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Electrical Activity in the Spinal Cord of the Chick Embryo, *in situ**

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Abstract. Unit electrical activity was recorded from single neurons in the lumbo-sacral spinal cord of 15-, 17-, and 19-day chick embryos, *in situ*. The dorsal columns showed relatively continuous single-unit activity. Below this lies an area of relative quiet 100–200μ deep. The ventral two thirds of the cord was the most active region, being characterized by polyneuronal bursts and intermittently active single units.

The origin of motility of the chick embryo has been the subject of considerable conjecture. Kuo,[1] Schneirla,[2] and others have proposed that behavior of the embryo is caused by stimulation. Hamburger,[3] in contrast, has hypothesized that embryonic motility results from endogenous activity within the spinal cord. The available evidence supports the hypothesis of spontaneous (nonreflexogenic) motility. Motility, from its onset at $3^1/_2$-4 days up to 7 days of incubation, is nonreflexogenic because no adequate external stimulus is effective in evoking a response.[4] The nonreflexogenic nature of motility in embryos older than 7 days has been demonstrated by experiments utilizing spinal embryos which were deafferented by the removal of the dorsal half of the spinal cord at two days. These embryos showed normal patterns of leg motility at least up to 15 to 17 days.[5] Although the brain influences normal embryonic behavior,[6] and sensory input may influence it, these recent experiments imply that autonomous activity of interneurons or motor neurons in the ventral half of the cord is sufficient to sustain motility up to about 15 to 17 days. On the 17th day, the spontaneous, uncoordinated motility of early stages declines and is superseded by a series of coordinated movements which lead to hatching of the chick on day 21.[7] Sensory and brain inputs may be necessary for the execution of these late appearing, well organized movements.[8]

The behavioral experiments discussed provide only indirect evidence concerning the neural substrate of embryonic behavior. The neurophysiological basis of motility can be revealed by direct examination of the activity of the spinal cord of the embryo. Given an adequate description of the patterns and distributions of single-unit activity in the normal embryonic spinal cord, it should be possible through experimental intervention to show the relative contributions to behavior of sensory, brain, and local cord inputs. The purpose of this preliminary paper is to provide the required description of the normal activity of single neurons in

the spinal cord of chick embryos during the developmental period showing the transition from random to coordinated motility.

Materials and Methods. More than 200 embryos of ages 15, 17, and 19 days were used. These ages are comparable to chick stages 41, 43, and 45.[9] The embryos were raised from fertile eggs of a Kimber strain, flock K137, obtained locally. The development of the embryos was synchronized by Gottlieb's technique.[10] The eggs were incubated in a forced draft incubator which was maintained at a temperature of 37° to 38°C and 70% relative humidity. The eggs were turned daily.

Apparatus: A Grass model P511 amplifier was used for all electrical recording. The total effective gain of the system as viewed at the oscilloscope was ×100,000. Electrical activity was recorded with an Ampex model FR-1300 tape recorder operating at 30 ips. Three-molar KCl-agar micropipettes with tip diameters between 4–6 μ and resistances of $^1/_3$–$^1/_2$ MΩ proved excellent for extracellular unit recording.[11] Occasionally, 25 μ tungsten filaments ensheathed in glass were used. The indifferent electrode, in all cases, was one of the stainless steel wires which were threaded through the skin and used to support the embryo in the shell opening. The electrode was manipulated by means of a hydraulic microdrive which was operated from outside of an electrically shielded, soundproof recording room.

Procedure: The embryos were prepared for recording by opening and removing the shell over the air chamber. An incision was then made in the inner shell membrane and chorioallantoic membrane with fine scissors. The amniotic membrane surrounding the embryo was torn open with forceps, giving access to the embryo. After the embryo was exposed, its dorsal lumbo-sacral area was located and brought to a position beneath the shell opening. The embryo was supported in this position by threading fine stainless steel wires through the skin anterior and posterior to the bulge identifying the glycogen body area of the lumbo-sacral spinal cord. The wires were taped to the outside of the shell. The skin and the connective tissue covering the vertebrae were removed with forceps and fine scissors, exposing the spinal cord and the glycogen body. The glycogen body was left intact when recordings were made in the region of dorsal root 25. Recording at dorsal root 27 necessitated the removal of the obstructing glycogen body with a small suction pipette. In all cases, a drop of mineral oil was placed in the incision to prevent drying of the exposed spinal cord. If embryonic motility was still present after the operation, minimal cord damage was assumed. Any embryo showing hemorrhage or cord damage was discarded. D-tubocurarine (25 μg/gm) was then injected into the leg muscles to immobilize the embryo. The embryo was placed in a chamber which maintained humidity and temperature at incubation levels. Twenty minutes were allowed for the embryo to acclimate before recording was begun. A single experiment usually lasted about 2 to 3 hr. Using the procedures described above, an embryo routinely could be kept viable for 10 to 12 hr.

The lumbo-sacral cord region was selected for study because it supplies the plexus innervating the leg and has been the site of previous experimentation.[5] The spinal cord was arbitrarily divided into three areas varying along the medio-lateral axis (Fig. 1). Area A is the most lateral region and includes the entrance points of the dorsal root fibers into the cord. Area B is located between area A and the central fissure which is occupied by the glycogen body. Area C is located between area B and the central fissure and is the most medial area.

The embryo was positioned so that a nearly vertical dorsal-to-ventral electrode pass was possible. The electrode was placed on the surface of the cord in one of the three cord areas. The point of contact on the cord surface (zero depth) was determined visually with a binocular microscope and electrically by listening for the click from an audio-monitor at the time of electrode-cord contact. The electrode was lowered in incremental steps of about 68 μ. A run was terminated if no activity was evident in eight consecutive increments. At least 1 min of activity was observed at each depth. During this time. a rating was given to activity at that site.

A rating technique was employed to yield an objective measure of the number of units (unit density) active at a particular cord locus. The procedure requires an observer to assign a value to the unit activity viewed on an oscilloscope according to the criteria given below:

Rating

0 No evidence of unit neural activity.
1 Very low level unit activity. No sustained firing of individual units.
2 Well-defined, sustained firing single unit.
3 Two or more well-defined, sustained single units.
4 Polyneuronal activity. Single-unit activity is obscured by the firing of numerous units.
β Qualitative designation of polyneuronal "burst" activity, characterized by abrupt, simultaneous discharge of several units. This rating is assigned *in addition* to above quantitative ratings of unit density (4 β, etc.).

Unit activity equivalents to these ratings are shown in Figure 2.

The rating assigned to a specific depth corresponds to the highest level of activity achieved during the observation period. This is important to note because some single units fire only periodically and high levels of polyneuronal activity seldom have a duration of more than a few seconds. The burst designation, β, allows the distinctive class of burst activity to be treated independently of the quantitative ratings of unit density. Only one burst must occur to justify a β rating.

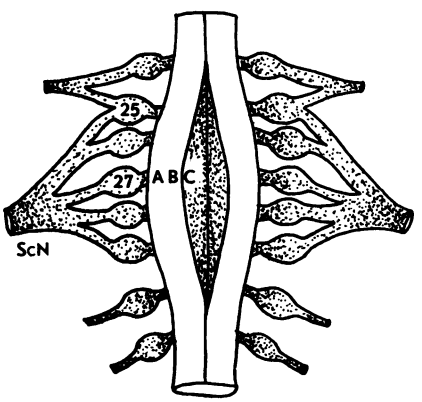

FIG. 1.—Diagram of the lumbo-sacral spinal cord of the 17-day chick embryo. The glycogen body has been removed, leaving a depression in the center of the cord. Recordings were usually made at area A, B, or C medial to dorsal root 27. ScN, sciatic nerve.

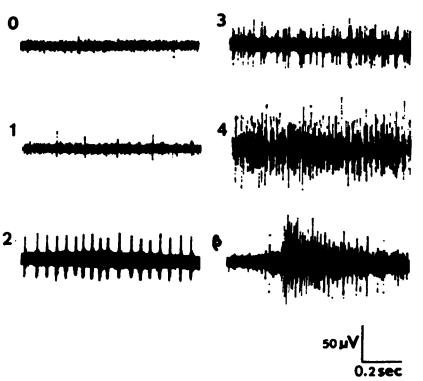

FIG. 2.—Samples of unit activity representing different ratings of unit density: *0*, no evidence of unit activity; *1*, minimal unit activity with no sustained firing; *2*, well-defined, sustained single-unit activity; *3*, two or more well-defined and sustained single units; *4*, polyneuronal activity which obscures individual unit activity; β, polyneuronal burst activity.

Activity profiles: Two types of activity profiles are constructed from the rating data:

1. *The density profile* is a distribution of average ratings of unit density (0–4) for each cord depth. Separate profiles are constructed for cord areas A, B, and C. Each profile represents the average ratings of 20 electrode passes from 5 to 6 embryos of each age.

2. *The burst profile* represents the percentage of electrode passes showing burst activity at each cord depth. These data were collected simultaneously with those reported in the density profiles.

Results. Preliminary observations revealed no important differences between the activity found in sites adjacent to dorsal roots 25 and 27 of 17-day embryos. The data reported here were collected exclusively from the area of dorsal root 27. Results from 17-day embryos are reported initially because they represent the general types and distribution of neural activity found in embryos of other ages.

17-Day Embryos. Cord area B: This area will be described first and in considerable detail because it possesses the general classes of activity found in other areas. Little activity was found on the dorsal surface of the cord as indicated in the density profile (Fig. 3). Only 3 of 20 cases showed any activity at the point of electrode contact. This extreme dorsal region corresponds structurally to the dorsal fasciculi. The average depth at which first activity was detected was 175 μ (Fig. 3). Further electrode penetration revealed a region of moderate activity with relatively short, if any, quiet periods. Regular and irregular patterns of interspike intervals were detected (Figs. 4*a*, 4*b*). Anatomically, this region corresponds to the dorsal column. We refer to it as the "sensory region."

Ventral to the sensory region, there is a relatively silent zone which is located at an average depth of 475 μ (Fig. 3). This region is distinct in most records of individual electrode passes, but some of its discreteness is lost in the density profiles because it is smoothed out by the averaging techniques employed. The silent region is 100–200 μ in depth and is located just below the dorsal columns.

The density profile shows the ventral two thirds of the cord to be the most active region (Fig. 3). This region is characterized by intermittently firing single units (Figs. 4*d*, 4*e*) and polyneuronal bursts (Figs. 2β, 4*c*). Continuously active units were found, but they were not exclusively located in this region. The activity periods of intermittently active single units lasted from a few seconds to over a minute and were usually separated by longer inactivity periods. The polyneuronal bursts started abruptly and usually trailed off into single units. Single bursts usually had a duration of one to three seconds with clusters of bursts often lasting longer. The intervals between bursts varied from a few seconds to several minutes. Some units fired only during burst activity. Others were found which began firing several seconds before burst discharges occurred. These units may have had some triggering function for burst activity. In the extreme ventral region of the cord, units were commonly found which fired synchronously with the heart beat. Some of these units changed their rhythm to participate in burst activity, afterward returning to their original pattern. A few such units ceased activity for periods up to several minutes and then returned to activity. The deepest ventral region contains units which fire continuously and show relatively regular intervals between spikes (Fig. 4*f*). The average depth of the last neural activity was 1260 μ (Fig. 3). The ventral two thirds of the cord is referred to as the "motor region." It is composed of motoneurons and a heterogeneous population of internuncial, commissural, and glial cells.

The percentage of electrode passes showing burst activity at each depth is represented by the open circles in the activity profiles (Fig. 3). The burst activity appeared at a depth of about 375 μ to 475 μ and extended to the bottom of

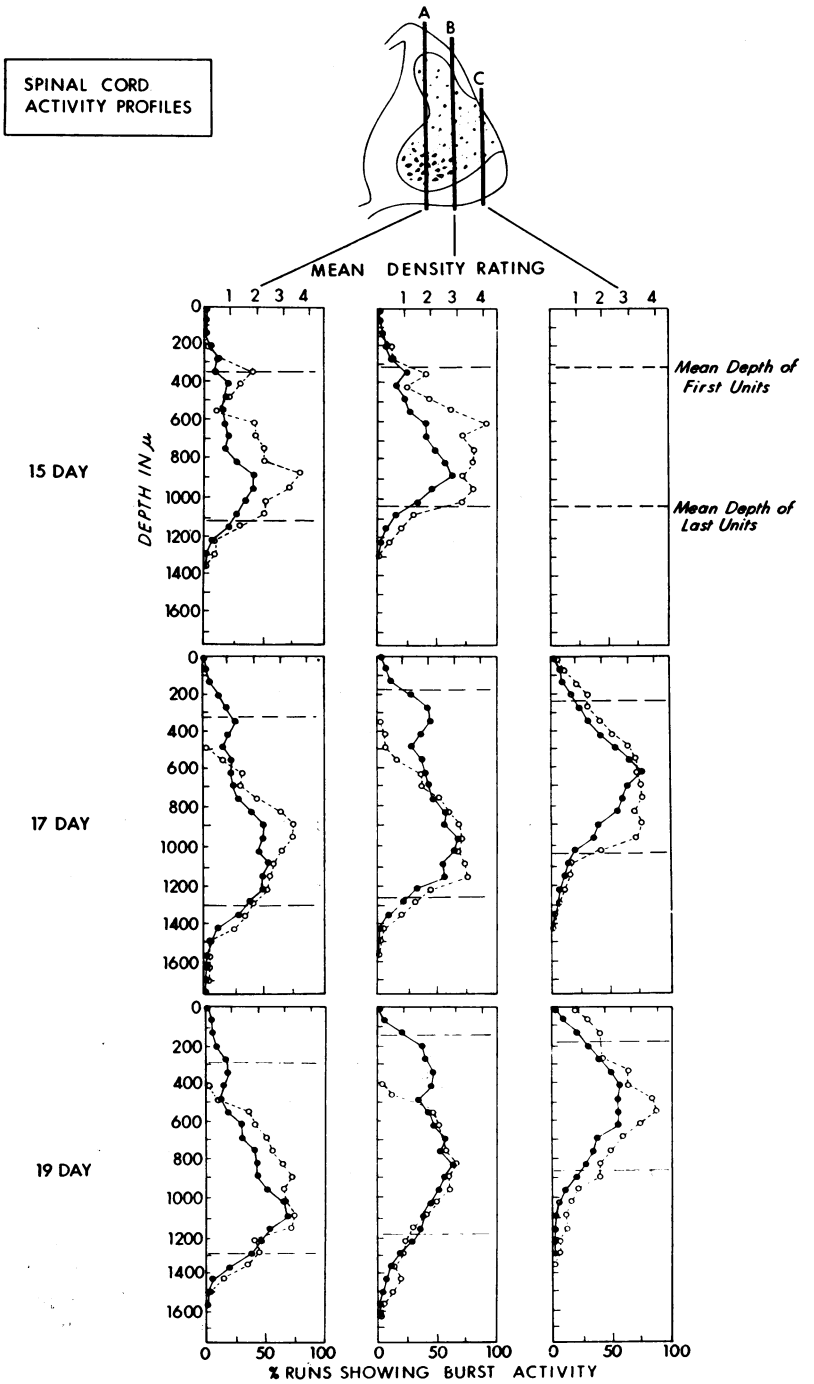

FIG. 3.—Profiles of unit density and burst activity. Activity profiles represent the distribution of unit activity at given depths of cord areas A, B, and C. ●, density of unit activity found at each cord depth based on the 0 to 4 scale given above the profiles. ○, percentage of electrode runs showing polyneuronal burst activity at each cord depth. Percentage scale is given below the profiles. Zero depth represents point of electrode-cord contact for all profiles.

the cord. Burst activity was most commonly observed at depths between 820 μ and 1150 μ.

Injury potentials occasionally resulted from electrode advancement. When they occurred in the dorsal columns, only the affected cell showed the typical injury pattern. In contrast, when injury potentials appeared in the ventral regions, they frequently evoked intensive firing in cells nearby, suggesting the possibility that the ventral cells are more closely coupled in function than those in the dorsal column. The firing rate of some injured cells in the ventral region was by far the highest observed in the cord.

All the profile data were collected with the KCl-agar micropipettes. A limited series of control observations was made using tungsten, stainless steel, and indium filled electrodes of various sizes. The general contour of the activity profile obtained was independent of the type and size of the electrode used.

Cord area A: The activity profile of area A is similar to that of area B (Fig. 3). No important differences were found between the types and distribution of single units or burst activity present in these two regions. Areas A and B also possess common histological features. Electrode passes through either of these regions would encounter portions of the dorsal column, the middle internuncial region, and the lateral motor column. Some structural differences do exist, however. Area A has more motoneurons than area B.

Cord area C: The density and burst profiles of area C resemble the ventral two-thirds of cord areas A and B (Fig. 3). The burst activity which is characteristic of the medial and ventral parts of these latter areas was found throughout the entire depth of area C. Histologically, area C includes the medial motor column and a heterogeneous collection of internuncial and glia cells.

15-Day Embryos. In 15-day embryos, difficulty was encountered in accurately determining the point of electrode-cord contact. The profile data from area C are not presented because of the uncertainty of depth measurements.

The profiles of areas A and B reveal a lower density of unit activity than shown in profiles of older embryos (Fig. 3). This probably reflects the generally greater problem of detecting and recording from units in embryos of this age. The profiles lack the clearly defined sensory and quiet regions depicted in the upper third

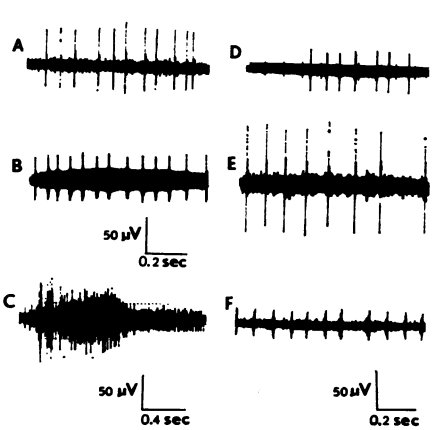

FIG. 4.—Examples of unit activity from cord area B of 17-day chick embryos. *A*, continuously firing unit with irregular interspike intervals, depth 408 μ. *B*, continuously firing unit with regular interspike intervals, depth 476 μ. *C*, polyneuronal burst activity trailing off into single unit, depth 1020 μ. *D*, burst firing single unit, depth 1088 μ. *E*, intermittently active unit in ventral cord with a rather slow firing rate, depth 1224 μ. *F*, continuously active unit with regular interspike intervals representative of the ventral-most activity, depth 1360 μ.

of the profiles of older embryos. This may result from a reduced level of precision with the rather delicate embryos of this stage and need not reflect a reduced level of sensory activity. The ventral two-thirds of the cord in areas A and B was found to be the most active region with respect to both single-unit and burst activity.

19-Day Embryos. The density and burst profiles of all areas of 19-day embryos resembled those of 17-day embryos (Fig. 3). These similarities are not necessarily an indication that the activity of 17- and 19-day embryos is identical. The actual level of neural activity of 19-day embryos may have been exaggerated somewhat in the profiles due to the greater ease of recording from units of this stage. Also, there may be subtle changes with age in the form or frequency of activity which would not be resolved by the rating technique. For instance, polyneuronal burst activity seemed to be less frequent in 19-day than in younger embryos although this is not reflected in the profile data.

Conclusions. Three basic regions of neuronal activity were identified in the embryonic spinal cord. The dorsal column (the "sensory region") showed the continuous unit activity usually associated with spinal interneurons.[12, 13] Although the rate of firing of single units varied, the activity of this region as a whole remained quite stable. A region of reduced activity 100 μ to 200 μ deep was located immediately ventral to the sensory region. The ventral two-thirds of the cord was characterized by polyneuronal burst activity and by intermittently firing single units. This region also contains numerous continuously firing units. The motor region showed large fluctuations of activity in contrast to the relatively continuous activity in the dorsal regions.

Unit activity of the ventral lumbo-sacral cord is of interest because this region contains the motoneurons and interneurons associated with the movement of the leg. Also, endogenous activity of the ventral half of the cord has been implicated as a source of spontaneous leg motility in embryos up to 15 to 17 days.[5] The polyneuronal burst activity was found exclusively in this ventral region and probably represents the type of unit discharge associated with leg movements. The frequency of this burst activity appeared to decline between 17 and 19 days. A corresponding decline of leg motility is also observed during this period. It is of interest that Boethius[14] has observed bursts of muscle potentials in the legs of chick embryos. These bursts had durations similar to those of the polyneuronal discharges observed in the cord and were found to be correlated with movements of the leg. It is, therefore, probable that these bursts of muscle potentials are a peripheral manifestation of the polyneuronal burst activity of the cord. This suggests that, during the 15- to 19-day period, a close functional coupling may exist between the bioelectric activity of spinal cord motoneurons and the overt motility of the embryo.

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