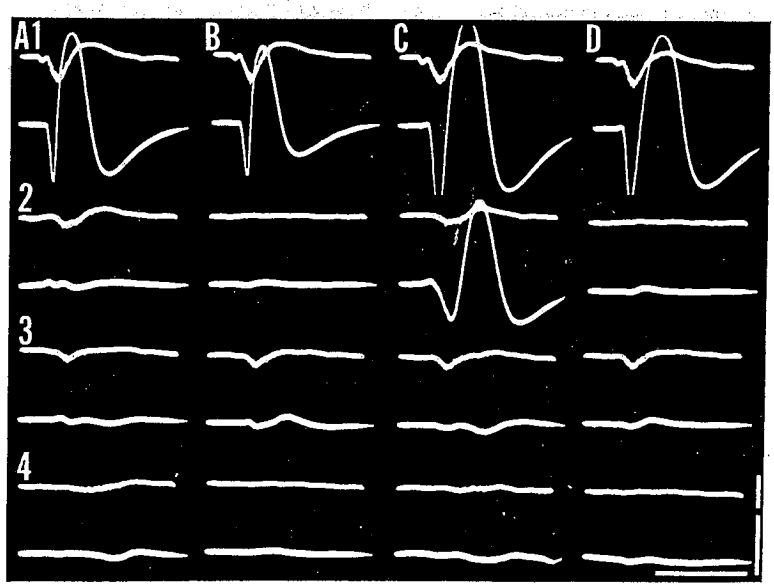

FIG. 9. Records from medullary pyramid (upper traces) and both postcruciate (A, B) and precruciate (C, D) arm cortices (lower traces) after strychnine treatment for 40 sec to both sites, just before (A, C) and immediately after (B, D) ablation of the opposite pericruciate cortex. Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength. Time: 50 msec; voltages: 0.5 mv (upper) and 2 mv (lower).

[3] Near-threshold stimulus strength refers to the corticofugal reflex, and hence to the thresholds of PT neurons to cutaneous stimulation.

Furthermore, at no time during the 1-hr effectiveness of the strychnine treatment was this picture seen to change.

If a larger amount of strychnine was applied, the above picture could be altered by the appearance of a strychnine spike and a corticofugal discharge to off-focus stimulation. Often, as shown in Fig. 5, the effect was confined to the contralateral inputs, but ipsilateral inputs could be made effective by the addition of yet more strychnine. Intravenous administration of strychnine yielded a similar picture, though the cortex became so active that the effect was difficult to follow. In every instance, however, the strychnine spike developed *after* the normal off-focus response had begun—in sharp contrast to the on-focus strychnine spike, which develops as an enhancement of the primary response itself. When a corticofugal discharge accompanied the late, off-focus strychnine spike, it occurred as a late, second burst of activity. In some cases, no corticofugal discharges accompanied these late, off-focus strychnine spikes. One such case is shown in Fig. 6A, as it appeared after several drops of strychnine had been applied. In this same preparation, as shown in Fig. 6B, spontaneous

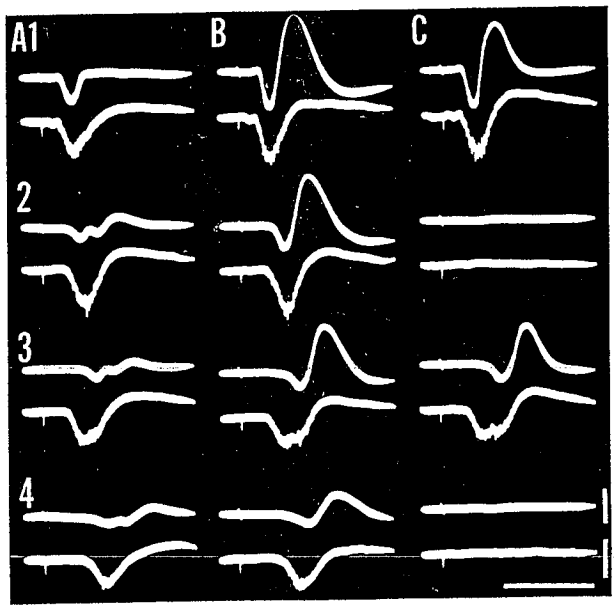

FIG. 10. Records from precruciate arm cortex (upper traces) and medullary pyramid (lower traces) before (A) and 3 min after (B) copious strychnine treatment, yielding late "off focus" strychnine spikes from hind limbs (3, 4). Responses evoked by ipsilateral stimulation (2, 4) abolished immediately after ablation of the opposite pericruciate cortex (C). Four paws (CFP: 1, IFP: 2, CHP: 3, IHP: 4) stimulated 1/sec at supramaximal stimulus strength. Time: 50 msec; voltages: 2 mv (upper) and 0.4 mv (lower).

strychnine spikes occurred without any accompanying corticofugal discharge, or they occurred before, during, or after such discharges. The temporal coupling between the two events was poor. In many preparations, the two events showed closer temporal coupling, though in no instance was the phasing between the two invariant.

Precruciate Cortex. The effects of topical strychnine on the evoked potentials that can be recorded just anterior to the cruciate sulcus, along the lateral third of its length, differ from those outlined above. The usual pattern of effects is shown in Fig. 7, where it is evident that strychnine enhances the surface responses to stimulation of either forelimb, whereas the hind limb responses remain unaffected. As with the strychnine effect in postcruciate tissue, that in precruciate tissue retained full effectiveness as the stimulus strength was reduced to near-threshold values (Fig. 7C and D). However, the strychnine spike induced by IFP stimulation often failed at two to four times threshold strength, a normal off-focus response appearing instead. The corticofugal reflex discharge evoked by CFP and by IFP stimulation increased slightly after the strychnine treatment, though it usually became more evident as the stimulus strength was reduced.

The idea that *sb* neurons—those neurons of precruciate arm cortex that respond to stimulation of either forepaw but neither hindpaw (14)—might be involved in the IFP-induced strychnine spike, and the knowledge that *sb* neurons depend upon the integrity of the opposite cerebral hemisphere for their responsiveness to ipsilateral cutaneous stimulation (30), suggested that removal of the opposite pericruciate cortex might eliminate the IFP-induced strychnine spike while sparing that induced by CFP stimulation. This expectation was met in every preparation tested. However, as shown in Fig. 8, not only did the surface response almost disappear—the corticofugal reflex discharge vanished. As shown in Fig. 8D, partial recovery occurred after some time, but full recovery was never observed. This diaschesis-like effect was confined to the ipsilateral inputs and was minimal or absent to contralateral inputs. Figure 9 dramatizes the difference between postcruciate (Fig. 9A and B) and precruciate (Fig. 9C and D) tissue (corticofugal reflex is upper trace of each pair). The CFP-induced strychnine spikes of Fig. 9A and C are slightly diminished after ablation of the opposite pericruciate cortex; in most preparations, they are unaffected. The IFP-induced strychnine spike of the precruciate cortex (Fig. 9C), absent in strychninized postcruciate cortex (Fig. 9A), is abolished by the ablation. The corticofugal reflex discharges to ipsilateral stimulation disappear, whereas to contralateral stimulation they remain unchanged.

When a large dose of strychnine is applied to precruciate cortex, strychnine spikes may be evoked by stimulation of any paw. As shown in Fig. 10, those induced by hind-paw stimulation may not attain full size;

nonetheless, they appear reliably to each stimulus (1/sec iterative rate). Furthermore, after ablation of the opposite pericruciate cortex, the strychnine spike to CHP stimulation continues unchanged (Fig. 10C). If the iterative stimulus rate is reduced to once every 3 sec, strychnine spikes may appear to some ipsilateral stimuli. Their appearance is irregular, 20–40 msec later than those seen regularly just prior to the ablation.

Interaction Effects. By triggering the master timer from each spontaneous strychnine spike, and stimulating CFP at variable times after the spike, it was possible to make observations on the course of recovery of the primary evoked response and of the CFP-evoked strychnine spike. In the presence of strychnine, both the spontaneous strychnine spike: CFP and CFP-evoked strychnine spike: CFP interactions followed the same time course as a normal CFP: CFP interaction for about 300 msec, after which time the CFP-evoked strychnine spike reappeared to the testing stimulus. In one preparation, it reappeared suddenly and almost full blown, whereas in two preparations, it was graded in size as a function of the C: T interval. The period of facilitation normally seen in a CFP: CFP interaction was masked by the strychnine spike.

Response to Sound. A medium-sized corticofugal reflex discharge can be evoked by hand claps or light flashes. Using hand claps, it was determined that no strychnine spike occurs on either side of the cruciate sulcus in direct association with the reflex. However, after some hand claps, a much later strychnine spike occurred, accompanied by a small corticofugal discharge. These late and sporadic strychnine spikes were observed on either side of the cruciate sulcus, depending on the site of strychnine application, and showed a variable latency (30–70 msec) when measured from the hand clap. The clap-evoked corticofugal reflex, on the other hand, appeared consistently with a latency of 20–22 msec, leaving no doubt about its origin.

DISCUSSION

The question posed in this study has been answered, to the effect that topically applied strychnine in an amount just sufficient to induce a strychnine spike acts primarily, if not exclusively, upon the superficial neurons (layers II and III) of the cerebral cortex, and has an indirect effect on some corticofugal neurons. That this conclusion is true can be understood by examination of Tables 2 and 3, which summarize the results of these experiments, and by recalling a few salient facts already known about the pericruciate cortex. First, however, it is necessary to review some definitions that may be used to separate cerebral neurons into distinct sets—definitions that contain no histological prejudice.

It has become customary to classify neurons primarily in terms of the size of their excitatory receptive fields, a procedure which has been shown

TABLE 1

DEFINITION OF NEURONAL RESPONSE TYPES (column) BY RESPONSIVENESS TO LIMB STIMULATION (row)

	CF	IF	CH	IH
sa	1	0	0	0
sb	1	1	0	0
sc	1	0	1	0
m	1	1	1	1

to impose maximum order [4] on all other neuronal response properties (25). A rough estimate of excitatory receptive field size can be obtained from electrical stimulation of the central footpad of each of the four limbs; the resulting patterns of responsiveness yield four major response types, termed *sa*, *sb*, *sc*, and *m* (11, 25, 29, 30). The definitions for each of these types, with reference to forelimb cortex, is shown in Table 1; an entry of 1 indicates a response probability equal to or greater than 0.5, and an entry of 0 indicates a response probability of zero. The *sc* neurons do not concentrate in pericruciate cortex, and hence do not enter into the arguments presented here. The other possible response types, not shown in Table 1, are practically nonexistent in normal, chloralose-anesthetized cats. [Note also that the *sa* category in Table 1 is the same as the *s* category used in the past (11, 25, 28–30). This change is made to improve the specificity of the terminology, and to allow reference to the *s* category in general, which includes the *sa*, *sb*, and *sc* types. We will hereafter adhere to this new and more specific terminology.]

The salient facts about pericruciate cortex are the following: (a) In an extracellular microelectrode sample, *s* and *m* neurons turn up in about equal numbers (29). (b) In postcruciate cortex, the *s* category is occupied almost exclusively by *sa* neurons (29), with some *sc* and a few *sb* neurons. (c) In precruciate cortex, the *s* category is shared about equally by *sa* and *sb* neurons (14, 29). (d) About half of the *m* neurons send their axons into the medullary pyramids, to contribute to the corticofugal reflex discharge (27, 29). (e) Neurons of the *s* category, and especially those of the *sa* type, are preferentially isolated by a microelectrode in layers II and III of the cortex (25, 28, 29). (f) Neurons of the *m* category are preferentially isolated by a microelectrode in deep layer III through layer VI.

With these facts in mind, we can see from Table 2 that a strychnine spike occurs only when *sa* neurons are activated in postcruciate cortex; no strychnine spike occurs when only *m* neurons are activated. From

[4] This is single-variable order; the addition of other variables to the criteria of classification imposes greater order.

TABLE 2

PATTERN OF NEURON RESPONSE AND STRYCHNINE EFFECT IN POSTCRUCIATE CORTEX EVOKED BY CUTANEOUS STIMULATION

Input	Neurons responding	Primary response	Strychnine effect	Effect on corticofugal reflex	
				Strong S	Weak S
CFP	*sa*, *m*	Large	Strong	None	Strong
IFP	*m*	Small	None	None	None
CHP	*m*	Small	None	None	None
IHP	*m*	Small	None	None	None

Table 3, we can see that a strychnine spike occurs when the *sa* plus *sb* neurons, or the *sb* neurons, are activated; again, no strychnine spike occurs when only *m* neurons are activated. The strychnine spike is usually smaller when the *sb* neurons are activated in isolation from the *sa* neurons, that is, after IFP stimulation.

The *m* neurons respond to stimulation of each of the four limbs, and they respond in much the same manner to each of these inputs in non-strychninized cats (14, 25, 27, 29). Therefore, if they are involved in the production of the strychnine spike, then the action of strychnine is either limited to the presynaptic terminals that are activated by CFP stimulation (postcruciate) or by both CFP and IFP stimulation (precruciate), or it is limited to the region of postsynaptic membrane to which these terminals synapse. There is no other way of accounting for the association found in this experiment and shown in Tables 2 and 3. This explanation is so unlikely and the explanation in terms of *s* neurons is so attractive that we reject the idea that *m* neurons are significantly involved in the production

TABLE 3

PATTERN OF NEURON RESPONSE AND STRYCHNINE EFFECT IN PRECRUCIATE CORTEX EVOKED BY CUTANEOUS STIMULATION

Input	Neurons responding	Primary response	Strychnine effect	Effect on corticofugal reflex	
				Strong S	Weak S
CFP	*sa*, *sb*, *m*	Large	Strong	None	Strong
IFP	*sb*, *m*	Medium	Moderate	None	Moderate
CHP	*m*	Small	None	None	None
IHP	*m*	Small	None	None	None

of the strychnine spike that results from moderate topical application of strychnine. Massive doses of this alkaloid, by either topical or intravenous routes, may create a different effect. However, the strong interference by spontaneous strychnine spikes in such circumstances, coupled with a lack of certainty concerning the site of action of intravenously administered strychnine, precludes a clear analysis.

Corticofugal Reflex. At strong stimulus strengths there is usually no detectable change in the corticofugal reflex discharge. This is a bit surprising, inasmuch as some pyramidal tract neurons belong to the *s* category, and might thus be expected to show an effect. Perhaps they are too deep in the cortex. On the other hand, weakening the cutaneous stimulus revealed a clear enhancement of the corticofugal reflex discharge. As summarized in Tables 2 and 3, this enhancement appeared only when *s* neurons were also activated by the stimulus; no enhancement was detectable in response to inputs that fail to activate *s* neurons in the strychnine-treated site. On the basis of the finding that *s*a neurons in postcruciate tissue exert a facilitatory action on the *m* neurons in their immediate neighborhood (19), and that a similar effect seems to hold for the *s*a and *s*b neurons in precruciate tissue (14), this result was expected. The evoked activity of the *s* neurons, enhanced by the action of strychnine, results in a stronger facilitation of the *m* neurons—and of *m* neurons of the PT in particular. This would result in a larger corticofugal reflex discharge from the strychnine-treated tissue, which could only become manifest at weak stimulus intensities, when it is not masked by intense corticofugal discharge from nontreated tissue. This result is to be expected, whether or not the strychnine has any effect on the PT, *m* neurons.

The foregoing pattern of effects is in complete accord with this explanation, but the range is not. Statistically, *s* neurons have higher thresholds to cutaneous input than the *m* neurons (14, 29), and the great majority of the PT neurons belong to the *m* category. Thus as the CFP stimulus strength was reduced, and the number of responding *s* neurons thereby diminished, the specific enhancement of the corticofugal reflex discharge should also have diminished. Most often, it did not. The most likely reason is that the thresholds of the cerebral *s* neurons were reduced by the strychnine, thereby extending the effective range of their facilitatory action on *m* neurons of the PT, to very weak cutaneous stimulus strengths. An examination of the thresholds of cerebral *s* neurons before and after strychnine treatment shows that this indeed occurs (Mann and Towe, unpublished observations).

Two reservations must be kept in mind when evaluating the foregoing arguments. Because of their large axons and their characteristic burst discharges, the large, deep-lying PT neurons make a major contribution

to the corticofugal reflex discharge. The more numerous superficial PT neurons, because of their small axons and brief discharge, may go almost undetected in the corticofugal discharge. Thus, this indicator strongly favors the large, deep-lying PT neurons. When the cerebral cortex is exposed and the corticofugal reflex suddenly diminishes in size, it is unclear whether the large PT neurons are preferentially affected. Strychnine could conceivably bring the affected neurons back into the pool of responding neurons, thereby confounding the interpretation. This did not seem to occur in response to topically applied strychnine, though it may easily have been involved in the profound response to intravenous administration of strychnine.

Route of Entry. The deduction that neurons of the *s* category—*s*a and *s*b neurons—are primarily if not exclusively responsible for the effects that follow brief topical application of strychnine suggests that proximity to the site of strychnine application is of prime importance. The work of Ramon-Moliner (19, 20) on cortex in the posterior lip of the cruciate sulcus shows that the neurons of layer II contribute overwhelmingly to the apical dendritic arborizations in layer I, and that these same neurons crowd together in double the density found in any other cortical layer. The first significant concentration of *m* neurons occurs deep in layer III, between 0.7 and 1 mm below the pial surface (29); calculation from our existing data base, after correcting for electrode sampling bias (26), suggests that layer III *m* neurons reside about three times as far from the pial surface as the average *s*a neuron, and represent only 15% of the neurons that reside within one millimeter of the pial surface. It is, thus, not surprising that these *m* neurons show no direct strychnine effect at the doses employed in this study. Only the enhanced facilitatory action of *s* neurons onto *m* neurons could be detected through the measures employed in this study. In all probability, strychnine readily enters any of the dendritic arborizations in layer I; whether it does so in its charged state is unknown. However, with a significant fraction of the strychnine molecules being positively charged, they would not only diffuse down the apical dendrite, but would also be mildly iontophoresed in the electrical field of the EPSP. By either route, the most superficial *s* neurons would preferentially "collect" the strychnine and be most powerfully affected by it. By leaving the strychnine-soaked pledget in place for many minutes, enough strychnine might be made available to ultimately affect the deeper lying *m* neurons.

It was found that after the addition of more strychnine to the cortex, a strychnine spike could be produced by stimulation of any limb. In this situation, the strychnine spike to off-focus stimulation [5] began 10–30 msec

[5] The term "off focus" here refers to those inputs which fail to activate any *s* neurons in the strychninized tissue, be they *s*a, *s*b, or *s*c neurons.

after the normal off-focus primary response had begun, and was accompanied by additional corticofugal activity. Its latency varied markedly on repeated trials, though its amplitude and configuration were more constant. It is highly probable that these events result from indirect activation of *s* neurons, which in turn reactivate some *m* neurons; an answer to this question awaits further study. Certainly the erratic behavior of corticofugal discharge in relation to spontaneous strychnine spikes makes it difficult to explain such spikes on the basis of PT neuron activity. Further, the absence of a strychnine spike in conjunction with the corticofugal reflex discharge evoked by hand clap, even in precruciate cortex where the responding neurons reside (6), dissociates PT neuron discharge from the strychnine spike. When a strychnine spike does follow some hand clap, it is of longer latency and may or may not be accompanied by corticofugal activity.

In summary, the effects of topically applied strychnine sulfate are uniquely associated with the activity of *s* neurons, but not with *m* neurons. The specific effects on the discharge of pyramidal tract *m* neurons can be ascribed to enhancement of the known facilitatory action of *s* neurons onto *m* neurons. The effects thus far observed could be a simple consequence of a change in membrane conductance, such as would occur by blocking potassium channels. These effects are effectively restricted to neurons of the *s* category because such neurons are concentrated in large numbers right under the site of strychnine application.

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