Unit Activity in the Isolated Spinal Cord of Chick Embryo, *in Situ*

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Abstract. Unit electrical activity was recorded from single neurons in the isolated lumbo-sacral spinal cord of 14- to 19-day chick embryos, *in situ*. Spinal cord transection was combined with transection of all lumbo-sacral dorsal roots. The spontaneous discharge of cells is confined, for the most part, to the lower two thirds of the cord. A quasilinear reduction in the number of spontaneously active units was found during the developmental period studied. Comparable results were obtained in decentralized cord with sensory inputs blocked by xylocaine.

In chick embryos, spontaneous motility (nonstimulated) has been observed.[12, 20, 24] The activity is characterized by periodicity and uncoordinated, convulsivelike movements of different parts. Its nonreflexogenic nature is shown up to 7 days by the unresponsiveness of the embryo to any adequate stimulation prior to that stage. For later periods, up to 17 days, evidence is provided by deafferentation experiments which show leg activity after isolation and deafferentation of the lumbo-sacral cord in chronic preparations made on the second day of incubation.[12] The hypothesis was proposed that all motility of the chick embryo up to 17 days has its source in self-generated discharges of the ventral half of the spinal cord.[10]

This view is justified by the observations of spontaneous discharges in isolated nervous tissue such as shown by isolated cortex using the "slab-technique."[4, 9, 13, 15, 16] Further evidence of spontaneously firing cells has been shown in a single-unit study of the isolated cortex.[8] Similarly, the presence of a high level of organized electrical activity (EEG) has been observed in the isolated forebrain of the developing chick embryo.[5] Whether these instances are specifically sensory or motor is not explicit. Spontaneous activity resembling EEG has been detected after chronic neuronal isolation of cat spinal cord *in situ*.[17] Spontaneously firing cells have also been found in the sympathetic preganglion of the upper thoracic cord.[1, 3, 14, 19, 23] Polosa[19] also observed this activity in a decentralized and deafferented segment of the spinal cord of the adult cat.

Neurons that can retain their characteristic property of conducting nerve impulses after long periods of isolation *in vitro* have been shown in cultured chick embryo spinal ganglion cells and mouse brain and spinal cord tissue.[6] In

long-term cultures of spinal cord of chick embryo, human, and rat, the repetitive spontaneous discharges continue for periods of minutes.[7]

The present experiments were undertaken to test the possible existence of autonomous discharges or "spontaneous electrical activity" of cells in the acutely isolated spinal cord of 14- to 19-day chick embryos *in situ*. If the lumbo-sacral cord (segments 23–30) in such a preparation is completely isolated by spinal transection (decentralization) and deafferentation, except for the blood supply, any electrical activity observed must be assumed to arise solely within the spinal cord and independently of influences from the higher centers or from peripheral sensory input.

Methods. Details of the procedure for preparing the embryo and the recording procedure have been described previously.[21] Seventy-two embryos, varying from 14 to 19 days of development,[11] were used for the acute surgical isolation of the lumbo-sacral cord roots 23–30. After the embryo had been immobilized with tubocurarine, the dorsal roots of both sides were exposed. The tissue is very fragile in earlier stages; special care was taken while transecting the dorsal roots with iridectomy scissors. Transection was made between roots 21 and 22 with a cataract knife (Fig. 1). After a successful operation, the blood supply remained intact as visualized on the dorsal surface of the cord. Embryos which began bleeding were discarded. Isolation was verified after each experiment. In another set of experiments, 0.5 cc of 0.5% xylocaine (Lidocaine—Invenex) was injected into the leg muscle in lieu of deafferentation following the spinal isolation, as a control for surgical trauma associated with dorsal root transection. In both experimental sets, six animals were used for recording at each of the following ages: 14, 15, 16, 17, 18, and 19 days.

Three M KCl-agar electrodes of 4 to 6 μ tip with resistance between $1/3$ and $1/2$ M Ω were used for extracellular recording. Electrodes were inserted into the left side of the cord at a fixed point, between dorsal roots 25 and 26. The landmark on the dorsal surface of the cord for electrode penetration was a point caudal to a large-sized blood vessel, the position of which was constant in relation to the cord and the glycogen body in all embryos. The angle of penetration of the electrode was roughly constant in all cases (Fig. 1). The electrode was referred to an indifferent electrode on the skin.
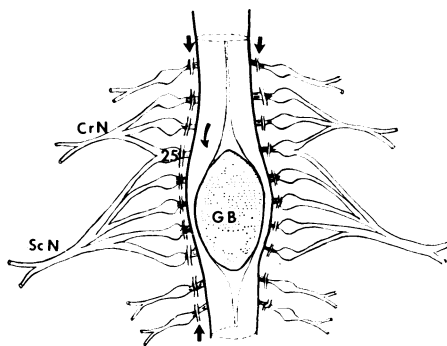

FIG. 1.—Diagram of the isolated lumbo-sacral spinal cord of 17-day chick embryo. Straight arrows point to the site of transection of the dorsal roots; curved arrow indicates the approximate electrode position. Spinal cord was cut at upper and lower extremities (*dots*). *GB*, glycogen body; *CrN*, cruralis nerve; *ScN*, sciatic nerve.

Results. The electrical activity in the spinal cord of the normal embryo (17 days) is shown in Figure 2. In comparison, the isolated and deafferented spinal cord showed almost no activity in the upper third of the cord, and the over-all activity in the lower two thirds of the cord was reduced as compared to normal.[21] Almost all active cells were considered to discharge "spontaneously"; we adopted the operational definition of Amassian[2] for cells of cat cortex—i.e., the cells, when they are initially detected, show a low amplitude and an increase
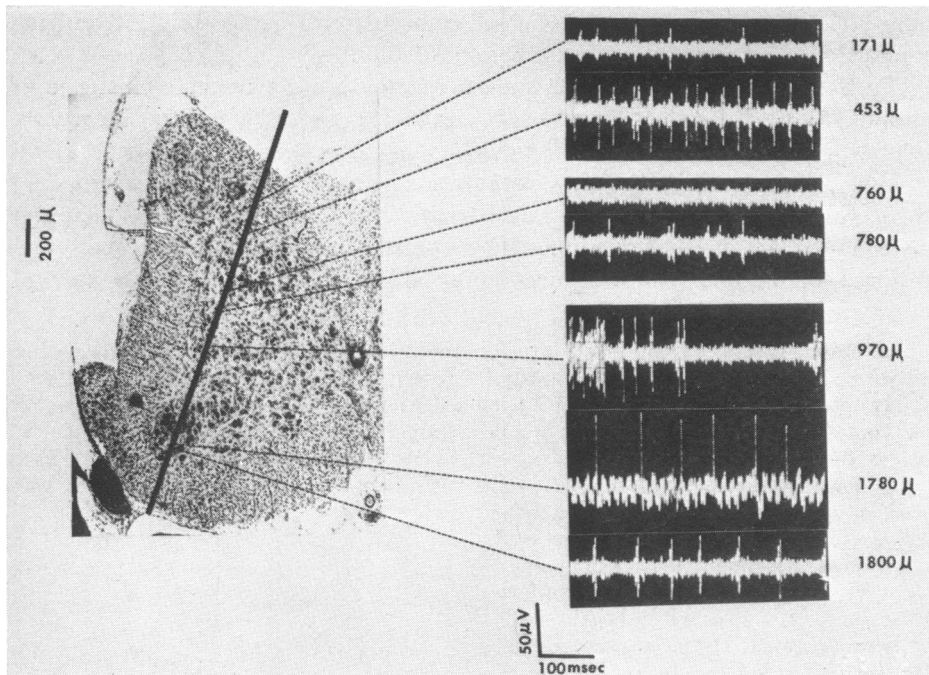
Fig. 2.—Electrode track plotted on the transverse section of the spinal cord of 17-day normal chick embryo at the level of dorsal root 25. Records at right show the activity picked up at the electrode tip at various points along the track. Upper units represent the sensory region. Lower four units are in motor column.

in amplitude as the microelectrode is slowly advanced toward them. They maintain a relatively consistent discharge over a period of time and do not exhibit typical high-frequency injury discharges. They are observed in the absence of any known stimulus.

The number of active units located depended upon the stage of the embryo; it decreased progressively in older embryos (Figs. 3*A* and *B*). Units were found at all depths, from 400 μ down to 2 mm below the surface; most units were located around 800 to 1200 μ. In a few units, the electrical activity was intermittent, active periods being interspersed with silent intervals. In other cases, the initiation of the activity was a large burst (probably a number of cells firing simultaneously) which trailed off into isolated single units. However, most units showed relatively continuous discharges.

In order to be sure that acute trauma caused by transecting the spinal cord and deafferentation does not abolish the leg motility, a few 14- to 17-day embryos were decentralized and deafferented but not curarized. Some motility in the leg returned within 15 min after the operation.

In each stage, six animals with surgical deafferentation at the lumbo-sacral level and decentralization, and six with xylocaine block and decentralization were studied. All penetrations were made at the level of the 25th and 26th dorsal spinal roots.

The microelectrode penetration in 14-day embryos resulted in the detection of an average number of 5.9 ± 0.46 and 6.5 ± 0.73 spontaneously active units per pass for the deafferented and xylocaine treated embryos, respectively. Typically, the first activity was seen at about 500 μ from the surface in experimental embryos as contrasted with the appearance of the first activity at 150 to 200 μ in normal embryos (Fig 2). Figure 4 shows the six units picked up on a single pass in a 14-day embryo. It should be noted that no activity was observed beyond 840 μ in a pass of 1359 μ. Comparable results were obtained when xylocaine was injected into both legs, prepared with spinal transection but no deafferentation (Figs. 3A, 3B, 4). The total number of active units did not
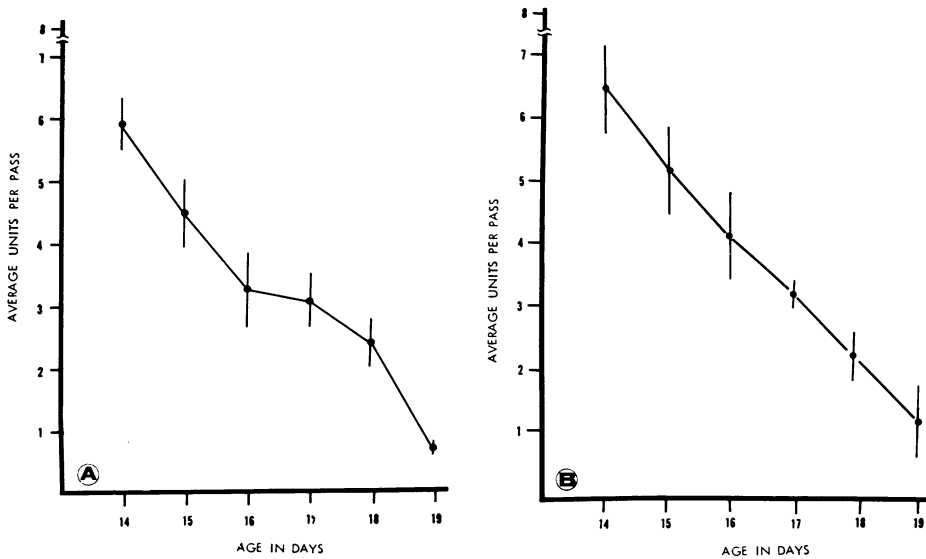

FIG. 3.—(A) and (B) Average units per pass in decentralized and deafferented embryos (A) and decentralized and xylocaine-treated embryos (B) as a function of developmental stages. Bar represents ±1 probable error.

vary markedly between deafferented embryos and embryos treated with xylocaine. There was, however, a variation in the depth at which units were found. This could be expected on the basis of the sampling technique employed in these experiments. The depth at which a given unit is found is associated more with a single pass than with a specific area of the spinal cord, and the results cannot be generalized to the entire cross section of the isolated cord. The results show, however, that fewer units are encountered on a single pass through any given part of the cord than were found in a similar pass in an intact animal.

Figures 5 through 9 show the activity of *all* units detected in selected individual passes in deafferented and xylocaine treated embryos at ages 15–19 days. It is clear that the number of units detected declines progressively during this period. Some features of the firing patterns are worth noting: (a) Most units
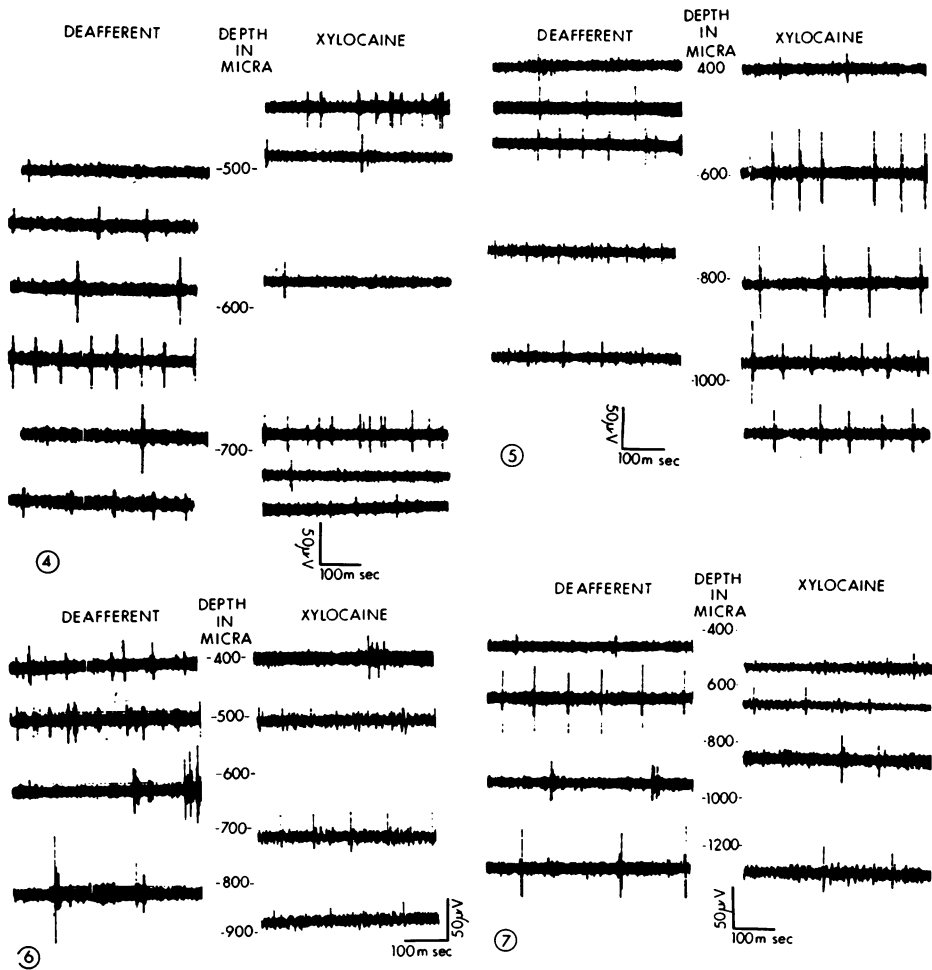
Fig. 4.—Recordings from 14-day embryos. Left column shows units found in decentralized and deafferented lumbo-sacral spinal cord (marked *Deafferent*) and the right column shows all units found in decentralized cord with xylocaine.

Fig. 5.—Recordings of spontaneous discharges in 15-day embryos. Details are the same as Fig. 4.

Fig. 6.—Recordings of spontaneous discharges in 16-day embryos. Details are the same as Fig. 4.

Fig. 7.—Recordings of spontaneous discharges in 17-day embryos. Details are the same as Fig. 4.

fire continuously at regular intervals, however, the rate may differ from unit to unit. (*b*) A few units fire in small bursts of 3–5 spikes with intermittent, longer silent periods. Most of these units, when found, can be held for a fairly long time. (*c*) In all experimental animals, units were invariably found near the bottom of the cord which fired synchronously with the heart beat. Such units were found also in all normal animals.[21] These units were not labeled as "spontaneous units."
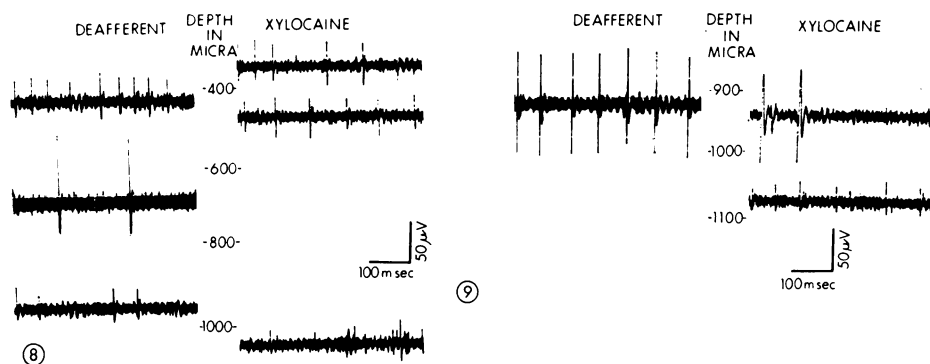
FIG. 8.—Recordings of spontaneous discharges in 18-day embryos. Details are the same as Fig. 4.

FIG. 9.—Recordings of spontaneous discharges in 19-day embryos. Details are the same as Fig. 4.

Discussion. These experiments show that the neuronal network of the developing spinal cord of chick embryos, in the absence of any afferent input, is capable of producing single-unit discharges. The most obvious question that arises from the observation of spontaneous activity is how the activity is generated in the absence of afferent inputs. The evidence that a few cells are still capable of firing after deafferentation and decentralization indicates that some other mechanism must be involved. It is conceivable that we are dealing with (a) internal milieu and environmental factors (metabolites, hormones, change in pH, respiratory influence, etc.); or (b) the release of excitatory transmitters from presynaptic terminals in the spinal cord due to acute transection. The last possibility seems unlikely in the light of observations that isolated cord preparations showed spontaneously firing cells up to 5 hr after the operations.

Sung and Truant[22] showed that in adult rats an injection of 0.2 ml of 2% xylocaine into the leg caused nerve block. It has been found that the duration of the block was approximately 120 min. Use of xylocaine in the present study has been restricted to blocking the sensory nerve activity in the leg and subsequently in the dorsal part of the isolated spinal cord. The activity found in the decentralized cord with xylocaine injected into the leg is comparable to the activity in the totally deafferented and decentralized cord (Figs. 3A and B). The recovery period of activity in the dorsal cord was about 1 hr.

The effects of the spinal and dorsal root lesions giving rise to the activity which we have observed deserve attention. Typically, lesions produce chaotic bursts of firing in single cells.[18] While we see bursts, they are relatively rare and differ little from bursts observed in normal embryos.[21] It is suggested here that the burst activity may result from the simultaneous discharge of several units and that the burst probably is the pattern of firing of certain cells rather than a result of lesion. It is also possible that burst activity may be characteristic of developing systems. An important point of the present findings is that most spontaneously active cells are located around the middle of the cord which

is a heterogeneous population of internuncial, commissural, and glial cells. So far, we have been unable to isolate the cell types responsible for this activity.

We have found that the number of spontaneously firing cells is not constant in all stages of development. There is a quasilinear decrease of these active cells in the developing cord between 14 and 19 days, which indicates some correlation with the maturation of the cord. This decrease may be due to dispersal of the cells in the maturing cord. It is more plausible that cells lose the capacity for spontaneous firing because of either progressive differentiation or increasing inhibitory influence. Because there is a large number of spontaneously firing cells in earlier stages, one cannot exclude the possibility that their spontaneity may be a phenomenon of development alone.

Hamburger et al.[12] completely deafferentated the lumbo-sacral spinal cord of 2-day chick embryos by removing the dorsal half of the cord, including the precursors of spinal ganglia. At the same time, the spinal cord was transected at the thoracic level. The motility of the leg was observed in these chronic preparations at different stages of development. They reported that, at least up to 15 days, embryos showed motility patterns in the leg similar to those of embryos with thoracic gaps of the same stages. A sharp decline of motility was reported in 17-day embryos. Contributing to this decline was the deterioration of the residual basal plate showing a depletion of motor cells probably due to transneuronal degeneration. The observations of Hamburger et al. on chronic animals and the present findings on acute preparations are not directly comparable. The present findings, however, indicate an indirect support of the main body of their hypothesis—that at least up to 15 days of age sensory input is not a prerequisite for the triggering and maintenance of leg motility.[10, 12] Moreover, the markedly reduced spontaneous electrical activity in the 19-day embryos suggests that the initiation of discharges in the later stages may become increasingly dependent on stimulation by various central or peripheral inputs required by the later, more coordinated prehatching activity.

While we have demonstrated the existence of spontaneously firing cells in the isolated spinal cord of the chick embryo, we do not deny the importance of inputs to these cells from either the peripheral sensory apparatus or higher levels of the central nervous system. Experiments presently underway are designed to show the relative contribution of these two groups of inputs.

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