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Ontogenetic Studies of the Primary Evoked Response and Strychnine Spike in Motor–Sensory Cortex of Kittens

M. D. MANN AND K. A. FOLLETT[1]

Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska 68105

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The development of the primary evoked potential in the motor–sensory cerebral cortex and the corticofugal reflex discharge in the medullary pyramids were studied in the first 2 weeks of postnatal life in kittens. In α-chloralose-anesthetized, paralyzed kittens at 1 day of age, the primary evoked potential produced by electrical stimulation of the contralateral forepaw was usually a purely negative response of 50-ms latency, poor frequency-following capability, and slow time course. During the first 2 weeks, the response became positive–negative as the latency shortened to 24 ms. During the same period, the frequency-following capability improved and the duration of the potential decreased. The cortical responses to stimulation of the other three paws were smaller, longer-latency negative potentials, but reliably evoked even in the youngest animals. The corticofugal reflex was not seen in any animal less than 14 days of age and was seldom seen in 14-day-old kittens. Topical application of strychnine enhanced the amplitude of the cortical response to stimulation of the contralateral forepaw, but had no effect on responses to stimulation of the other three paws. No effect on response latency was seen. The strychnine effect developed in about 15 s as in adults, but lasted two to four times longer. Both positive and negative components of the primary response were enhanced by strychnine, and, in animals that had no positivity in control responses, one was induced, remaining in the record for at least 11 h. As far as can be determined from these studies, the effect of topical strychnine is the same in neonatal and adult cat cerebral cortex.

INTRODUCTION

Strychnine, applied topically to the postcruciate forepaw cortex of adult cats, enhances the discharges of small-field or *s*a neurons as deduced from

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the enhanced surface-recorded primary evoked potential [due primarily to activity in *s*a neurons, (26)] and from the increased discharges in single *s*a neurons (12, 27). In this tissue, *s*a neurons are those that respond only to stimulation of the contralateral forepaw. The increase in the primary evoked potential is seen only in response to stimulation of the contralateral forepaw; no change is seen in responses to stimulation of the other three paws. Similarly, strychnine enhances the corticofugal reflex discharge recorded from the ipsilateral (with respect to the cortical site) medullary pyramids when the discharge is evoked by contralateral forepaw stimulation, but not when it is evoked by stimulation of any of the other three paws. This discharge is due primarily to activity in wide-field or *m* neurons which respond to stimulation of all four paws. Enhancement of the corticofugal reflex may be ascribed to the known facilitation of *m* neurons by *s*a neurons (29) which is increased when the *s*a neuron discharge is enhanced by strychnine. Thus, the effect of strychnine on *m* neurons is only indirect via *s*a neurons.

The influence of strychnine on *s*a neurons may result from a direct effect of the alkaloid on their membranes (12, 27) and the absence of a direct effect on *m* neurons may result from either their greater distance from the site of application or from some intrinsic difference in the membranes of *s*a and *m* neurons. On the other hand, the influence of strychnine on *s*a neurons may also be indirect, as suggested recently by Towe *et al.* (28). Thus, the strychnine effect may be exerted upon terminals of thalamocortical afferent fibers, setting up bursts of orthodromic and antidromic spikes that lead to production of the paroxysmal depolarization shift (7, 16, 24). The effect on *s*a neurons would be synaptic and therefore indirect. An influence on thalamocortical fiber terminals easily explains more of the effects of strychnine on both evoked responses and single units than direct effects on *s*a neurons and is therefore the more likely explanation at this time.

The differential effects of strychnine on sets of neurons in the cortex could be used as a test of the hypothesis that *s*a neurons develop later than *m* neurons in ontogeny. This hypothesis is derived from observations in the mouse, rat, hamster, rabbit, and cat that neurons destined for adult layers II and III (i) result from later mitotic activity of cells in the ventricular zone than do those in layers V and VI, (ii) migrate into the outer layers of cortex after layers V and VI have formed, and (iii) mature after those in deeper layers (2, 3, 5, 13, 14, 17, 25). These conclusions are based on studies of Golgi-stained sections and studies of [^{3}H]thymidine-labeled tissues. In the adult postcruciate cortex, *s*a neurons are concentrated in layers II and III, whereas *m* neurons are concentrated in layers V and VI and deep layer III. [A more detailed discussion of the hypothesis may be found

in Mann (11).] Supposing that layers II and III do not form until birth or shortly thereafter in cats, Mann (11) deduced that *m* neurons should be found in the immediate postnatal period, *s*a neurons not becoming active until later.

There exists no direct evidence regarding the action of strychnine on immature animals; however, differences in penicillin effects on immature and mature neurons appear to be mainly due to differences in excitability that are also characteristic of untreated neurons (19). If the action of strychnine on neurons in the immature cortex is similar to that in adults, i.e., if it affects the same neurons or parts of neurons in the same way, and if the hypothesis is correct, then topical strychnine should cause no strychnine spike and no enhancement of the corticofugal reflex discharge in newborn kittens. Alternatively, the strychnine spike should occur after stimulation of any paw and there should be an enhancement of the corticofugal reflex discharge evoked by stimulation of any paw. The first result would be expected if strychnine acts on the specific thalamocortical axons that influence *s*a neurons (28) or if the differential effects are due to different properties of membranes in *s*a and *m* neurons. The second result would be expected if the differential effects depend on proximity to the surface.

In our experiments, the development of the primary evoked potential and the corticofugal reflex discharge were studied in kittens in the first 2 weeks of the postnatal period. Strychnine effects on these responses were studied in the same period and compared with those in adults in which differential effects on *s*a and *m* neurons are known to occur.

METHODS

Thirty-one kittens, anesthetized with α-chloralose (Sigma, 50 to 60 mg/kg, i.p.) and paralyzed with decamethonium bromide as needed (Burroughs-Wellcome), were used in this study. Chloralose was chosen as the anesthetic agent because it was used in studies of adult responses to strychnine with which the current experiments were to be compared (12, 27) and because *m* neurons are silenced and the corticofugal reflex eliminated by barbiturates (8, 11). The *s*a and *m* classification of cortical neurons was developed using chloralose anesthesia, but it can be applied, and both *s*a and *m* neurons can be recognized, in unanesthetized cats [see (11) for a review]. Although chloralose is classified as a convulsant agent, it is clearly different from agents such as strychnine, bicuculline, and picrotoxin. Unlike those agents, it does not produce enhancement of the primary evoked response when applied topically in barbiturate-anesthetized adult cats (M. D. Mann, unpublished observations). Clearly, it deserves a different classification. Only kittens born in the laboratory were used so that accurate

ages of 1, 4, 7, and 14 days were known. At 1 day, the animals weighed 103.9 ± 27.8 g (mean ± SD), at 4 days 113.0 ± 58.9, at 7 days 179.7 ± 42.6, and at 14 days 240.5 ± 124.3 g. All animals were taken from the mother, anesthetized, and maintained at 38.5°C using a servo-controlled DC heating pad with thermoprobe taped to the animal's abdomen. They were respired using a Palmer positive-pressure respirator at 40 strokes/min, with volumes of 1.5 to 4 cc/stroke. Atelectasis was prevented by incorporating a sigh (blocking the expiratory port of the respirator for two complete strokes) every 5 min.

In most animals, the medullary pyramids were exposed through a ventral approach, removing the basioccipital bone and reflecting the dura mater. The right pericruciate cerebral cortex was exposed, taking care to minimize blood loss from the overlying profusion of blood vessels. The cortex was immediately covered with a polyethylene sheet which was removed only during recording. The corticofugal discharge was recorded from the medullary pyramid ipsilateral to the site of cortical recording with a sharpened insulated stainless-steel electrode positioned using visual control. The potential evoked in the medial lemniscus by electrical stimulation of the contralateral forepaw was also used as an indicator of electrode position (27). The surface-evoked potential was recorded from the postcruciate forepaw focus (11) using a flame-polished 0.5-mm silver wire electrode. The recording position was determined using both the shape of the primary evoked potential (27) and the position relative to the cruciate, ansate, and coronal sulci, the usual recording site being about one-fifth of the distance caudally from the cruciate toward the ansate. Recordings were made with two Grass P15B amplifiers, set at 0.3 Hz and 10 kHz half-amplitude frequency response. The output of the amplifiers was led to a Tektronix 565 oscilloscope for photographic recording, using a Grass C4 kymograph camera.

Bipolar needle electrodes were inserted into the pads of each paw. The paws were stimulated through these electrodes with square pulses of 0.1-ms duration and various amplitudes. Unless otherwise stated, records shown were obtained with 25-mA pulses. A Tektronix 2600 pulse generator was used to control frequency and duration of pulses, and amplitudes of the constant-current stimuli were controlled by a Tektronix 2620 photon-coupled stimulus isolator.

A 2% strychnine sulfate solution was used to soak a 1.5 × 1.5-mm square of paper towel which was placed on the cerebral surface at the recording site for 20 s. The square was then removed, the electrode replaced on the treated area, and recording resumed. All times were referenced to the time of contact of the strychnine with the cortical surface. Although no accurate estimate of the amount of strychnine in the tissue can be made, the same

application procedure was used in all animals, making them roughly equivalent. In experiments where a more accurate estimate of the speed of action of strychnine on the tissue was desired, a drop of solution was allowed to run down the recording electrode while it was in contact with the cerebral surface. Recording could then be done continuously, before and after application of strychnine.

RESULTS

Normal Responses

The Corticofugal Reflex Discharge. We were unable to record a corticofugal reflex discharge in response to stimulation of any paw in any kitten less than 14 days of age and in all but two at 14 days of age. Figure 1, column 5, shows the corticofugal reflex in an adult cat for stimulation of the contralateral forepaw (A5, lower trace), the ipsilateral forepaw (C), the contralateral hind paw (E), and the ipsilateral hind paw (G). These responses are similar to those shown by Towe and Mann (27). Examples of the corticofugal reflex in one of the few 14-day-old kittens in which one could be recorded are shown in Fig. 1, column 4. In both animals that had a corticofugal reflex, this discharge was of approximately the same magnitude for stimulation of each paw. In adults, the response to contralateral forepaw stimulation is usually larger.

The Normal Primary Evoked Potential. The primary evoked potential recorded in the postcruciate forepaw focus in the 1-day-old animal was usually a purely negative potential or a negative potential preceded by a small positivity. An example of the surface-recorded primary response is shown in Fig. 1A1. In older kittens, the positivity of the primary response became more prominent, whereas the negativity became smaller and longer in duration. Examples for a 4-day-old animal are shown in Fig. 1A2, for a 7-day-old animal in Fig. 1A3, and for a 14-day-old animal in Fig. 1A4. Responses were somewhat variable in configuration from animal to animal despite the careful positioning of the recording electrode with respect to the tip of the cruciate sulcus. In animals with purely negative primary responses, the cortical surface around the anatomically determined recording site was explored for regions where a positive–negative configuration could be recorded, but none were found within a radius of 2 to 3 mm.

The latency of the primary evoked response was shorter in older animals than in younger ones. Table 1 summarizes measurements of (i) latency of the first change in potential (either positive or negative), (ii) latency of the first negative change in potential, and (iii) peak to peak amplitude of the primary response in kittens and adult cats. At 1 day of age, the mean

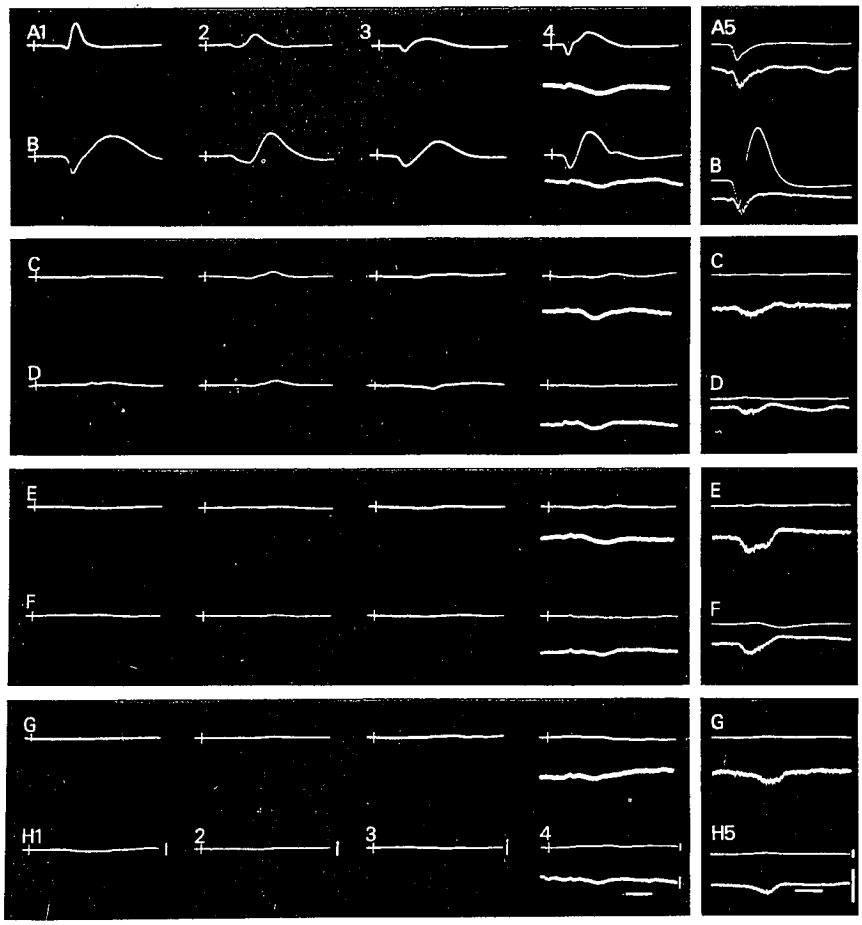


FIG. 1. Responses from the postcruciate forepaw focus due to stimulation of the paws in kittens and cats before and 1 min after treatment with strychnine. Responses recorded from the cortical surface in 1-day (column 1)-, 4-day (column 2)-, and 7-day-old kittens (column 3); and surface responses (upper traces) and corticofugal reflex discharges (lower traces) in a 14-day-old kitten (column 4) and an adult cat (column 5) are shown for stimulation of the contralateral (upper panel) and ipsilateral (second panel) forepaws and contralateral (third panel) and ipsilateral (lower panel) hind paws before (A, C, E, G) and after (B, C, F, H) strychnine application to the recording site. A small lemniscal response in the pyramidal tract recordings shows that the electrode position did not change with time. Calibrations in H: time, 40 ms (H4) and 20 ms (H5); voltage, 500 μV (H1 to 4, upper), 100 μV (H4, lower), 1 mV (H5, upper), 500 μV (H5, lower).

latency of the first change in potential was nearly 50 ms. By 14 days, the latency decreased to half this value, i.e., to 24 ms, and thereafter decreased to the adult value of 8 to 10 ms. The latency of the first negative change

TABLE 1

Changes in Latency and Amplitude of the Primary Evoked Response with Age[a]

	1 Day		4 Days		7 Days		14 Days		Adult	
	C	S	C	S	C	S	C	S	C	S
Latency of first change in potential, ms	49.5 ± 2.65 (4)	49.0 ± 3.56 (4)	44.8 ± 6.85 (4)	43.1 ± 6.51 (4)	39.8 ± 8.33 (6)	41.7 ± 8.79 (6)	24.2 ± 5.26 (5)	25.4 ± 7.09 (5)	9.21 ± 1.85 (7)	9.14 ± 1.89 (8)
Latency of first negative change in potential, ms	57.2 ± 4.57 (4)	74.6 ± 11.90 (4)	59.5 ± 10.21 (4)	76.1 ± 9.96 (4)	62.7 ± 12.39 (6)	69.4 ± 12.15 (6)	47.2 ± 9.68 (5)	55.0 ± 11.58 (5)	20.2 ± 5.94 (7)	19.6 ± 4.58 (8)
Peak to peak amplitude, mV	0.86 ± 0.57 (5)	1.67 ± 1.20 (5)	0.80 ± 0.14 (5)	2.01 ± 0.84 (5)	0.84 ± 0.44 (6)	1.97 ± 1.40 (6)	1.22 ± 0.48 (5)	3.19 ± 0.82 (5)	3.64 ± 1.00 (8)	8.51 ± 3.76 (9)

[a] Number of animals in parentheses; values are means ± SD.

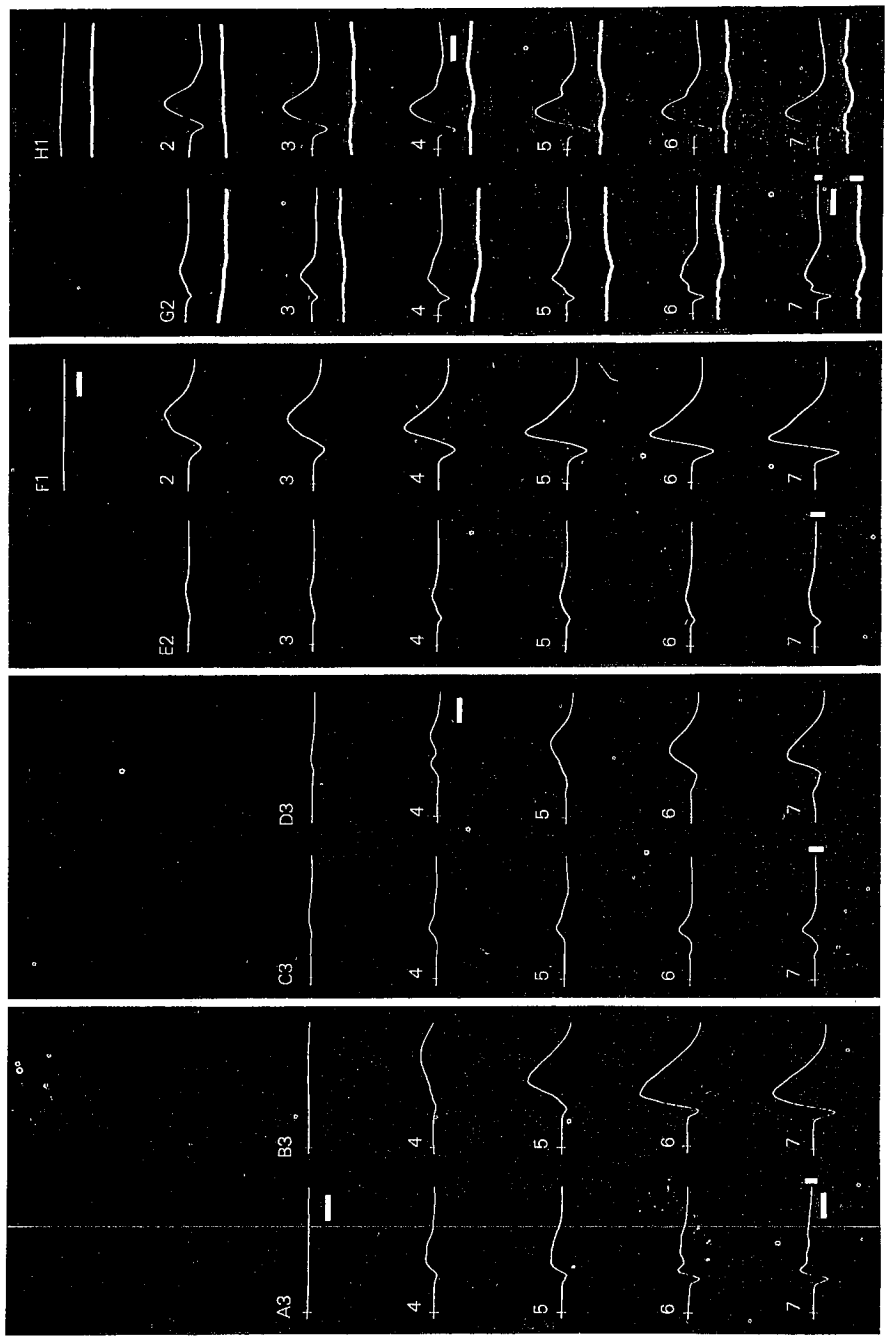
in potential increased from the 1-day mean value of 57 ms during the first postnatal week, attaining 63 ms on day 7 and then declining to the adult value of 20 ms. The insertion of the positivity preceding the surface negativity accounts for the lengthening of the latency at 4 and 7 days.

During development, the peak to peak amplitude of the primary evoked response increased steadily from a 1-day mean of 0.86 to 1.22 mV in 14-day-old animals and 3.64 mV in adults. With respect to changes in amplitude, the age series in Fig. 1, row A, is not entirely typical, but it is typical with respect to changes in latency as discussed previously. The records of Figs. 1 to 4 were assembled because the kittens were all from the same litter of four.

As stimulus strength was increased from below threshold, the primary response appeared and then grew until it attained its maximum amplitude. The response appeared in older kittens at weaker strengths than in younger ones. Figure 2 illustrates this observation in kittens at 1 day (column A), 4 days (column C), 7 days (column E), and 14 days (column G). In the youngest kittens, the primary response was not evoked by 2.5-mA, 0.1-ms pulses (A3), but appeared when stimulus strength was increased to 5 mA (A4). In contrast, a response was apparent in surface recordings from older kittens at 2.5 mA (4 days, C3) or 2.0 mA (7 days, E2; 14 days, G2). In adults, the primary response was evokable with stimuli of strengths less than 1.0 mA. In some kittens, the response appeared and then, with increasing stimulus strength, grew gradually to a maximum (Fig. 2, columns C and E), whereas in others the growth of the response had a more all-or-nothing character (Fig. 2, columns A and G). These patterns did not appear to be related to age; both appeared in animals at each age tested.

The ability of the evoked response to follow iterative stimulation was enhanced with age. At 1 day, the response was reduced in amplitude at a stimulus interval of 10 s compared with responses at longer intervals. As the stimulus interval was shortened from 10 to 1 s, the amplitude of the response was reduced by 50% or more (Fig. 3A1–4). A similar result was obtained in 4-day (B1 through 4)- and 7-day-old animals (C1 through 4). By 14 days of age, the response became relatively insensitive to changes in the interval within this range (D1 through 4), but it was still more sensitive to greater reductions in interval (i.e., intervals less than 1 s) than was that in the adult (E1 through 4).

Off-Focus Potentials. As in adults, off-focus stimulation (stimulation at sites outside the topographic skin focus, in this experiment stimulation of paws other than the contralateral forepaw) usually led to a small, but reproducible potential at the cortical surface in the forepaw focus. Sample records from cats at various ages are shown in Fig. 1 for ipsilateral forepaw stimulation (row C), for contralateral hind paw stimulation (row E), and


H1
G2
F1
E2
D3
C3
B3
A3
2
3
4
5
6
7

for ipsilateral hind paw stimulation (row G). Similar results were obtained in all animals studied.

Strychnine Effects

Strychnine Effects on the Primary Response. Strychnine application to the recording site produced an enhancement of the primary response evoked by stimulation of the contralateral forepaw in every animal studied regardless of age. The magnitude of the enhancement (Table 1) varied between 200 and 260% with a slight but not systematic increase with age. This enhanced primary response is the strychnine spike. Both the positivity and the negativity of the response increased in both amplitude and duration in most animals; animals without an initial positivity developed one. The surface negativity was usually enhanced more than the positivity, but not always.

The latency of the first change in potential of the primary response (Table 1) was not altered by strychnine application in any animal. On the other hand, the latency of the first negative change in potential was increased in kittens, reflecting the enhancement of the duration of the surface positivity. This effect was most pronounced in younger animals in which the positivity was small or absent in control records. A slight but insignificant decrease in latency of the first negative change in potential was usually seen in adults.

The relationship between the amplitude of the strychnine spike and stimulus strength roughly paralleled the relationship for the control primary response (Fig. 2). The threshold strength for a surface-recorded response to contralateral forepaw stimulation was not altered in kittens or adult cats. Strychnine reduced the median response thresholds in single neurons in adults, but this effect was produced primarily by selectively reducing the higher thresholds rather than reducing all values, thus producing little change in the threshold of the primary response (12). Presumably, strychnine has a similar effect in kittens. As in adults, the strychnine spike sometimes appeared to be graded in amplitude with increasing stimulus strength and sometimes it appeared to be more all-or-nothing (27).

FIG. 2. Changes in surface-recorded primary response in kittens at 1, 4, and 7 days of age (three left panels) and in primary response and corticofugal reflex discharge in a 14-day-old kitten (right panel) with changes in strength of stimulation of the contralateral forepaw at 1/5 s before (columns A, C, E, and G) and after strychnine application (columns B, D, F, and H). Stimulus strengths in rows 1 to 7 were 1, 2, 2.5, 5, 7, 15, and 25 mA. Calibrations in row 7: time, 40 ms; voltage 500 μV (A, C, E, and G, upper trace) and 100 μV (G, lower trace).

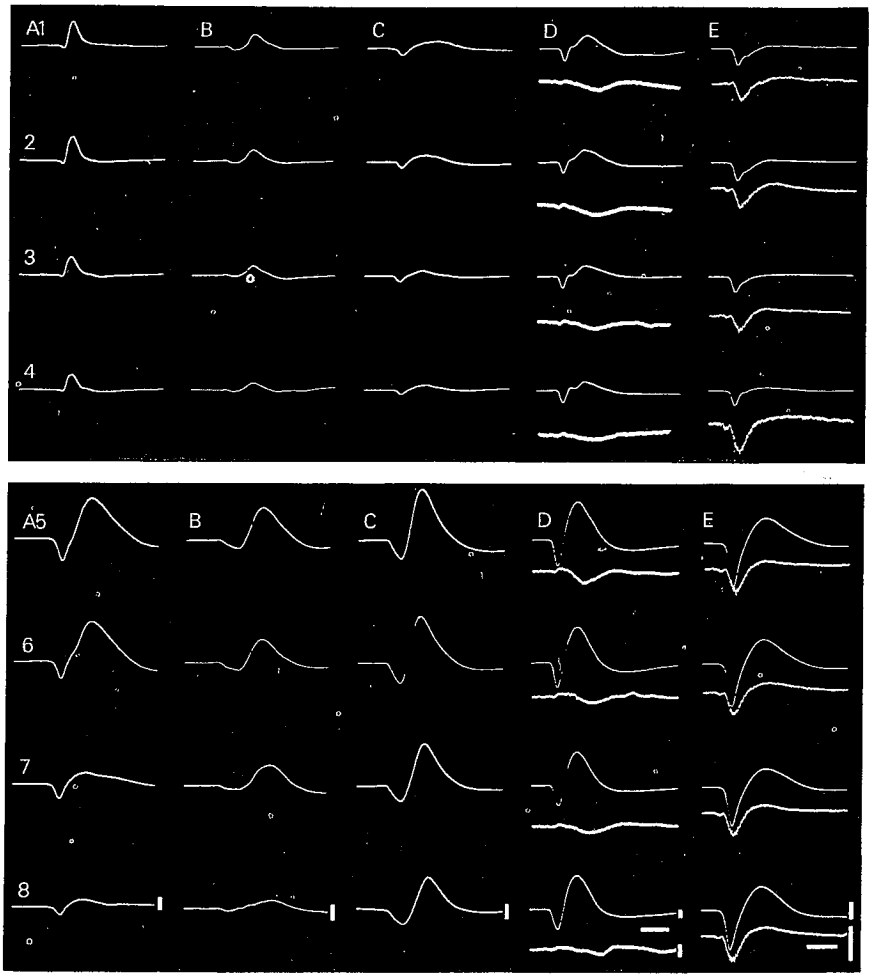


FIG. 3. Changes in surface-recorded primary response in kittens at 1, 4, and 7 days of age (columns A through C) and in primary response and corticofugal reflex discharge in a 14-day-old kitten (column D) and an adult cat (column E) with changes in the frequency of stimulation of the contralateral forepaw at supramaximal strength (25 mA) before (rows 1 through 4) and after (rows 5 through 8) strychnine application. Stimulus frequencies: 1/10 s (rows 1 and 5), 1/5 s (rows 2 and 6), 1/2 s (rows 3 and 7), and 1/s (rows 4 and 8). Calibrations in row 8: time, 40 ms (A through D), 20 ms (E); voltage 500 μV (A through C, D upper trace), 100 μV (D, lower trace), 2 mV (E, upper trace), 500 μV (E, lower trace).

Like the primary response, the strychnine spike in newborn kittens was frequency labile. Figure 3, rows 5 through 8, illustrates spikes in animals of different ages for stimulus intervals of 10, 5, 2, and 1 s, respectively.

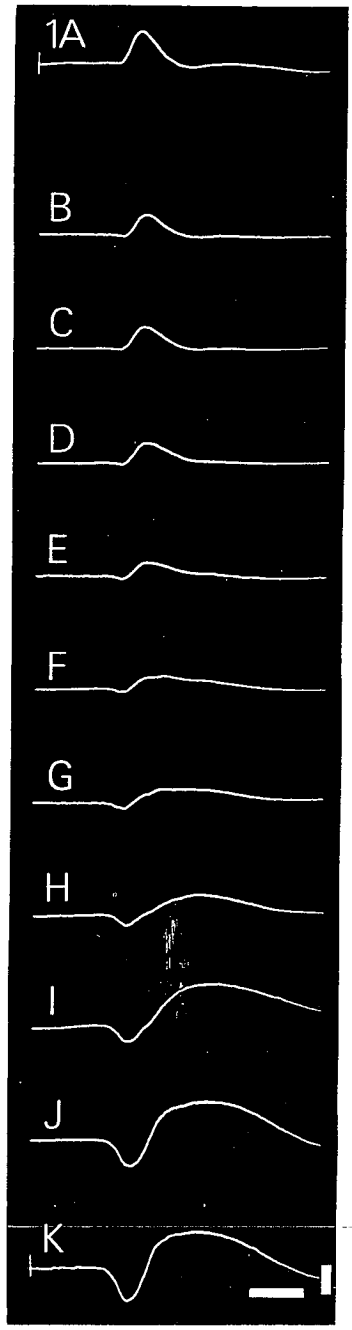
Strychnine responses in 1- and 4-day-old animals were depressed even at longer intervals (A6, B6), whereas responses in older animals were minimally reduced even at intervals of 1 s (D8). The adult strychnine spike was reduced less than 10% in amplitude when the stimulus interval was shortened from 10 to 1 s.

Speed of Action. In adult cats, strychnine exerted its influence on the primary response in less than 30 s, probably of the order of 15 s. The speed of action in kittens appeared to be just as rapid. Figure 4, column 1, shows the responses to stimulation of the contralateral forepaw in a 4-day-old kitten before (A) and immediately after application of a drop of strychnine to the recording site. There is a clear change in the configuration of the primary response at 20 s (E) and perhaps also at 15 s (D). This record is typical of the time course observed in a number of animals. Occasionally, we found an animal (kitten or adult) in which strychnine produced only a small change in the primary response. In these animals, the small effect also occurred with a much longer time course. Why some animals are fairly immune to the effects of topical strychnine is not clear; perhaps the pia in these animals is particularly impenetrable.

Duration of Action. The duration of action of topical strychnine in adult cats seldom exceeded 1 h, never 2 h. This is illustrated in Fig. 5, column E, where the primary response had nearly recovered by 45 min (E7) and had recovered completely by 2 h (E8). In contrast, the duration of action was much longer in kittens. None of the responses in Fig. 5A–D had recovered by 2 to 4 h after application. In fact, there is some question whether or not there was ever complete recovery in animals with purely negative control primary responses in which a preceding positivity was induced by strychnine. The 2-day-old animal in Fig. 4, column 2, was observed for 11 h after a single 20-s application (G) and showed little diminution of the positivity. We did not follow kittens longer than 11 h.

Alternation. In adult cats not treated with strychnine, stimulation of the contralateral forepaw at 2/s produced a primary response and corticofugal reflex discharge on each trial not very different from that produced at 1/5 s. However, when the stimulus frequency was increased to 3/s, the amplitudes of both the primary response and the corticofugal reflex were reduced but relatively constant from one stimulus to the next (Fig. 6A–C). The strychnine spike and corticofugal reflex followed stimulation at 1/5 s and 2/s (Fig. 6 D, E), but, at 3/s or 4/s, the responses alternated between a configuration near the 1/5-s strychnine configuration and one near the control 3/s configuration (Fig. 6F). This behavior was reported previously for both strychnine (28) and bicuculline application (9).

In kittens, the control primary response was reduced drastically in amplitude when the frequency of stimulation was increased from 1/5 s to 2/


1A
B
C
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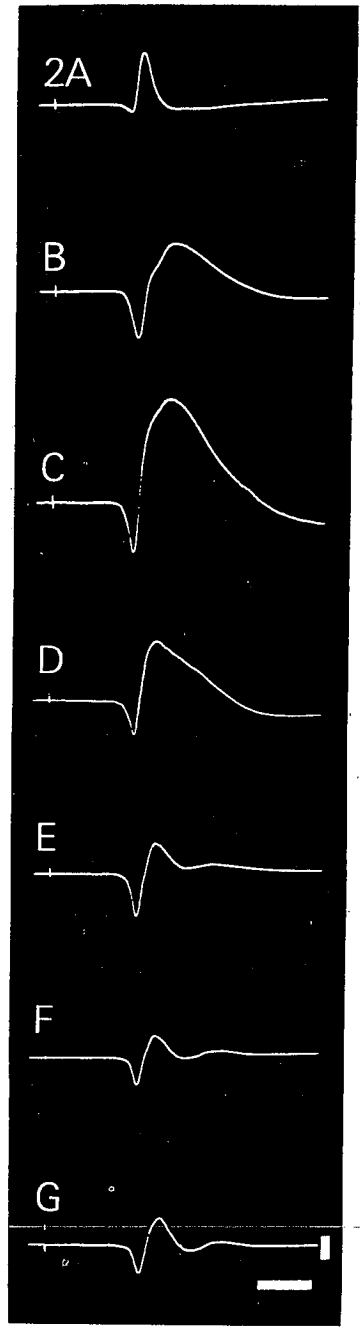
2A
B
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s (Fig. 7 A, B), and was still further reduced at 4/s (C). There was no evidence of a tendency to alternate. The strychnine spike was variable from trial to trial even at 1/5 s (Fig. 7D), but did not alternate. On the other hand, clear alternation occurred when the stimulus frequency was 2/s, although the alternation was between a response smaller than the 1/5-s strychnine spike and a response much larger than the 2/s control primary response (Fig. 7E). When even higher frequencies were used, a double alternation occurred in which a large response was succeeded by an extremely small response (smaller than that for control 4/s stimulation in Fig. 7C) which was succeeded by an intermediate response (Fig. 7F).

Strychnine Effects on Off-Focus Responses. There was usually no influence of strychnine application on the off-focus responses in the postcruciate forepaw focus of kittens or adult cats. This is illustrated in Fig. 1, rows D, F, and H, for ipsilateral forepaw, and contralateral and ipsilateral hind paw stimulation, respectively. As in adults (27), it was possible to produce enhancement of all responses, both on- and off-focus, by applying copious amounts of the alkaloid.

DISCUSSION

In kittens at birth there are already a primary evoked potential and off-focus evoked potentials in the postcruciate forepaw focus although they are later, slower in time course, more labile with higher stimulus frequencies, and different in configuration from the adult potentials. During the first 2 postnatal weeks these parameters shift more or less gradually in the direction of the adult condition reflecting increasing conduction velocity and efficiency in synaptic transmission and perhaps changing amounts or distributions of excitatory and inhibitory postsynaptic processes within the central nervous system (21). In these respects, the results reported here do not differ greatly from those reported previously (6, 15, 20, 22, 23).

The occurrence of a spontaneous strychnine spike in neonatal cortex has been reported for rabbits (4) and cats (23), but no reports of evoked strychnine spikes have appeared. We found an evoked strychnine spike with the adult positive–negative configuration in neonatal pericruciate cortex at 1 day of age. Like the adult strychnine spike, the neonatal event is evoked

FIG. 4. Development and duration of the strychnine spike in kittens. Column 1: control primary response in a 4-day-old kitten to contralateral forepaw stimulation at 25 mA and 1/5 s (A); responses at 5, 10, 15, 20, 25, 30, 40, 50, 60, and 70 s after strychnine application (B through K). Column 2: control primary response in a 2-day-old kitten to the same stimulus (A); responses at 1, 10, and 60 min, 5, 8, and 11 h (B through G) after strychnine application. Calibrations: time, 40 ms; voltage, 500 μV.

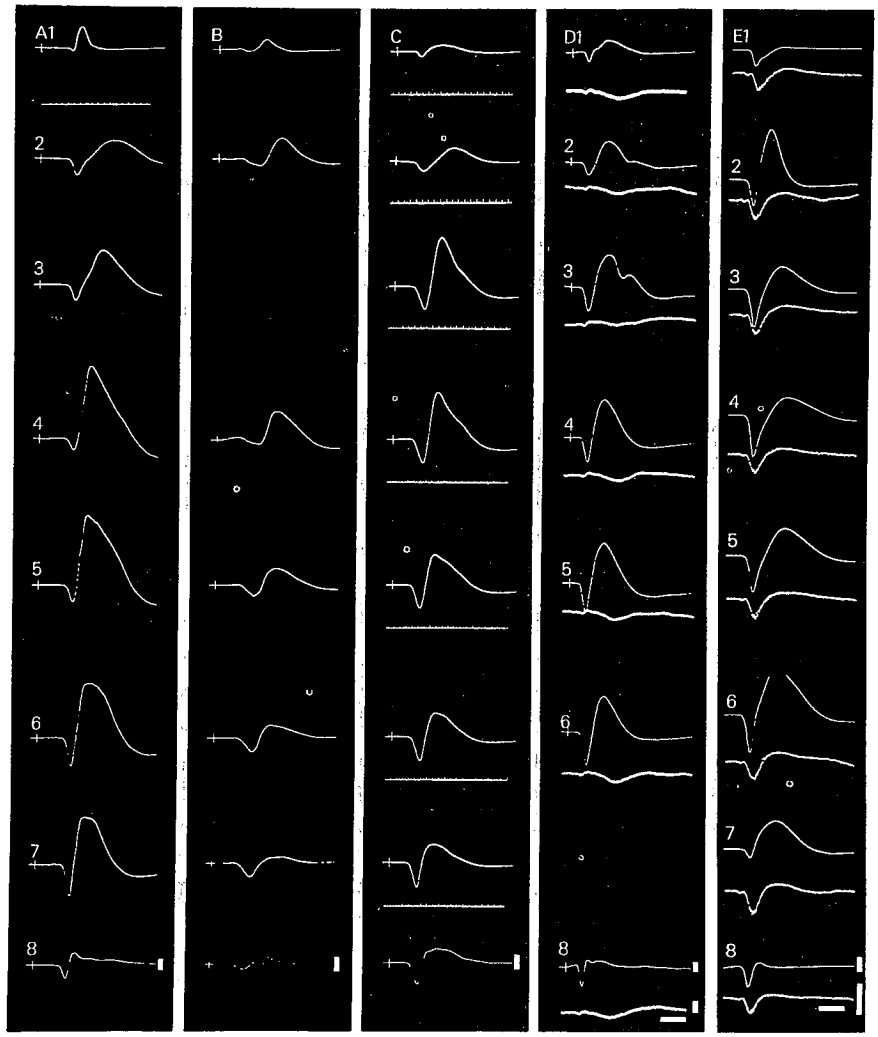

FIG. 5. Time course of the strychnine effect on the primary response in 1-, 4- and 7-day-old kittens (columns A through C) and on the primary response and corticofugal reflex discharge in a 14-day-old kitten (column D), and an adult cat (column E) evoked by contralateral forepaw stimulation at 25 mA and 1/5 s. Control responses are presented in row 1; responses after strychnine application are presented in rows 2 through 7 for 1, 5, 15, 25, 33, and 45 min and in row 8 for 2.5+ (A), 2+ (B), 4+ (C), 3.5+ (D), and 2 h (E). Calibrations in row 8: time, 40 ms (D), 20 ms (E); voltage, 500 μV (A through C and D upper), 100 μV (D, lower), 2 mV (E, upper), and 500 μV (E, lower).

by brief electrical stimulation of the contralateral forepaw, but not by stimulation of the other three paws and it exhibits an alternation behavior at higher stimulus rates although the minimum rate at which alternation

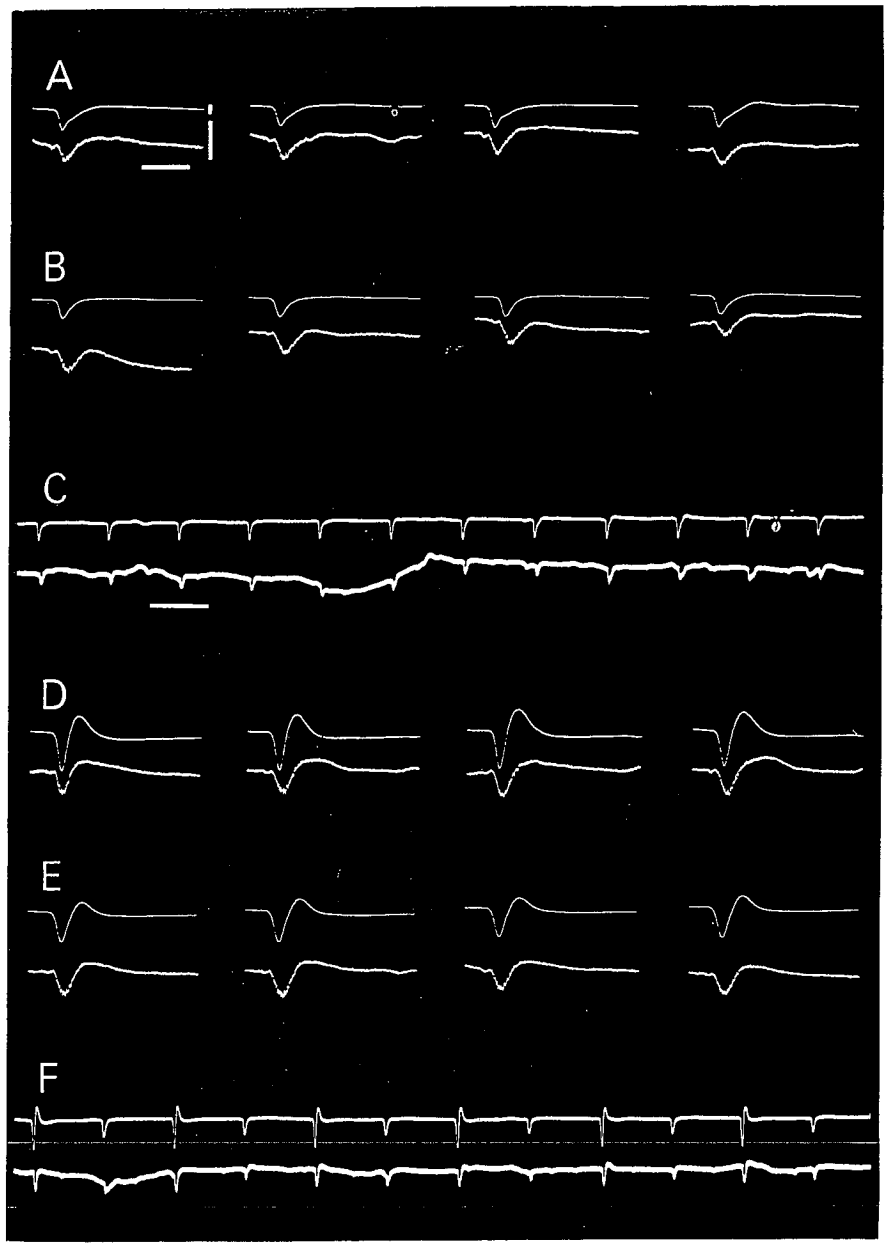

FIG. 6. Alternation in the adult strychnine spike. Consecutive surface-recorded primary responses and corticofugal reflex discharges evoked by contralateral forepaw stimulation at 1/5 s (before, A; after strychnine, D), 2/s (before, B; after, E), and 3/s (before, C; after, F). Calibrations: time, 30 ms (A), 200 ms (C), voltage, 1 mV (upper traces), 500 μV (lower traces).

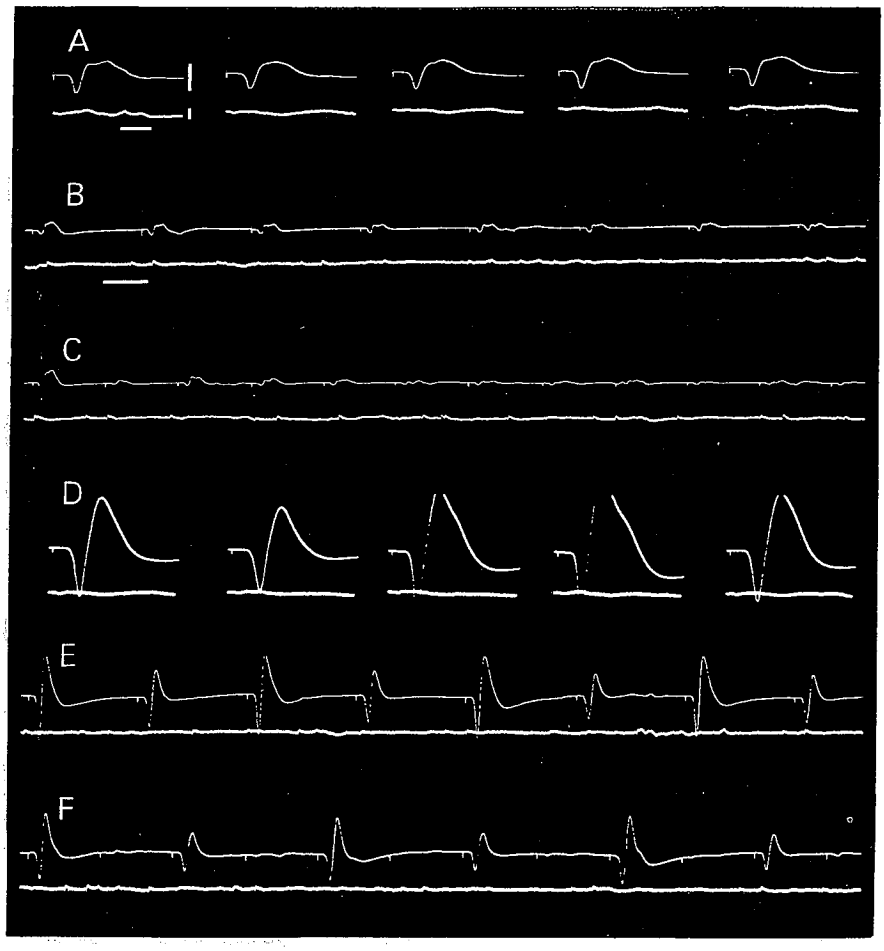

FIG. 7. Alternation in a 14-day-old kitten. Consecutive surface-recorded primary responses and corticofugal reflex discharges evoked by contralateral forepaw stimulation at 1/5 s (before, A; after strychnine, D), 2/s (before, B; after, E), and 4/s (before, C; after, F). Calibrations: time, 50 ms (A), 200 ms (B); voltage, 500 μV (upper traces), 100 μV (lower traces).

occurs is lower than in adults. Unlike the adult strychnine spike, the neonatal event is of longer latency and duration and has greater frequency-lability, conditions that change gradually toward adult form during the first postnatal weeks in parallel with similar changes in control responses.

The Hypothesis. We had hoped that the observations above coupled with observations of the corticofugal reflex discharge would allow us to conclude that *m* neurons develop sooner and migrate before *s*a neurons in ontogeny. However, our inability to record a corticofugal reflex during the first 2

postnatal weeks does not allow such a conclusion. We were surprised not to find a corticofugal reflex because of previous reports that corticofugal axons exist in the pyramids at birth (10, 17), that responses due to direct cortical stimulation are recorded in the pyramids at birth (20), and that field potentials attributed to pyramidal activity are present in the brain stem at birth (17). Perhaps the reflex normally is present in the neonatal brain but it is even more fragile in newborns than in adults (27), being totally eliminated by opening the cranium until sometime after 2 weeks of age. However, in some animals we attempted to record the response before opening the cranium and still found no response. Possibly small changes in cerebral oxygenation or carbon dioxide concentrations could prevent the corticofugal reflex, but it was absent in both artificially respired and normally respiring animals. Variation of respiratory parameters did not reveal a reflex in kittens and therefore, we believe this is not an adequate explanation. On the other hand, the corticofugal reflex discharges may simply be too asynchronous [certainly more so than the response to direct cortical stimulation (20)] to lead to a significant killed-end recording, a conjecture supported by the initiation of myelination and increase in axon diameter in the pyramids that takes place only after 2 weeks of age (10, 20). Whatever the reason for the absence of the reflex, it is clear that the effects of strychnine on neonatal *m* neurons and the time course of their development can be studied only in single-unit recordings. This is a difficult study because yields of neurons are never great in young animals, usually two to three units per kitten to 30 days of age (1, 10).

What, then, can we say about the original hypothesis that *m* neurons are ontogenetically older than *s*a neurons? Perhaps the hypothesis is incorrect. The existence of a neonatal strychnine spike and absence of a corticofugal reflex may, on first analysis, be taken to indicate the presence of *s*a neurons and the absence of *m* neurons. However, it is known that *m* neurons, more accurately pyramidal tract neurons (most of which are *m* neurons in adults), already respond to stimulation of the contralateral forepaw in the immediate postnatal period (1). Perhaps they have received only their contralateral forepaw input at this time, the off-focus input arriving later. Still, the existence of off-focus evoked potentials indicates that these inputs are already present in the cortex at birth, although they may not be capable of discharging the cells.

Recently, Tyner *et al.* (30) demonstrated that apparent *s*a neurons (they respond only to contralateral forepaw stimulation) can be converted to *m* neurons (they respond to stimulation of any paw) by intravenous administration of naloxone or picrotoxin and then converted back to *s*a neurons by diazepam. They suggest that some neurons in motor–sensory cortex (perhaps all neurons) have the capacity to switch back and forth from *s*a to *m*, and that the condition of a cell's receptive field at any time is a

function of the animal's behavioral state (C. F. Tyner, M. Y. Spiegelstein, and M. L. Howell, personal communication).

However, the conversion of *s*a to *m* neurons or the reverse does not explain the effects of topical strychnine in adults or kittens. In adults, receptive fields are stable under the conditions of these present experiments (11). Even if they changed, conversion of *s*a to *m* neurons should enhance the off-focus evoked potentials and corticofugal reflex discharges (provided that some of the *m*s are pyramidal tract neurons); conversion of *m* to *s*a neurons should produce no effect (or perhaps a slight reduction, because *s*a neurons discharge fewer spikes) on the primary evoked response. The similarity of the pattern of the results in kittens to those in adults suggests that the effect of strychnine is the same and exerted on the same elements, namely *s*a neurons. A likely conclusion, therefore, is that the cat is precocious with respect to the rat and mouse, and *s*a neuron migration is a prenatal event—we have simply studied the tissue too late.

Alternation. Alternation behavior of the strychnine spike at stimulus frequencies of 3 to 4/s has been interpreted to indicate that the strychnine spike is really composed of two separable events: an enhanced normal synaptic event and an additional strychnine event, possibly a paroxysmal depolarization shift (9, 28). A longer refractory (or subnormal) period for the strychnine event would yield alternation like that seen in adults. A similar explanation would apply to simple alternation in kittens if the enhanced normal event was much larger than that in adults relative to the amplitude of the strychnine event. The smaller alternate response in kittens is much larger than the control response that occurs at the same stimulus frequency. If this explanation is correct, then most of the lability of the control primary evoked potential in kittens at higher frequencies of stimulation is the result of synaptic failure within the cortex itself and at the synapses affected by topical strychnine.

However, such a simple two-event model will not account for the double alternation observed at even higher frequencies. Both events would occur with the first stimulus, giving a large strychnine spike. For the second stimulus to give essentially a normal 4/s response (cf. Fig. 7C, the second response onward and Fig. 7F, the second response and every third one thereafter) and the third stimulus an intermediate response, there must be at least three events, each with a different refractory period or some interaction between the events.

Site of Strychnine Action. The rapidity with which the topical strychnine exerts its effects on cortical neurons in both adults and kittens suggests that the alkaloid works very near the cortical surface. Strychnine, like other convulsant agents (18, 31), probably has moved only 100 to 200 μm into the tissue by the time its action is observed [arguments summarized in (28)]. Clearly, in both kittens and adult cats it must act on elements in

layer I or layer II. In this region of the adult brain, and presumably also the kitten brain, are found axon terminals of thalamocortical and other afferent fibers and dendritic processes, primarily of more superficial neurons; i.e., *s*a neurons. It may be that strychnine influences *s*a neurons preferentially because of their proximity to the site of application, but even at long times after application there is no direct effect on the deeper *m* neurons. Perhaps the strychnine is metabolized completely in the more superficial layers. In precruciate tissue, where *m* neurons are located statistically more superficially, direct effects on *m* neurons have been observed (12, 27). However, the model of Towe *et al.* (28) suggests that topical strychnine influences *s*a neurons in postcruciate tissue primarily because their thalamocortical afferent fibers have collaterals in layer I, a testable hypothesis.

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