

Electrophysiology of the Fetal Spinal Cord

I. *Action potentials of the motoneuron*

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ABSTRACT Responses from motoneurons were recorded with microelectrodes, from the spinal cords of kitten fetuses and newborn kittens between 40 days' gestation and a few days after birth. As in the adult animal, intracellularly recorded action potentials by either ortho- or antidromic shocks have two components, "A" and "B" or IS and SD. The action potentials of the adult and immature motoneuron differ mainly in the afterpotentials which are absent in the fetal cell in "good" condition. Repeated stimulation or deterioration of the cell resulted, however, in the appearance of depolarizing and hyperpolarizing afterpotentials. No major differences were found in the mode of anti- or orthodromic invasion of the adult and fetal motoneuron, but the degree of invasion of the soma-dendritic complex may be somewhat less in the fetal cells. The ventral root discharge by dorsal root stimulation could be obtained in the fetus 3 weeks before birth. This reflex discharge was concluded to be monosynaptic. Excitatory postsynaptic potentials, probably monosynaptically activated, could be recorded from inside motoneurons by stimulation of dorsal root or peripheral nerves. The most remarkable change during prenatal development was an increase in the speed and efficacy of the excitatory synaptic potentials which showed a marked change during the last weeks of prenatal life.

INTRODUCTION

Development of the central nervous system (CNS) which is closely related to the problems of learning and memory provides us with many interesting questions. However, the electrophysiological studies so far made on this subject have been confined to recording of mass responses.

During the past fifteen years the motoneurons of the cat spinal cord have been the subject of intensive probing by intracellular microelectrodes (Brock *et al.*, 1952; Eccles, 1953, 1957; Frank and Fuortes, 1955). The electrical properties of the motoneuron are now well known and a large amount of information is available. There is also considerable literature on the prenatal

development of reflex activity and the morphological changes in the fetal cat. These studies make the spinal cord of the fetal cat a logical place to seek changes at the single cell level during the maturation of the CNS. Though several authors have reported on the ventral root (VR) reflex in neonatal kittens (Malcolm, 1953, 1955; Skoglund 1960 *a, b, c*; Arutyunyan, 1961) the earliest intracellular recording was by Wilson (1962) who obtained spikes and synaptic potentials from an 8 day old kitten.

In this study it is hoped that some of the questions on the development of the CNS can be answered by determining at what stage of development basic mechanisms of cellular interaction first appear, as indicated by excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs), and post-spike hyperpolarizing potentials. In the adult motoneuron the spike potential can be split into two components, "A" and "B" or IS and SD, which result from sequential invasion of parts of the cell (Fuortes *et al.*, 1957; Coombs *et al.*, 1957). Roles of the different parts of the motoneuron in generation of spike potentials and also in the integrating process in the cell may be revealed by studying the ontogenetic order in which the components of the spike appear.

TECHNIQUE

Intracellular potentials were recorded from motoneurons of fetal cats by the following techniques.

I. *Electrode and Amplifier*

The glass micropipettes used were filled with 1 to 2 M potassium citrate and measured over 50 megohms. The use of potassium citrate instead of potassium chloride, described by Boistel and Fatt (1958) and Kandel *et al.* (1961), is thought to prevent interference with inhibitory hyperpolarization of the motoneuron.

The recording and stimulating techniques used were standard except for the display circuit developed by Mr. J. Coombs.¹ By synchronizing electronic switches in both horizontal and vertical amplifiers of a single beam oscilloscope, dual traces were obtained with independent "X" and "Y" inputs. For vertical display the upper trace was condenser-coupled with a 100 msec. time constant and the lower trace direct coupled. The two traces were usually at the same gain. Stimuli were delivered to peripheral nerves, dorsal roots (DRs), ventral roots (VRs), or directly to the spinal cord; stimulation was usually not oftener than once per second.

II. *Preparation of the Animal*

During this series of experiments 60 kittens of various ages and 50 pregnant cats were used, together with about 80 fetuses. The pregnant cat was anesthetized by either hexobarbital sodium (evipal) 100 mg/kg or pentobarbital sodium (nembutal) 30

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mg/kg. Usually the pregnant cat was given progesterone a few days before operation to decrease the chance of uterine contractions. Fetuses were removed by aseptic Cesarean section, usually one at a time, and the incision closed. The mother cat could survive repeated operations and it was possible to use most of the fetuses found in a pregnant cat. This required anesthesia for up to 4 days, using automatic intravenous injection of 4 per cent glucose solution mixed with nembutal (0.4 mg/cc) and penicillin (1000 units/cc) at the rate of about 20 cc in 10 min. every hour. Some of the fetuses were sacrificed for histological control by injecting 10 per cent formalin solution into the heart.

Once out of the uterus, the fetus was immediately given artificial respiration with 98 per cent oxygen, 2 per cent CO₂. The fetus near term was then anesthetized by evipal, 10 mg/100 gm, and immobilized by gallamine triethiodide (flaxedil) 0.2 mg/100 gm. The younger fetuses were given a mixture of evipal and flaxedil, or only flaxedil in the case of the very immature fetus. This method allowed the fetus to survive outside of the uterus for from a few hours (in the case of the fetus of 40 days' gestation) to more than 7 hours (in the case of the fetus near term). It was very difficult to keep alive a fetus of less than 40 days' gestation (less than 15 gm).

In the fetus over 50 days old, four peripheral nerves were usually dissected: tibialis (TIB), common peroneal (PER), and lateral and medial gastrocnemius (GSL and GSM) nerves. The dissection of the nerves was not always successful and it was rare that all four nerves gave recognizable reflexes in the fetus younger than a week prenatal.

Care was taken to keep the temperature of the fetus at about 38°C (monitored by a needle type thermistor). However, difficulties in controlling body temperature, amount of artificial respiration, and level of anesthesia as well as unsatisfactory technique in dissection (as mentioned above) prevented our getting very consistent results.

III. *Criteria for the Selection of Records*

In this series of experiments, records typical of the various stages of development were selected. In the fetus near term the intracellular recording was stable up to 1 hour, while in younger fetuses stable recording was difficult and impaled cells rapidly deteriorated. Though the resting potential was monitored throughout the experiment, this was not enough for selection of results because the high resistance of the electrodes used resulted in erratic dc recording. Data were selected primarily on the basis of spike potential amplitude and cells with spikes of more than 40 mv were selected as normal. An exception was made in the case of the easily recognizable giant extracellular response (Granit and Phillips, 1956; Freygang and Frank, 1959). This eliminated a majority of the cells impaled, as they were considered to be in a deteriorating condition. Some of such records were included in the results when particular information obtained from the unit was thought not to be distorted by the deterioration of the cell.

IV. *Criteria Used for Determination of Fetal Age*

To obtain fetuses of known gestation age, cats were bred in the laboratory. Even so, the practical necessity of repeated mating introduced some uncertainty as to the exact

age of any particular fetus. Considerable variation was found in the weight and length of fetuses of the same age and even among fetuses from the same cat. In this report, fetuses are classified according to gestation age regardless of their maturity. Days of gestation of a given fetus were counted simply from the days when a female was exposed to a stud cat. In this report 65 days was used as the gestation period. Scott *et al.* (1957) gave 65 ± 4 days as the mean for cats.

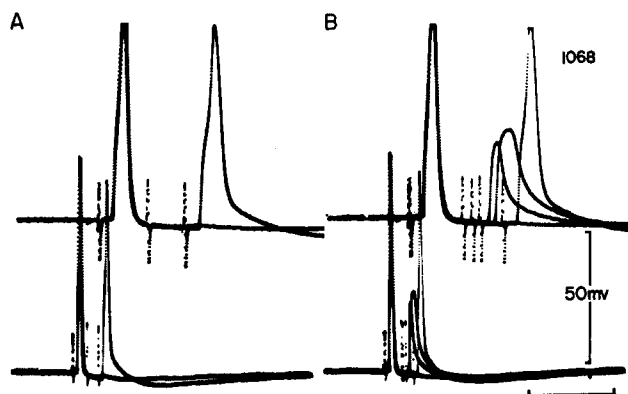


FIGURE 1. Spike potential recorded from a motoneuron in a newborn kitten. Paired shocks were delivered to the VR at different intervals. In A two and in B four sweeps were superimposed. Voltage calibration bar applies to both sweeps. Unless otherwise mentioned, horizontal bar is 10 msec. for upper and 30 msec. for lower trace in this and following records. Sharp transients are stimulus artifacts. (Note: three of the spikes on the fast traces are off scale. Amplitudes, however, are indicated by the slow traces.)

RESULTS

I. Spike Potential

A typical intracellular response obtained from a motoneuron of a newborn kitten is shown in Fig. 1. Multiple sweep records show the response to paired VR shocks of decreasing intervals. The first spike was 2.5 msec. in duration measured at its foot as compared to the range of 1.2 to 2.1 msec. given by Frank and Fuortes (1955). A slight inflection may be observed on the rising phase of the spike potential at the level of 30 mv and only a small hyperpolarizing afterpotential (HAP) is visible. The second spike differed remarkably from the first one by having a depolarizing afterpotential (DAP) of about 8 mv which turned into an HAP 5 msec. later.

As in the case of adult motoneurons (Brock *et al.*, 1952; Coombs *et al.*, 1957; Fuortes *et al.*, 1957), paired VR shocks within the critical stimulus interval revealed the sequential invasion of "A" and "B" regions of the cell. The "A" component of the second spike became larger in amplitude the later it fired, as in the case of the adult cell.

The motoneuron of the kitten fetus could be activated by orthodromic as

well as by antidromic stimuli. Records in Fig. 2 were obtained from a fetus presumed to be about 1 week before birth. Shocks of progressively increasing intensity were delivered to gastrocnemius (GS) nerves. As seen in A and B, a weak shock gave rise to an EPSP about 20 msec. after a shock was given to the nerve. Apparently the small spike in C is an "A" component and the bigger spike in D an "AB" component. The delay between "A" and "B" components became much shorter with increased intensity of the shock. The EPSP shown in A and B of this record had a rather smooth rising and falling phase suggesting that the EPSP was obtained by synchronous impingement on the cell. In F two sweeps were superimposed, one with only an EPSP and the other with a spike firing from the EPSP. It is seen that when an "AB"

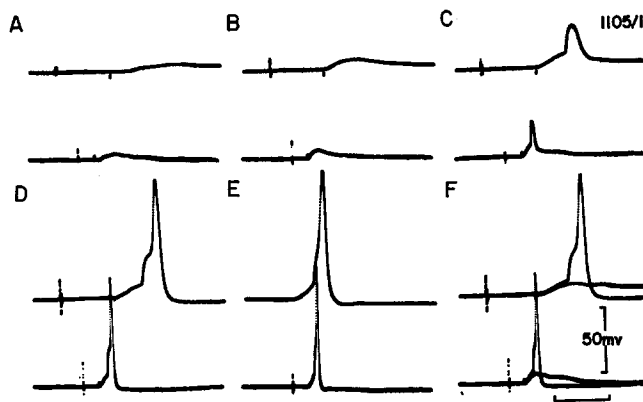


FIGURE 2. Monosynaptic EPSP and spike potential from a motoneuron in a fetus approximately a week before birth. Intensity of shocks given to GS was increased from A through E. In F two sweeps were superimposed to show the relation between EPSP and spike potential.

spike was fired, the falling phase of the spike potential wiped out the EPSP, while the time course of the EPSP was little affected by the "A" spike alone (Fig. 2C). In this cell the time from beginning of synaptic potential to firing of spike potential was from 3 to 6 msec. compared with less than 1 msec. in the adult cell (Coombs *et al.*, 1955).

II. Components of the Spike Potential

It is generally agreed that in the adult motoneuron the "A" component represents the spike potential in the initial segment or axon hillock, while the "B" component represents the invasion of spike potential into the soma membrane which may include some part of the basal dendrite (Frank and Nelson, 1961). It is of interest to know how early this "A-B" break can be observed in the development of the fetal motoneuron.

The responses in Fig. 3 were obtained from a fetus of 40 ± 2 days' gesta-

tion. It was not possible to dissect roots in such small fetuses and the shock was delivered through a pair of platinum wires placed on the surface of the cord. Though there was no direct evidence, the most probable locus of recording was a motoneuron, as motoneurons are the largest cells in the cord. Two records, A and B, were taken successively. The response started about 10 msec. after the shock, from a slowly rising potential (presumably postsynaptic), which gave rise to a spike potential of about 30 mv.

It is more likely that the small amplitude of this spike potential is due to poor impalement of the cell rather than that it is an indication of characteristic spike size in young fetuses. Motoneurons measure 10 to 20 microns at this stage of development (our unpublished observation). The long duration of the spike may also be due to the deterioration of the cell. On the rising phase of the spike in A an inflection was observed at the level of 10 mv and in B

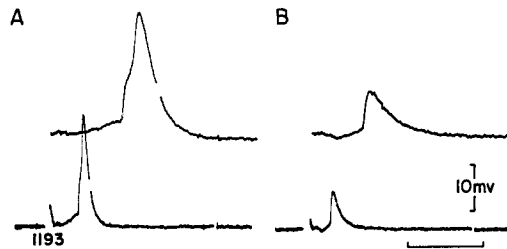


FIGURE 3. Spike potential from a fetus of 40 ± 2 days' gestation. The response, probably from a motoneuron, following stimulation of the spinal cord, shows two components, "A" and "B". Discontinuity of sweep at beginning of trace was due to stimulus artifact.

only a small potential of 10 mv was recorded. As in the case of preceding records, it seems appropriate to conclude that these two components in the spike are the "A" and "B" components found in the adult cat. It may be noted that the "A" spike rose rather rapidly and fell slowly back to the base line.

With orthodromic activation of the motoneuron the "A" and "B" components could be seen in a fetus of 48 ± 1 days' gestation (Fig. 4). In A a graded EPSP of a few millivolts was obtained 10 msec. after the DR shock. In B the amplitude of the EPSP increased to 5 mv and a slow spike ("A" component) appeared on the EPSP. The "A" spike characterized by its flat top had an amplitude of about 20 mv and duration of 5 to 6 msec. measured at the foot of the spike. In C a full size spike potential was fired from the EPSP. Two humps developed on the rising phase of the spike potential, one showing the take-off of "A" spike from EPSP and the other the transition from "A" to "B" component. The "AB" spike was about 2 to 3 msec. in duration which is much shorter than the duration of the "A" component in B.

The "A" spikes recorded from fetal motoneurons could be divided into two types, one of which responded in a more or less graded manner as shown in Fig. 1 and the other which could follow fairly fast repetitive stimuli and was nearly constant in amplitude. As shown in Fig. 1 the amplitude and configuration of the "A" spike of the former type depend upon the stimulus

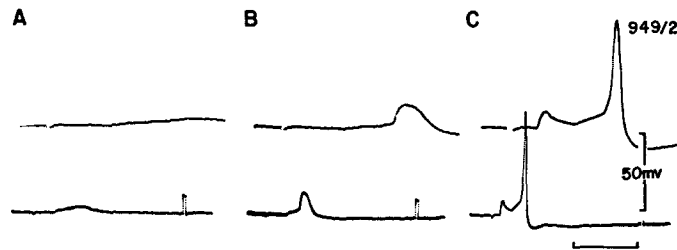


FIGURE 4. EPSP and spike potential recorded from a motoneuron in a fetus of 48 ± 1 days' gestation. Intensity of DR shock was increased from A through C. In C stimulus spread gave rise to the response immediately after the artifact, probably through the VR.

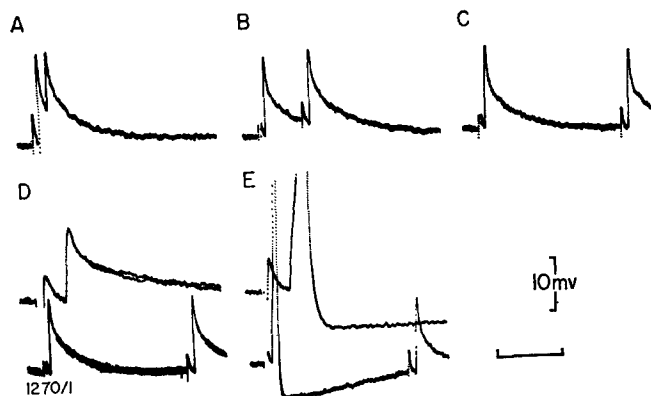


FIGURE 5. Second type of "A" spike showing constant amplitude, a steep rising phase, and a slow exponential falling phase from a fetus of 51 ± 1 days' gestation. Horizontal bar is 10 msec. for upper trace in D and E; 30 msec. for all other traces.

interval. This has been interpreted (Fuortes *et al.*, 1957) as a gradual change in size of the "A" area. An "A" spike of the second type is shown in Fig. 5, which was recorded from a fetus of 51 ± 1 days' gestation. The "A" spike showed a rather steep rising phase followed by a slow decay to the base line. As seen in Fig. 5A a pair of shocks delivered to the VR 5 msec. apart gave rise to two nearly identical responses. Records in B and C show that longer shock intervals had no effect on the shape of the second "A" spike. This type of "A" spike responded very well to repeated stimulation, as shown in D in which pairs of shocks were delivered about five times per second. In E the first shock gave rise to an "AB" spike, while the second shock produced only

an "A" spike. However, we found that this type of "A" spike is rarely followed by a "B" spike.

Properties of "A" spikes of this type suggest that only a small area of membrane is involved with a rather sharp line of separation from the area responsible for the "B" spike. Our observation that this type of "A" spike was often recorded from young fetuses suggests that the particular geometrical

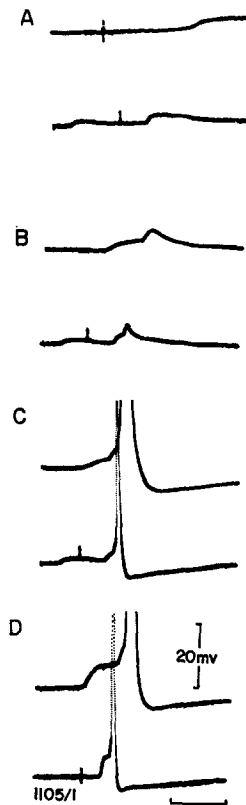


FIGURE 6. Small or local response from a motoneuron in a fetus approximately 1 week before birth. In A, B, and C two shocks were given to peripheral nerves, one to GSL and the other to GSM. The sharp transient on the fast sweep shows the artifact of the second shock in A, B, and C. The first shock was delivered at the start of the slow sweep. Each GS gave rise to an EPSP. In D one shock was delivered to one of the GSs which gave rise to an EPSP and a spike potential.

shape of the developing fetal cell may be responsible for the difference in "A" spikes.

Another component of the response of the fetal motoneuron is a small pre-potential or local potential often observed superimposed on an EPSP. A typical example of this component is shown in Fig. 6, which was recorded from a fetus approximately 1 week before birth. In A two EPSPs are seen and in B a small local response is observed on the second EPSP which gave rise to a spike potential in C. Two steps on the rising phase of the spike potential clearly indicate that the spike potential took off from the small local response. In D, the EPSP was obtained by a shock to one of the GS nerves. Again two steps on the rising phase give evidence of the small local response.

These small local responses were often obtained riding on the long lasting EPSP produced by PER or TIB stimulation but could also be seen on the EPSP activated monosynaptically by GS stimulation. In some cells clear "A-B" separation accompanied the small response indicating that a similar sequential invasion occurred even when the spike potential took off from the prepotential.

III. *Afterpotentials*

It was a consistent finding that when a cell was in good condition, the afterpotential was very small in amplitude. In Fig. 7A (recorded from a fetus of 61 ± 1 days' gestation) the response to the first VR shock returned to the base line without showing any afterpotential. The second shock, delivered 7 msec. later, gave rise to a spike potential with a DAP followed by an HAP. The DAP started from a distinct inflection point at a level of 10 mv measured

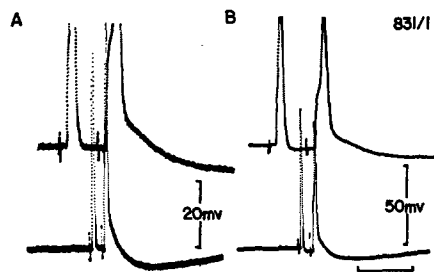


FIGURE 7. Spike potentials following paired shocks to the VR in a fetus of 61 ± 1 days' gestation. A was recorded immediately after, and B a few seconds after penetration of the microelectrode into the cell. Note appearance of an HAP following the first spike in record B.

from the resting level and after 5 msec. turned into an HAP rising to about 10 mv amplitude

It may be concluded that the first stimulus modifies the membrane parameters in some way so that the second spike now is followed by afterpotentials. Fig. 7B was recorded a few seconds after Fig. 7A. The small HAP of the first spike suggests that the resting potential had decreased due to deterioration. Afterpotentials followed the second spike as before. Such afterpotentials due to repeated stimulation could be seen in the responses from a fairly young fetal cell.

IV. *Giant Extracellular Response*

Freygang and Frank (1959) reported large positive-negative extracellular responses from the motoneuron of the cat spinal cord and referred to them as "giant extracellular responses." Except for the generalized field effect from many cells firing synchronously at a distance, all extracellular responses obtained in this study showed a positive phase followed by a negative phase with amplitudes of from a few millivolts to tens of millivolts. Responses exceeding 30 mv were not uncommon. When the so-called giant extracellular

response was obtained, the recording was more stable than when inside the cell.

Fig. 8 is a typical response from a fetus of 52 ± 1 days' gestation. The response to the VR shock showed a positive phase with an inflection on its rising slope which turned sharply into a negative phase, returning rather slowly to the base line. In C the second shock gave rise to a smaller response ("A" spike) of predominantly positive phase followed by a much smaller

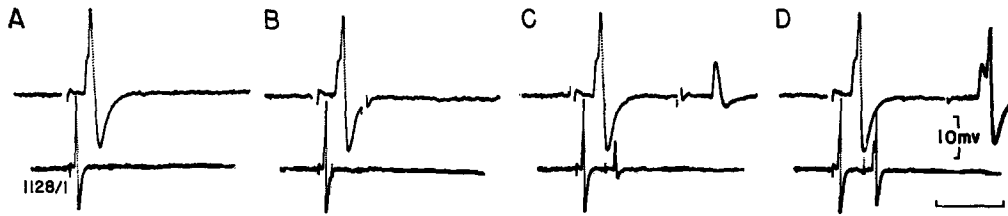


FIGURE 8. Giant extracellular response from a motoneuron in a fetus of 52 ± 1 days' gestation. Single shocks in A and double shocks in B, C, and D were delivered to the VR.

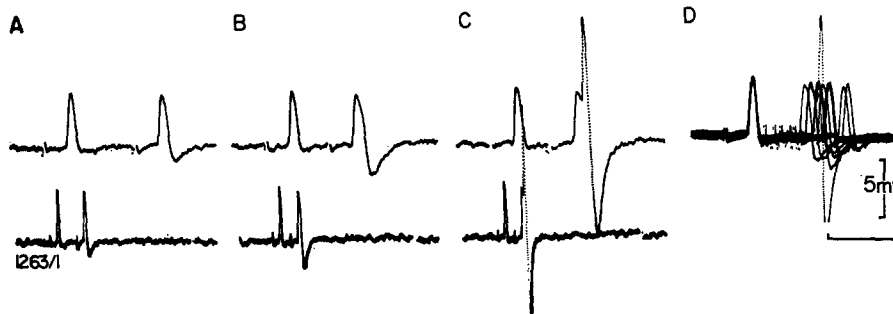


FIGURE 9. Giant extracellular response from a cell which usually fired only "A" spikes in response to VR shocks, recorded from a fetus of 55 ± 2 days' gestation. Shock interval was decreased from A through C. In D several sweeps were superimposed to show change in the negative phase of the response.

negative phase. At the critical stimulus interval for invasion of the "B" area, the transfer from "A" to "B" resulted in a delay as shown by the dip on the rising phase of the second spike in D.

In Fig. 9 (recorded from a fetus of 55 ± 2 days' gestation) a single VR shock elicited only an "A" spike; such a cell provided us with an opportunity to observe the shape of the "A" component of the giant extracellular response undisturbed by a preceding spike. In A the first response was of nearly monophasic positive configuration, while the second had a hump on its shoulder which turned into a negative phase. When the second shock was given earlier as in B, the hump on the shoulder of the second spike was more conspicuous and also the negative phase was larger. At some critical point

the second "A" spike produced a "B" spike with a negative phase of considerable amplitude. Record D, obtained by superimposing several sweeps, shows that the amplitude of the negative phase of the second "A" spike depended on the timing of the second shock and increased and then decreased as the second shock was delivered earlier.

These observations suggest that the negative phase of the giant extracellular spike in the fetal motoneuron is not simply a passive property of the membrane making immediate contact with the electrode as suggested by Freygang and Frank (1959).

V. Change in Shape of Spike and EPSP during Prenatal Development

As shown in Fig. 10 the responses from a 2 day old kitten motoneuron are typical of the adult cell. The EPSP from a kitten of this age reached maximal

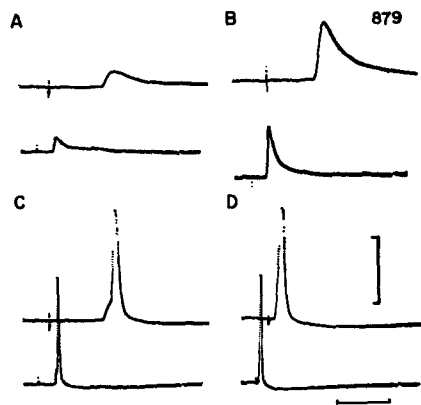


FIGURE 10. Monosynaptic EPSP and spike potential from stimulation given to TIB in a 1 to 2 day old kitten. B is a high gain recording and D is a spike potential from antidromic shock. Vertical bar is 50 mv for A, C, and D; 20 mv for B.

amplitude within 1.6 msec. after the beginning of the response and the maximal slope of its rising phase was 13 v/sec. According to Coombs *et al.* (1955) the maximal slope of the adult EPSP was 50 v/sec. with a rise time of 1.0 msec. The shape of the EPSP in this record strongly suggests that it was activated by a highly synchronous afferent volley through the monosynaptic pathway.

The spike potential had an amplitude of 80 mv and the duration measured at its foot was about 1.6 msec., which was comparable to the duration of the spike potential of adult cells given by Frank and Fuortes (1955).

Fig. 11 shows responses from a fetus within a week of term. In this record shocks were delivered to the DR but the resulting EPSP had a sharp rise and a smooth fall-off suggesting that it was also produced by a synchronous impingement, probably monosynaptic in nature. The EPSP reached its maximal amplitude within 1.8 msec. and the maximal slope of the rising phase was about 8 v/sec. The duration of the spike potential of 1.2 msec. is comparable

to that of the adult motoneuron. The spike potential was 60 mv in amplitude and its threshold was about 10 mv, judging from the inflection point on the rising phase.

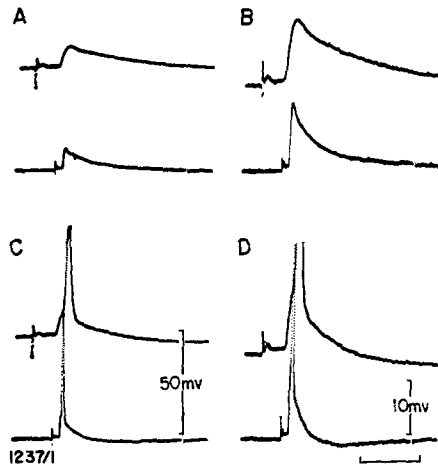


FIGURE 11. EPSP and spike potential from DR stimulation in a fetus within a week of term. B and D are high gain recordings.

Fig. 12 shows graded EPSPs and a 12 mv threshold spike produced by DR stimulation in a fetus of 50 ± 1 days' gestation. The EPSP, with a latency of about 10 msec., had very slow rising and falling phases compared with EPSPs shown in records of Figs. 10 and 11. Though it was masked by the

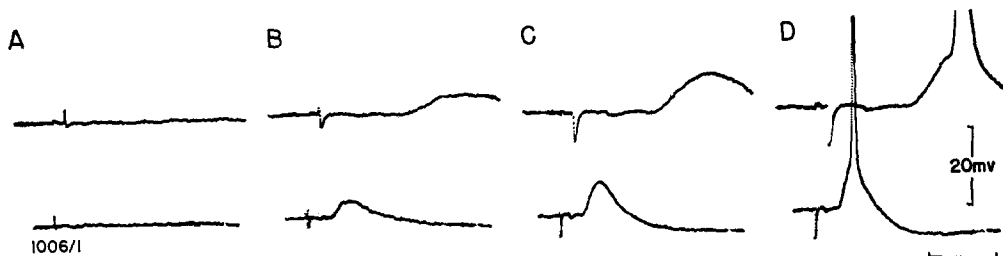


FIGURE 12. EPSP and spike potential from a fetus of 50 ± 1 days' gestation. Intensity of shock increased from A through D.

spike potential, the EPSP in Fig. 12 D seemed to have reached its maximal amplitude within 5 msec. with maximal slope of about 2 v/sec. It appears that, though its wave form is very slow, the early part of the EPSP was activated by a monosynaptic volley which became less synchronous because of slow conduction in the afferent collaterals and, possibly, also due to slow reaction at the site of transmitter release. The EPSP shown in C was followed by an afterhyperpolarizing phase of considerable amplitude which was frequently observed in the fetus of this age. This may be due to the inhibitory component of the postsynaptic potential activated by a shock to the DR or

may be due to a long lasting high conductive state as in the case of the squid giant synapse (Hagiwara and Tasaki, 1958).

VI. *Repetitive Firing of the Fetal Motoneuron*

According to Skoglund (1960 *a*) it is not possible to differentiate afferent fibers on the basis of strength of stimulus to the peripheral nerve. Skoglund (1960 *a*) and Hursh (1939) attribute this to a uniform fiber conduction velocity at birth. Though it is true that discrimination of fibers was difficult, the afferent

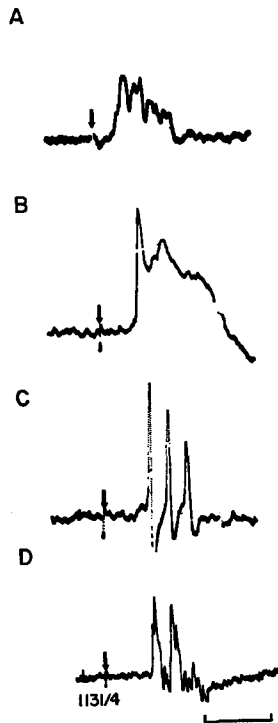


FIGURE 13. (A) is a recording of responses from afferent fiber; (B) of EPSP from motoneuron; (C) of extracellular spikes from the motoneuron; and (D) of VR reflex from a shock to TIB in a fetus of 54 ± 1 days' gestation. The EPSP in B was about 10 mv. Horizontal bar is 30 msec.

volley recorded from the DR had several peaks indicating the presence of several fiber groups with different conduction velocities. In the case of GS stimulation, afferent fibers could follow repetitive shocks as fast as TIB or PER, but the VR reflex barely followed shocks given once per second in the fetus of about 50 days' gestation. However, shocks given to TIB or PER could often evoke repetitive firing of the motoneuron, and the VR reflex from these nerves could follow repetitive stimulation much faster than in the case of GSs.

Fig. 13 shows the relationship between the long lasting synaptic potential and the afferent volley produced by a single stimulus delivered to TIB. The afferent volley (A), EPSP (B), spikes (C), and VR reflex (D) were recorded

from a fetus of 54 ± 1 days' gestation. The afferent volley was recorded from the DR which was cut close to the cord after the other records (EPSP, spikes, and VR reflex) were obtained. The afferent volley (A) recorded from the DR had four major peaks probably representing four groups of fibers of different conduction velocities. The latency of the first peak was 13 msec., corresponding roughly to a conduction velocity of 1 to 2 m/sec. This is much slower than the 10 m/sec. observed by Skoglund (1960 *c*) in the newborn kitten. B is a long lasting EPSP with multiple peaks characteristic of activation by PER or TIB stimulation. C shows three spike potentials, probably fired from the three peaks on the EPSP, recorded extracellularly from a motoneuron. As is apparent from the VR reflex in D, three out of four peaks in the afferent volley were transmitted through the intraspinal pathway and appeared in the VR. Even in a fetus of 1 to 2 weeks before birth, an EPSP obtained by shock to PER could follow fairly high frequency stimuli which usually resulted in a prolonged compound EPSP due to summation and also in repetitive firing of the motoneuron. Possibly the repetitive firing was the result of continuous impingement on the cell by an afferent volley composed of several components arriving successively. In the adult cat the deep peroneal motoneuron often fires repetitively in response to stimulation of muscle or cutaneous nerve, and such "repetitive cells" are characterized by large and long compound synaptic potentials similar to the ones observed in Fig. 13B (Wilson and Burgess, 1962).

At this stage of development differences exist between the pure muscle nerve such as GS and the mixed nerve such as TIB or PER, and the fetal motoneuron is capable of firing repetitively in response to stimulation of the mixed nerve as it does in the adult cat.

DISCUSSION

I. *Nature of the Potential Recorded*

Except for the giant extracellular responses it may reasonably be assumed that the records shown are intrasomatic as both axons and dendrites of the fetal cord are thin and present very small targets for the microelectrode.

Our results may have been influenced by the experimental conditions. Detachment of the fetus from the mother, the use of artificial respiration, or the occasionally inadequate control of anesthesia or body temperature might all have affected neuronal responses. For example, anoxia has a profound effect on the fetal nervous system (Windle and Becker, 1940). Also pulsation and the relatively small size of the neurons often resulted in unstable recording.

II. *Components of the Spike*

Similarities between immature and adult motoneuron spikes with regard to "A" and "B" components suggest that the same sequence of invasion occurs

in both. That is, for orthodromic as well as for antidromic excitation, the axon hillock fires first and the soma subsequently. These conclusions apply to fetuses as young as 40 days' gestation.

In the adult cat motoneuron a typical "AB" spike potential is followed by a brief phase of DAP and then a longer lasting HAP (Eccles, 1957). In the fetus, on the other hand, no DAP comparable to that in the adult was recorded from a cell in good condition following a single stimulus. With repeated stimulation a DAP similar to the one observed in the adult cell began to accompany the spike potential. This phenomenon could be observed in the fetus at as early as 50 days' gestation the cells of which have a very different shape from the adult cell (Windle, 1930, and our unpublished observation). The DAP observed in the fetus started from a rather sharp inflection on the foot of the spike potential and was absent when the cell had deteriorated. This can be explained by assuming a strong conductance change following the spike toward an equilibrium potential of about 60 mv (inside negative) as in the adult cell (Eccles, 1957). On the basis of this explanation, the absence of a DAP in fetal motoneurons could mean either an equilibrium potential near the resting potential for the postspike conductance change or the absence of such a change. The appearance of the DAP with multiple stimulation suggests that an area of membrane such as the soma or bases of the dendrites not normally invaded has become active and exhibits the DAP phenomenon. Similarly, the DAP may indicate activity in the dendrite as found in some neurons (Hild and Tasaki, 1962). A preceding invasion of the soma may influence the degree of excitability of the dendrite and cause some part of the dendrite not previously activated to become invaded.

Another interesting difference between the adult and fetal cell is the type of "A" spike shown in Fig. 5. Some of the motoneurons of the early fetus differ from those of the adult cell by having a sharp demarcation between axon hillock and soma and between dendrites and soma (Windle, 1930, and our unpublished observation). As development continues, these demarcations become less sharp until it becomes quite difficult to make any clear distinction in the adult cell. The shapes of these cells might fit with the finding that some "A" spikes are relatively constant in amplitude, may fail to elicit "B" spikes, and show an exponential falling phase like that of the dendrite-free ganglion cells of *Aplysia* (Tauc, 1962).

The giant extracellular response from the motoneuron of the adult cat spinal cord was analyzed in detail by Freygang and Frank (1959), who concluded that this type of response was to be expected from inactive membrane close to the pipette. The present results obtained from the immature motoneuron are quite similar to those from the adult cell except that we could record a giant "A" spike not disturbed by a preceding soma invasion (Fig. 9). Though failure of invasion of "B" area or soma might have been due to deterioration of the cell or to external pressure of the electrode, it is an in-

interesting observation that the "A" spike which was not affected by a preceding "A" area invasion showed a very small negative potential, while the second "A" spike showed a negative phase which could be correlated with the appearance of the hump on the "A" spike. This may imply that the negative phase of the "A" spike was an indication of partial invasion of the soma area. The large diphasic "B" spike would then have to be attributed to a further invasion of the soma-dendrite area.

III. *Synaptic Activation of the Fetal Motoneuron*

Several authors (Malcolm, 1953, 1955; Skoglund, 1960 *a, b, c*; Arutyunyan, 1961; Wilson, 1962) have reported on the recording of the VR reflex by stimulation of DR or peripheral nerves in the kitten. Their results agree in that the monosynaptic pathway is already functional at birth. Wilson (1962) recorded intracellular responses from a motoneuron of an 8 day old kitten and concluded that the reflex discharge was monosynaptic. He also observed a marked similarity between intracellular responses of kitten and adult. Despite the long latencies observed, the shape of the EPSP shown in some records strongly suggests that these EPSPs were activated by a highly synchronous monosynaptic volley.

According to Wilson (1962) the central delay of the reflex in a 3 day old kitten was 1.8 to 2.3 msec., while in a fetus 3 weeks prenatal we found the latency to be 10 to 20 msec. Though this central delay time includes conduction time in both the DRs and VRs, it illustrates a feature of central transmission in the immature animal. The slow central transmission in the immature spinal cord was mainly due to the slow rise time of EPSP as well as to the slow conduction in the afferent collaterals as suggested by Wilson. Though there is some objection to the conclusion of Hursh (1939) that the slow conduction velocity in the immature animal was compensated well by the smaller dimension of the animal (Skoglund, 1960 *a*), the slow rise time of synaptic potential seems to be the limiting factor in the fetal CNS. The slow rise time of the synaptic potential can be attributed to the asynchronous impingement and also to the slow reaction at the site of transmitter release.

Morphologically, monosynaptic reflex arcs could first be observed in the fore leg in a cat embryo of 23 days' gestation (Windle, 1934). Furthermore, in a 23 day old embryo, Windle observed "a significant number of axon and collateral terminations of primary sensory fibers in close approximation to the dendrites and cell bodies of primary motor neuroblasts." According to Coronios (1933) the extension or flexion reflex of the hind leg of the kitten fetus was observed at "fertilization age of 28 to 29 days." These behavioral and morphological studies show that the reflex arc is established 2 to 3 weeks earlier than could be shown electrophysiologically.

IV. *Change in Spike and Synaptic Potential during Development*

One of the primary interests of this study was to find possible changes during pre- and postnatal development of the spinal cord at the single cell level. However, this has the inherent difficulty that the younger the animal the less stable the recording, making it extremely difficult to sample typical responses from each stage of development. As described under Technique the amplitude of the spike potential was used as the main criterion in selecting records. Table I lists several parameters of the responses shown in this paper which were the result of considerable selection. In this table records from a

TABLE I

	Fetus No.				Adult
	949	1006	1237	879	
Age	17 to 18 days before birth	15 to 16 days before birth	Within a week of term	2 days after birth	1-2*
Spike duration, <i>msec.</i>	2.6	3	1.2	2	1-2*
Spike height, <i>mv</i>	70	More than 50	60	80	
Slope of EPSP, <i>v/sec.</i>	1-2	2.4	7.8	13	50‡
Rise time of EPSP, <i>msec.</i>	6	5	1.8	1.6	1.0‡

* Frank and Fuortes, 1955.

‡ Eccles, 1957.

kitten and three fetuses are listed: two fetuses 2 to 3 weeks prenatal, one a week prenatal, and the fourth a 2 day old kitten. Though the days of gestation were nearly the same in the two youngest animals, one (No. 949) weighed less than 30 gm and the other (No. 1006) 50 gm, suggesting that the former fetus was studied earlier in development. The cells shown in this table produced spike potentials of more than 50 mv; they were assumed to be normal, and not severely damaged by impalement of the electrode. As is apparent from the table, as much as a week before term the duration of spike potential was less than 2 msec. which was comparable to the duration of spikes from adult motoneurons (Frank and Fuortes, 1955). Even in the fetus of less than 30 gm the spike duration was about 3 msec. The slope of the EPSP rising phase in a fetus about 3 weeks prenatal was less than 2 v/sec., increasing to 8 v/sec. in a fetus a week prenatal and up to 13 v/sec. in the 2 day old kitten. The rise time of the EPSP also steadily shortened from 6 msec. in a fetus 3 weeks prenatal to 1.6 msec. in the 2 day old kitten.

These observations reveal two interesting points: (a) Generally speaking, the duration of the spike potential (probably of the soma) remains nearly the same during the late prenatal stage. It could be suggested that the membrane of the soma, as far as its ability to generate spikes is concerned, had acquired properties similar to those of the adult cells at a fairly early age. (b) The EPSP, on the other hand, was very slow at an early stage of development but steadily decreased in rise time, this change being most remarkable in the last weeks of prenatal life. (This is somewhat in contrast to the Renshaw type IPSP which does not undergo such a remarkable change during the same period, as described by Naka, 1964.)

It is also interesting to note that, contrary to the increase in the speed of EPSP in the late prenatal period, the fetuses are known to become less responsive to external stimuli in the late prenatal period than they were at earlier stages (Windle, 1940). As we could find no indication that motoneurons have developed a high threshold, it seems to suggest the establishment of supraspinal control of the motoneuron at this period of prenatal life. Windle also pointed out that there is little spontaneous motor discharge in the fetus of this late developmental stage in the absence of afferent impulses. If this is the case, it can be suggested that the synaptic mechanism of the spinal cord, especially that of the excitatory pathway, increases its efficacy without any apparent external stimulus.

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