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DEVELOPMENT OF SPINAL REFLEXES IN THE RAT FETUS STUDIED *IN VITRO*

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SUMMARY

1. The onset and development of spinal reflex activity was investigated using the isolated spinal cord of the rat fetus. The potential changes generated in motoneurones were recorded extracellularly from L3 ventral roots.

2. A spike potential was recorded from the ventral root at embryonic day 13·5 in response to stimulation of the cord surface close to the ventral root. The discharge persisted in Ca^{2+}-free solution but was blocked by tetrodotoxin.

3. At embryonic day 14·5, trans-synaptically evoked discharges were detected in motoneurones.

4. Stimulation of the dorsal root was first effective in eliciting reflex discharges at embryonic day 15·5. The reflex response then consisted of a prolonged depolarization upon which were superimposed small spikes, and was probably polysynaptic.

5. A spike potential, presumably a monosynaptic reflex, was generated at the end of fetal life. This discharge appeared first at embryonic day 17·5 in a primitive form.

6. Between embryonic day 16·5 and 17·5, stimulation of the dorsal root of different segments (L1–L6) elicited responses similar to those induced by the corresponding (i.e. L3) dorsal root stimulation. These inter-segmentally induced responses were then reduced in size toward the birth. However, in the presence of strychnine, a train of spike discharges of similar shape to the segmentally induced response was also evoked by stimulation of the dorsal root at L4 or L5. These spikes disappeared during further post-natal development.

7. It is concluded that synapses in the segmental polysynaptic pathway become functional in a retrograde sequence with respect to the direction of normal reflex impulse flow. The reflex responses, elicited by stimulation of the dorsal roots of different segments, are suggested to be suppressed first by the development of inhibitory mechanisms and then by neuronal cell death or by elimination of the synapses responsible for generating the inter-segmental reflexes.

INTRODUCTION

Electrophysiological studies of spinal cord development have been carried out in the cat fetus (Naka, 1964*a*, *b*) and the young kitten (Eccles & Willis, 1963, 1965; Eccles, Shealy & Willis, 1963; Kellerth, Mellström & Skoglund, 1971; Mellström, 1971*a*, *b*; Skoglund, 1960*a*, *b*, *c*; Wilson, 1962). In these studies, the electrical

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characteristics of the motoneurone and of its excitatory and inhibitory synaptic inputs were investigated. A monosynaptic reflex discharge was already observed in the youngest fetus examined, and facilitation and inhibition of the reflex discharge were also shown. These studies, however, did not investigate the electrophysiological characteristics of the cord at a stage when synapses were just beginning to function, because it was difficult at such an early stage to keep the fetus alive out of the uterus during the experiment (Nake, 1964*a*).

There have also been studies *in vivo* using chick embryos, in which only spontaneous burst discharges and unit activities were recorded from the cord (Provine & Rogers, 1977; Provine, Sharma, Sandel & Hamburger, 1970; Sharma, Provine Hamburger & Sandel, 1970).

In studies *in vitro*, the rat fetal spinal cord has been cultured in a sliced form and investigated from a synapse-free stage to a stage at which complex bioelectric activity could be recorded (Crain & Peterson, 1963, 1964, 1967; Peterson, Crain & Murray, 1965), but in these preparations, however, most of the neuronal connexions had been severed.

To study the development of spinal cord electrophysiologically, a preparation is required which is relatively intact and survives during the course of an experiment. Recently, a suitable preparation namely isolated spinal cord of new-born rats has been developed (Otsuka & Konishi, 1974, 1976). With this preparation, stable recordings of monosynaptic and polysynaptic reflex discharges are possible for many hours. In addition, it is possible to investigate the effects of drugs and ions in controlled concentrations (Konishi & Otsuka, 1974; Otsuka & Konishi, 1974, 1976). By applying this method to the fetal spinal cord, the onset and development of spinal reflexes have been studied in the present experiments. A part of the present results has appeared in a preliminary form (Saito & Ohga, 1977).

METHODS

Wistar rats were used in the present experiments. Fetuses at various developing stages were prepared using the methods described by Vaughn & Grieshaber (1973). Several female rats were placed together with a male in a cage from 9 p.m. until 9 a.m. the next morning. When sperm was found in the vaginal smear, the age of the fetus was designated as embryonic day 0·5.

The fetuses were removed by caesarean section from mother rats that had been stunned and bled to death. The fetus was decapitated and fixed supinely in a dissection chamber filled with a nutrient medium and bubbled with 95% O_2 and 5% CO_2. Under a binocular stereoscopic microscope, the thoracic and abdominal cavities were opened and emptied. By removing the ventral body the spinal cord was exposed and isolated together with the dorsal and ventral roots and dorsal root ganglia. The isolated spinal cord was hemisected sagittally and placed in an experimental chamber that was filled and perfused with a nutrient medium of the following composition (mM): NaCl, 124; KCl, 5; $MgSO_4$, 1·3; $CaCl_2$, 2·4; KH_2PO_4, 1·24; $NaHCO_4$, 26 and glucose, 10. This medium was also bubbled with a 95% O_2 and 5% CO_2 gas mixture and kept at 30 ± 1 °C.

New-born rats and fetuses near the end of the embryonic stage, which breathed after the removal from the uterus, were anaesthetized with ether before decapitation. The mother rats and new-born rats were killed usually between 10 and 11 a.m.

The potential changes generated in the motoneurones at the lumbar segments were recorded extracellularly from the ventral root by the method of Otsuka & Konishi (1974) with slight modifications. From embryonic day 15·5, lumbar segments could be distinguished from the thoracic segment by the lowest rib. The recording electrode was a glass suction electrode. The tip of the electrode was connected by a polyethylene tube to a glass capillary that had ap-

proximately the same inner diameter as the ventral root in order to ensure that the ventral root could be introduced tightly into the electrode. This recording electrode was connected through a chlorided silver electrode to a preamplifier and then to an oscilloscope. The chamber was grounded through a reference calomel electrode. Under these conditions, stable DC recording was possible for more than 10 hr. Electrical stimulation (a single rectangular pulse of 0·03 or 0·3 msec and supramaximal intensity) was applied to the dorsal root through another suction electrode. In some cases, the spinal cord was directly stimulated between a glass capillary electrode filled with the nutrient medium and a chlorided silver plate-electrode beneath the preparation (a single rectangular pulse of 1 msec at supramaximal intensity). Drugs were added to the perfusing solution to give the final required concentration.

Under the present experimental conditions, there was no sign of development of reflex responses after the isolation of the spinal cord; the evoked potential did not alter in wave form though it usually increased in amplitude during the early period of the experiment presumably due to the improvement of sealing between root and electrode.

RESULTS

Onset and development of segmental reflexes

Synapse formation on the motoneurones. The youngest fetal stage examined was embryonic day 13·5. In these preparations, the dorsal root ganglia were attached to the cord and the dorsal and ventral root were barely visible under a binocular stereoscopic microscope. The lumbar enlargement was not yet developed.

When the lumbar segment of the spinal cord was stimulated with a single shock in close proximity to the ventral root, a spike potential with a short latency was recorded from the ventral root (Fig. 1*Aa*). This potential was not abolished in a Ca^{2+}-free, Mg^{2+}-elevated (7 mM) medium containing glycol-etherdiamine-*N*,*N*,*N'*,

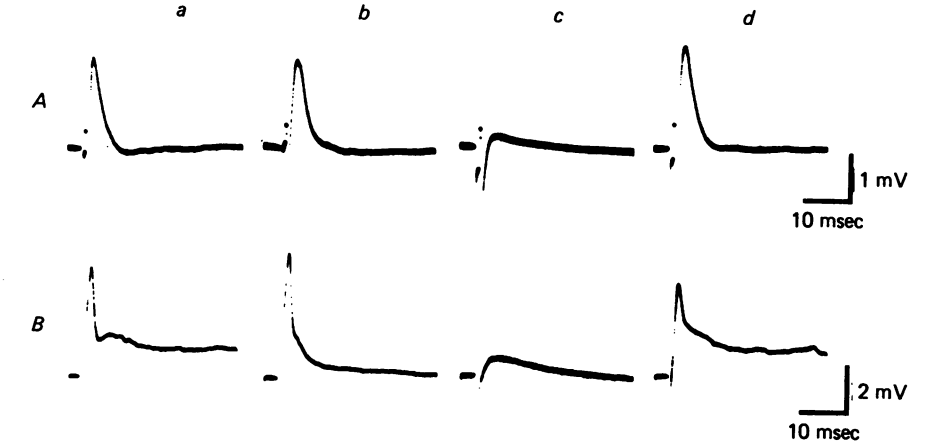

Fig. 1. Electrical activity of motoneurones at embryonic day 13·5 (*A*) and 14·5 (*B*). The spinal cord was directly stimulated in close proximity to the ventral root with a single square pulse (1 msec, supramaximal intensity) using a capillary electrode 100 μm in diameter. In *A* and *B*, the recordings show the evoked responses observed in normal Krebs solution (*a*), 0-Ca^{2+}, high-Mg^{2+} (7 mM) solution containing 1 mM-GEDTA (*b*), normal Krebs solution with tetrodotoxin (2×10^{-8} g/ml.) (*c*), and after returning to normal solution (*d*). The records are shown as a single oscilloscope sweep. Ca^{2+}-sensitive depolarization following spike potential is seen at embryonic day 14·5 but not at 13·5.

N'-tetraacetic acid (GEDTA; 1 mM), but disappeared after application of tetrodotoxin (2×10^{-8} g/ml.) (Fig. 1*Ab*, *Ac*). This suggests that the spike potential was not evoked trans-synaptically, but was generated by a direct activation of the motoneurones. Consistent with this physiological observation, is the report that in the cervical spinal cord of the rat fetus at embryonic day 12·5 neuroblasts in the motor column develop into multipolar shapes (Windle & Baxter, 1935–1936) and their axons grow into the ventral roots (Vaughn & Grieshaber, 1973).

By embryonic day 14·5, stimulation of the cord surface in immediate proximity to the ventral root evoked a spike potential similar to that observed at embryonic day 13·5 but this was followed by a depolarization on which small discharges were superimposed (Fig. 1*Ba*). This depolarization (half-decay time of 662 ± 198 msec; mean $\pm$ S.E. of mean, $n = 7$) was never seen in the four embryonic day 13·5 spinal cords examined, but was observed in all of the nine preparations at embryonic day 14·5. In contrast with the spike potential, the depolarization disappeared in Ca^{2+}-free, high-Mg^{2+} (7 mM) solutions (Fig. 1*Bb*), so that it is reasonable to assume that the depolarization represents the onset of synaptic transmission to the motoneurone. A similar depolarization, with superimposition of small spikes, was sometimes generated spontaneously. This spontaneous activity was also abolished after the removal of Ca^{2+} from the medium, suggesting again that it was evoked trans-synaptically. According to the recent electron microscopic study of the cervical segment of the rat fetus, immature synapses first occur at embryonic day 13·5 in the motor neuropil (Vaughn & Grieshaber, 1973).

Transmission from the primary afferent neurones to motoneurones was not observed at embryonic day 14·5, since stimulation of the dorsal root or the cord near the dorsal root was ineffective in producing any detectable potential changes in the ventral root.

Reflex discharges. Stimulation of the dorsal root or the dorsal root ganglion was first effective at embryonic day 15·5, in producing a prolonged depolarization and superimposed small spikes within the L3 ventral root (Fig. 2*A*). This response was characterized by a slow rising and falling phase each with a duration of several hundred msec or more, that varied from preparation to preparation (Table 1). These reflex responses seem to be polysynaptic in view of the report by Vaughn & Grieshaber (1973) that up to embryonic day 15·5, collaterals of the primary afferent fibres are predominantly involved in disynaptic pathway in the cervical segment.

The development of the segmental reflex response is demonstrated in Fig. 2. At embryonic day 16·5 (Fig. 2*B*), mean half decay time of the response is longer than at embryonic day 15·5, whereas mean time to peak depolarization is not statistically altered, although the conduction time within the cord is significantly shortened ($P < 0{\cdot}05$, Table 1).

At embryonic day 17·5, another short lasting depolarization appeared before the prolonged depolarization in eight out of nine preparations at this stage (Fig. 2*Cb*). With further development, this short lasting depolarization developed into one or several spike potentials with a concomitant reduction in amplitude and duration of the late prolonged depolarization (at embryonic day 19·5, Fig. 2*D* and *E*).

At the end of fetal life, the pattern of reflex discharges developed into the same as that observed in the new-born animal by others (Otsuka & Konishi, 1974), consisting

of an early spike potential and late small amplitude depolarization (Fig. 2*F*). The conduction time of the reflex response within the cord, time to peak depolarization, half-decay time of the late prolonged depolarization, and the relative amplitude of the first depolarization to the second one are shown in Table 1 at five embryonic days from 15·5 to 21·5.

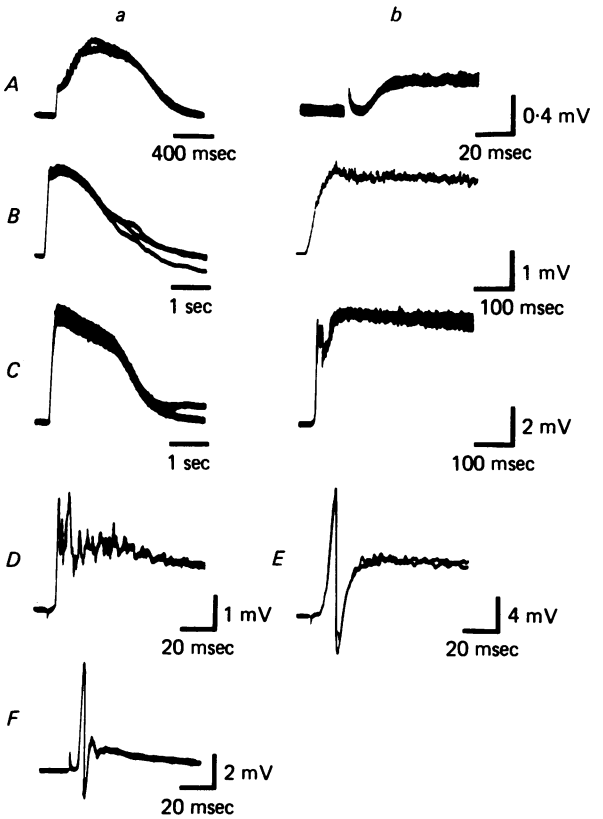

Fig. 2. Reflex discharges observed at various fetal stages; embryonic day 15·5 (*A*), 16·5 (*B*), 17·5 (*C*), 19·5 (*D* and *E*) and 21·5 (*F*). Stimulation (0·3 msec in *A* and 0·03 msec in B–F, supramaximal intensity) was delivered to the dorsal root at L3, and the reflex response was recorded from the corresponding ventral root. In *b*, the records are shown at faster sweep speed but at the same amplification to show latency and rising phase (different responses). Two to three sweeps are superimposed except in *Bb* and *D* (single sweep). Prolonged depolarization is evoked as the first reflex response at embryonic day 15·5 (*A*), then another short lasting depolarization appears before the prolonged depolarization at embryonic day 17·5 (*Cb*). A single or multiple spikes, and always a single spike followed by slow depolarization are seen at embryonic day 19·5 (*D* and *E*) and 21·5 (*F*), respectively.

Onset and development of inter-segmentally induced reflex

Reflex discharge induced by stimulation of the dorsal root of different segments. The potential changes generated in motoneurones in response to stimulation of the dorsal root from L1 to L6 were recorded from the ventral root of L3. At embryonic day 16·5, a similar pattern of response was evoked following stimulation of the dorsal root of

TABLE 1. Comparisons of segmentally induced reflex responses at five embryonic days concerning latency within the cord, time to the peak depolarization, half decay time of the prolonged depolarization and relative amplitude of the early peak to the second one

Embryonic day	Latency (msec)	Time-to-peak (msec)		Half-decay time (msec)	
					Relative amplitude of first peak to second one
		Time-to-early-peak (msec)	Time-to-late-peak (msec)		
15·5	10·8 ± 0·7 (5)	430 ± 131 (8)		611 ± 201 (8)	
16·5	8·2 ± 0·7*** (6)	277 ± 121 (7)		1346 ± 197*** (7)	
17·5	8·8 ± 0·8 (9)	15·9 ± 2·7 (8)	48·7 ± 4·5*** (9)	1282 ± 203 (9)	1·3 ± 0·2 (9)
19·5	5·5 ± 0·4* (10)	3·5 ± 0·5* 10)	15·9 ± 1·6* (10)	113 ± 50* (9)	2·0 ± 0·2* (10)
21·5	5·0 ± 0·3 (17)	2·1 ± 0·1* (17)	7·9 ± 0·9* (17)	—	3·4 ± 0·4** (17)

Values are mean ± S.E of mean (no. of preprations). *, **, *** indicate values significantly different from those listed just above them (compared by Student's t test, $P < 0.01$, 0·02 and 0·05, respectively).

TABLE 2. Comparisons of reflex responses recorded from the L3 ventral root following stimulation of the L1–L6 dorsal roots concerning latency within the cord, time to the peak depolarization, half decay time of the prolonged depolarization and appearance rate of the early depolarization

Embryonic day 16·5

	Latency (msec)	Time-to-peak (msec)	Half-decay time (msec)
L1	15·3 ± 2·9 (3)	195 ± 103 (3)	1200 ± 306 (3)
L2	13·3 ± 3·5 (3)	345 ± 169 (4)	1390 ± 213 (4)
L3	8·2 ± 0·7 (6)	277 ± 121 (7)	1346 ± 197 (7)
L4	11·0 ± 1·0 (2)	480 ± 232 (3)	1433 ± 33 (3)
L5	25·0 ± 15·0 (2)	570 ± 276 (3)	1447 ± 29 (3)
L6	36·0 ± 24·0 (2)	907 ± 372 (3)	1467 ± 114 (3)

Embryonic day 17·5

	Latency (msec)	Time-to-early-peak (msec)	Time-to-late-peak (msec)	Half-decay time (msec)	Appearance rate of early depolarization %
L5	17·9 ± 1·2 (8)	17·6 ± 0·4 (2)	53·4 ± 13·3 (8)	1603 ± 275 (8)	25
L3	13·2 ± 0·9* (9)	14·2 ± 11·1 (5)	44·4 ± 11·2 (9)	1458 ± 220 (9)	56
L3	8·8 ± 0·8* (9)	15·9 ± 2·7 (8)	48·7 ± 4·5 (9)	1282 ± 203 (9)	89
L4	12·9 ± 0·8* (9)	16·9 ± 2·6 (7)	54·2 ± 8·3 (9)	1896 ± 451 (9)	78
L5	14·6 ± 2·0 (9)	14·5 ± 1·4 (5)	69·1 ± 15·8 (9)	1224 ± 219 (9)	56
L6	20·0 ± 2·7 (8)	27·9 ± 10·1 (4)	192·8 ± 78·3 (8)	1388 ± 209 (8)	50

Values are mean ± S.E. of mean (no. of preparations). * indicates values significantly different from those listed just above them (compared by Student's t test, $P < 0.01$).

each segment (Fig. 3*A*). This consisted of a prolonged depolarization upon which was superimposed many small spikes. In each experiment, the latency of the response was longer as the segment stimulated was further from the recording site, though the mean values were not significantly different (Table 2).

In preparations at embryonic day 17·5, another transient depolarization appeared before the prolonged depolarization in response to stimulation of the different dorsal roots (Fig. 3*Bb*), as has been mentioned above in the case of segmentally induced

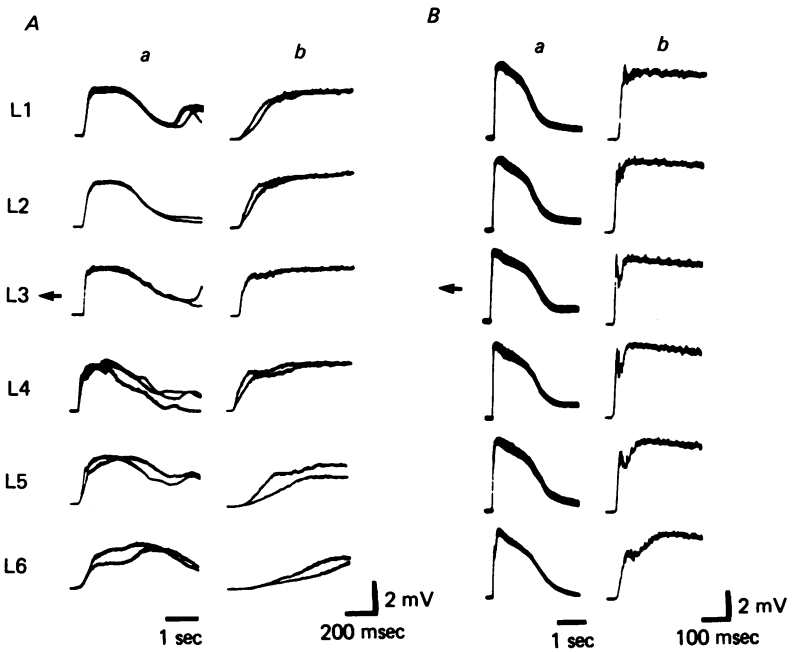

Fig. 3. Segmental (L3 arrow) and inter-segmental (L1, L2 and L4–L6) reflex discharges at embryonic day 16·5 (*A*) and 17·5 (*B*). Stimulation (0·03 msec, supramaximal intensity in this and the following Figures) was delivered to the dorsal root at L1–L6, and the reflex responses were recorded from the ventral root at L3. In columns *b*, records are shown at faster sweep speed. Two to three sweeps are superimposed in *A* (*B*; single sweep). At embryonic day 16·5, spontaneous discharges sometimes follow the reflex response (e.g. *Aa*, L1), but segmental and inter-segmental reflex discharges are somewhat similar in both amplitude and duration. This situation is similar at embryonic day 17·5, but another transient depolarization appears in inter-segmental responses (e.g. *Bb*, L4) as well as in segmental one.

reflex. This early depolarization was observed more frequently when the segmental or adjacent dorsal root was stimulated (Table 2).

As the fetus grew older, the early transient depolarization evoked by stimulation of the dorsal root at L3–L5 developed into a spike potential at embryonic day 19·5 (Fig. 4*A*). Stimulation of the L4 dorsal root induced larger responses than that of the L3 dorsal root (in six out of seven preparations). But the responses induced by stimulation of the L1, L2 and L6 dorsal roots were always smaller (Fig. 4*A*). Amplitude of the spike potential evoked by stimulation of the L4 or L5 dorsal root then

decreased in six out of eleven preparations (less than 60% of segmentally induced one), or remained large in five others (more than 60%) at embryonic day 21·5.

Effects of picrotoxin and strychnine. Picrotoxin and strychnine had little effect on the reflex discharge at stages before embryonic day 19·5, suggesting that pre- and post-synaptic inhibitory mechanisms are not functioning at this stage. From this stage on, the reflex response, especially the later part of the discharge, was enhanced by the application of picrotoxin (1×10^{-5} g/ml.). Strychnine (5×10^{-6} g/ml.) was,

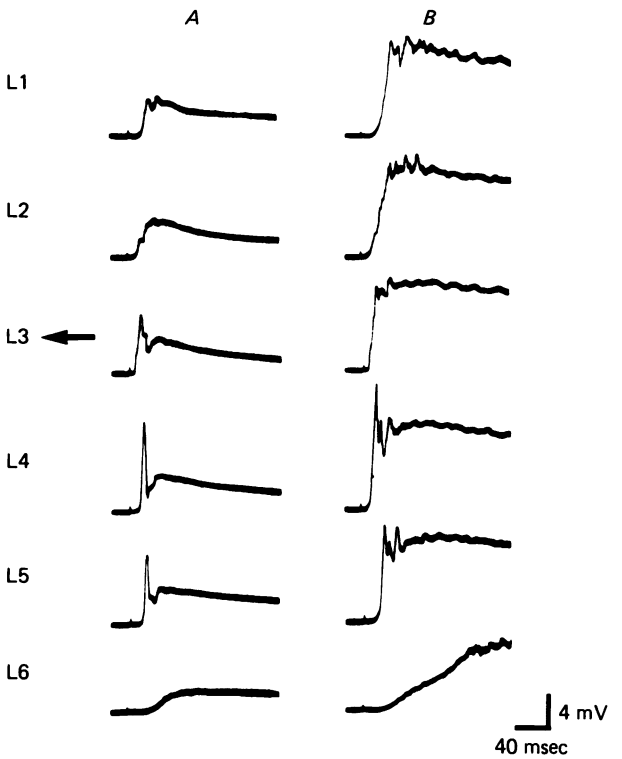

Fig. 4. Segmental (L3 arrow) and inter-segmental (L1, L2 and L4–L6) reflex discharges at embryonic day 19·5. Stimulation was delivered to the dorsal root at L1–L6, and the reflex responses were recorded from the ventral root at L3 in the absence (*A*) and presence (*B*) of strychnine (5×10^{-6} g/ml.). Records are shown in a single sweep. Spike potential larger than segmentally induced one are seen in inter-segmental reflex response (*A*, L4–L5). In the presence of strychnine all the responses are largely enhanced.

however, more effective than picrotoxin in potentiating the early part of reflex discharges. Fig. 4 illustrate such results at embryonic day 19·5. Strychnine enhanced reflex discharges especially those induced by stimulation of the dorsal roots of different segments; stimulation of the dorsal root of L3–L5 then elicited a train of large spike potentials. These observations suggest that at this time inhibitory neurones may become involved in suppression of the reflex response evoked by stimulation of the dorsal roots of different segments.

In new-born animals, the effects of stimulating dorsal roots of the different seg-

ments were also tested and compared to those seen at the end of fetal life. Within a few days of birth, inter-segmental reflex responses became much smaller than the segmental reflex (Fig. 5*A*). Trains of large spikes no longer resulted from stimulation of the dorsal root of neighbouring segments even in the presence of strychnine (Fig. 5*B*). This failure to elicit inter-segmental spike discharges was not due to an alteration in electrical characteristics of the motoneurones as an equivalent stimulus to the corresponding dorsal root still evoked a burst of spikes.

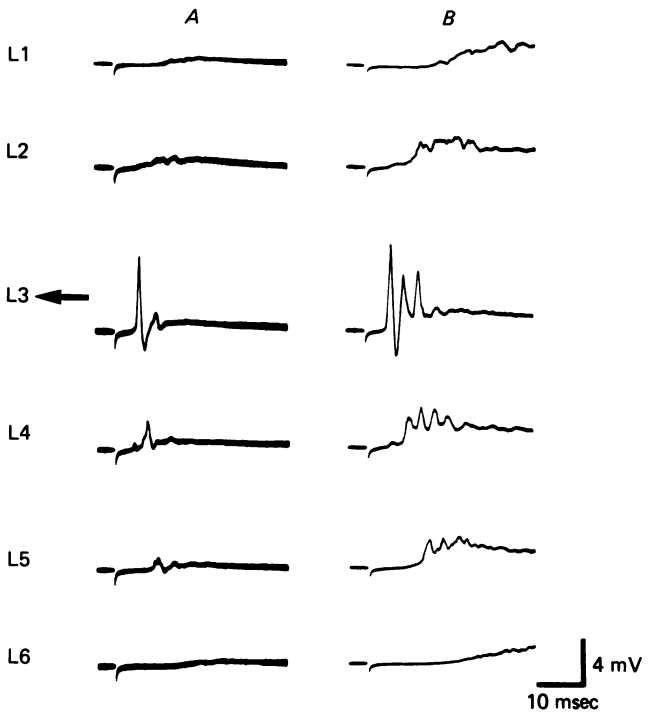

Fig. 5. Segmental (L3, arrow) and inter-segmental (L1, L2 and L4–L6) reflex discharges in a 4-day-old rat. Stimulation was delivered to the dorsal root at L1–L6, and the reflex responses were recorded from the ventral root at L3 in the absence (*A*) and presence (*B*) of strychnine (5×10^{-6} g/ml.). Inter-segmentally induced reflex discharges are very small, and are not markedly enhanced compared with the segmentally induced reflex discharges after the application of strychnine.

At four stages of development, the amplitude of inter-segmentally induced reflex responses have been compared with the segmentally induced response (Fig. 6). The height of the inter-segmentally induced responses (L4 and L5) had increased at embryonic day 19·5, and then gradually decreased with age (filled circles). In the presence of strychnine (open circles), the response was restored to almost the same size as that evoked by stimulation of the corresponding dorsal root at intermediate times (embryonic day 21·5, see responses to stimulation of the dorsal root of L4 and L5 in Fig. 6*C*), but not at later times (Fig. 6*D*; 4-day-old).

DISCUSSION

Characteristics of segmental reflex at early stages. The present experiments have shown that the onset of the reflex discharges occurred at embryonic day 15·5. The reflex response consisted of a prolonged depolarization with rising phase of 120–720 msec and half-decay of 100–1600 msec. This prolonged discharge may be caused by long lasting excitatory post-synaptic potentials (e.p.s.p.s) and an asynchronous firing of the motoneurones. Naka (1964*a*) has shown in motoneurones of the cat

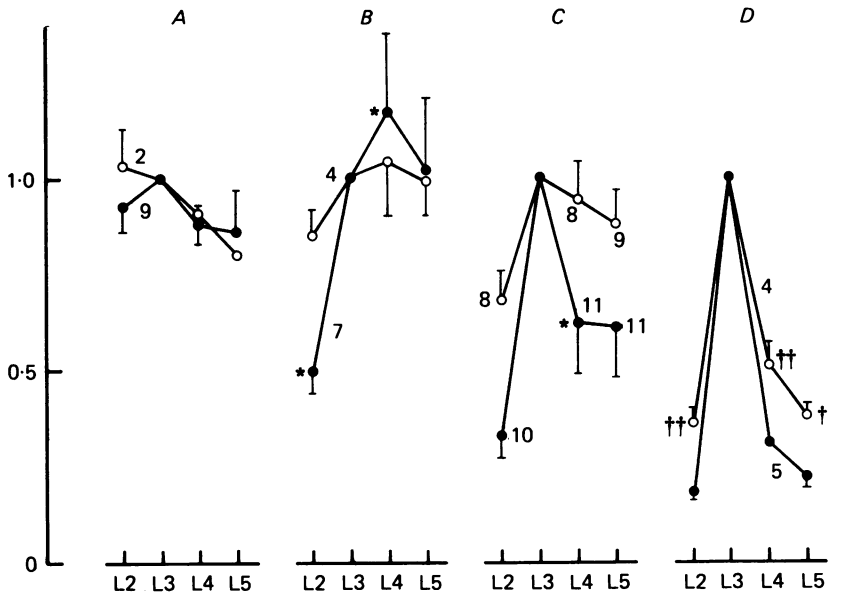

Fig. 6. Comparison of the amplitude of reflex responses induced by stimulation of the corresponding (L3) and neighbouring (L2, L4 and L5) dorsal roots at four stages during development; embryonic day 17·5 (*A*), 19·5 (*B*), 21·5 (*C*) and 4-day-old (*D*). Height of the inter-segmental spike discharge or short lasting depolarization (at embryonic day 17·5; *A*), in the absence (●) and presence (○) of strychnine (5×10^{-6} g/ml.), was normalized with respect to that of the segmental response. When the response did not show a clear peak (e.g. Fig. 5 *B* L1), maximal height of the response was taken. Circles with vertical bars represent mean ± S.E. of mean. Numbers associated with lines (*A*, *B* and *D*) or circles (*C*) refer to the number of preparations. * and † indicate significantly different ($P < 0{\cdot}01$) values from those at the former stage in the absence and presence of strychnine, respectively (††; $P < 0{\cdot}02$). Values were compared by Student's *t* test. Further explanation see text.

fetus that long lasting e.p.s.p.s (about 40 msec in duration) may be recorded following stimulation of the peroneal or tibialis nerves. These long lasting e.p.s.p.s consist of multiple peaks, suggesting that they were due to an asynchronous afferent barrage. In the rat fetus, however, the reflex discharge is much more prolonged, lasting sometimes for a few seconds. This discharge may therefore reflect a repetitive re-excitation of some neurones. These repetitively activated neurones are likely to be internuncial neurones, since activation of a motoneurone in a Ca^{2+}-free medium did not result in a long lasting depolarization.

Monosynaptic discharge. It has been reported that the monosynaptic reflex arc already functions in the spinal cord of the cat at fetal stages (Naka, 1964*a*) as well as at immediately post-natal stages (Kellerth *et al.* 1971; Mellström, 1971*a*, *b*; Skoglund, 1960*a*, *b*, *c*; Wilson, 1962). In the new-born rat, homonymous monosynaptic e.p.s.p.s have been observed by stimulation of the gastrocnemius nerve in the hind limb (Konishi & Otsuka, personal communication). In the present experiments, the early sharp spike evoked by stimulation of the dorsal root in late stage fetuses was similar to that induced in the new-born rat in both wave form and latency. The minimal central delay was 3·6 msec at embryonic day 21·5 which is similar to that in new-born rats (3 msec, Otsuka & Konishi, 1974) but shorter than that in the cat fetus (10 msec, Naka, 1964*a*). In view of the relatively short central delay and the close similarity of the wave form of the reflex response to that recorded in new-born rats and adult cats, it seems reasonable to consider the spike potential observed in the rat fetus at this stage to be a monosynaptic discharge. This monosynaptic reflex discharge was first apparent at embryonic day 17·5 but only in a primitive form seen as an early short lasting depolarization.

Development of inter-segmental reflex. The present experiments have shown that reflex spike discharges may also be evoked by stimulation of the dorsal roots of different segments during embryonic development. There are several possible mechanisms that can be suggested to explain this observation.

First, motoneurones within different segments might interact electrically with each other, or else motoneurones within segments L4 or L5 might send branched axons to the L3 rather than only to the L4 or L5 ventral root. This is, however, hardly tenable since stimulation of L4 or L5 ventral roots did not elicit any response in the L3 ventral root. In addition, activation of motoneurones within segments L4 or L5 by stimulation of the cord surface close to the ventral root evoked a calcium insensitive spike potential in the corresponding ventral root but not in the L3 ventral root. Secondly, impulses in the primary afferent fibres of the L4 or L5 dorsal root might activate ephaptically those of the L3 dorsal root to induce spike potentials in the L3 ventral root. This interpretation, however, is also unlikely since an ephaptic interaction between primary afferent fibres of different segments has yet to be shown; stimulation of the dorsal root of different segments evoked only the dorsal root potential V previously described by Lloyd & McIntyre (1949) which disappeared in Ca^{2+} free medium.

A more likely explanation is that the primary afferent terminals of L4 or L5 dorsal root might also innervate L3 motoneurones either directly, or indirectly, in a similar manner to those of the L3 dorsal root, so that the effect of stimulation of the L4 or L5 dorsal root is similar to that of the corresponding dorsal root stimulation. These afferent fibres are likely to extend close to the L3 motoneurones; in a Ca^{2+}-free medium stimulation of the cord close to the L3 ventral root elicited a spike potential in the dorsal roots at L4 and L5 as well as at L3 (although the response had almost disappeared at the L2 dorsal root). Such long-ranging primary afferent fibres have also been reported in the adult cat (Conradi, 1976; Imai, 1964; Imai & Kusama, 1969; Sprague, 1958; Sprague & Ha, 1964), and these fibres are suggested to connect with motoneurones at different segments.

The spike potential in response to stimulation of the L4 or L5 dorsal root decreased

at the end of fetal life in about half of the preparations. In the presence of strychnine, however, the response was restored to almost the magnitude of that evoked by stimulation of the corresponding dorsal root. This indicates that an inhibitory neuronal mechanism has become effective in suppressing the inter-segmentally induced spike discharge at this stage.

In new-born animals older than a few days, stimulation of the dorsal root of L4 or L5 no longer elicited spike discharges comparable to those evoked by stimulation of the corresponding dorsal root, even in the presence of strychnine. This suggests that the excitatory inputs on motoneurones originating from neighbouring dorsal roots have largely disappeared by this period. This may be consistent with the suggestion that heteronymous monosynaptic e.p.s.p.s are more frequently observed in the spinal motoneurones of the kitten than in the adult cat (Eccles *et al.* 1963). The mechanism involved in the disappearance of the inter-segmentally induced spike discharges may be neurone death or the elimination of synapses, or both. The elimination of the synapses during development has been known in the course of post-natal life in mammals. For example, in the new-born rat and kitten, temporary formation of surplus synapses and their elimination have been described at neuromuscular junctions (Bennett & Pettigrew, 1974; Brown, Jansen & Van Essen, 1976; Redfern, 1970; Rosenthal & Taraskevich, 1977) and at neuro-neuronal junctions (Conradi & Skoglund, 1969; Crepel, Mariani & Delhaye-Bouchaud, 1976; Lichtman, 1977; Ronnevi & Conradi, 1974).

Neurone death in the mammalian spinal cord has been reported to take place during either the fetal stage or in early post-natal life; the number of motoneurones in the mouse spinal cord declines between embryonic day 11–14 (75% reduction, Flanagan, 1969) or during post-natal day 2–3 (20% reduction, Romanes, 1946). In another study, axon counts show that the number of fibres in the ventral root decrease steeply during post-natal day 1–2 in the cervical segments (55% reduction) but not in the thoracic segments in the rat (Fraher, 1974). These findings in the new-born animals may suggest a possible mechanism underlying the present observations. It can, however, only be offered as a tentative suggestion in the absence of detailed knowledge of the situation in the rat lumber spinal cord.

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