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Development of Spinal Cord Bioelectric Activity in Spinal Chick Embryos and Its Behavioral Implications

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SUMMARY

Embryonic behavior of the chick is the product of spontaneous multiunit burst discharges within the ventral spinal cord. The present study describes the ontogeny of spinal cord burst discharges in embryos which were deprived of brain input by removing several neural tube segments of 2-day embryos at cervical or mid-thoracic levels. Characteristics of bioelectric activity present in both intact and chronically transected cords are: a) the appearance of spike discharges; b) the organization of unit discharges into synchronized multiunit bursts; c) the establishment of intracord synchronization of burst discharges over wide expanses of cord tissue; d) an increase in burst duration and complexity at 7 days due to the appearance of the burst afterdischarge; e) an increase in the amount of burst activity from 6 to 13 days followed by a decline until hatching at 21 days; f) a shift from periodic to irregular patterns of burst activity at 13 days; and g) the existence of the cord burst discharge as a correlate of embryonic movement.

Several differences were found between burst activity from chronic spinal and intact embryos: a) cervical spinal embryos were significantly less active than controls from 15 through 19 days; and b) long sequences of unusual repetitive burst afterdischarges appeared in chronic spinal embryos by 13 days.

The results indicate that the transected embryonic spinal cord is remarkably autogenous in function, although patterns of activity unique to the transected cord appear and increase in prominence during later stages of incubation.

INTRODUCTION

Behavioral studies of chronic spinal chick embryos reveal the inherent capacity of the immature spinal cord to support the ontogeny of behavior. The most comprehensive studies of behavior development of spinal chick embryos have been provided by Hamburger and colleagues. In a series of studies they demonstrated that chronic spinal embryos which had never formed brain connections showed a pattern of ongoing jerky and apparently unorganized motility which

is typical of the behavior of intact control embryos during most of prenatal development (Hamburger and Balaban, 1963; Hamburger, Balaban, Oppenheim, and Wenger, 1965; Oppenheim, 1975). The autonomous nature of spinal cord activity was elegantly demonstrated in a subsequent experiment in which embryos deprived of both brain and sensory input showed normal patterns of motility up to late incubation stages (Hamburger, Wenger, and Oppenheim, 1966). The contribution of the brain to the development of structure and function in the spinal cord has also been evaluated by investigating spinal embryos (Windle and Orr, 1934; Windle and Austin, 1936; Visintini and Levi-Montalcini, 1939; Rhines, 1943; Hamburger and Balaban, 1963; Hamburger, Balaban, Oppenheim, and Wenger, 1965; Hamburger, Wenger, and Oppenheim, 1966; Oppenheim and Narayanan, 1968; Hamburger, 1973; Oppenheim, 1975).

Electrophysiological investigations have shown that multiunit burst discharges within the ventral portion of the embryonic spinal cord are synchronous with embryonic movements or peripheral motor-nerve discharges in curarized preparations (Ripley and Provine, 1972; Provine, 1976; Stokes, 1976). These studies provide direct evidence that embryonic movements in the chick are neurogenic, hence inferences concerning the occurrence of behavioral events can be made on the basis of spinal cord bioelectric data and vice versa.

In the present study, we examine the development of the multiunit burst discharges in embryos which have been deprived of brain input by removing several cervical or thoracic segments of the neural tube at very early stages (1½–2 days) before descending supraspinal fiber tracts reach the cord (Oppenheim, 1975). The form of burst envelopes and the amount and periodicity of multiunit burst discharges obtained from curarized thoracic- and cervical-gap embryos will be compared with those present in the spinal cords of intact embryos in order to assess the degree of functional autonomy possessed by the immature spinal cord. The role of the brain input in spinal cord development will also be considered.

METHODS

The numbers of control embryos and embryos with thoracic and cervical spinal gaps which were examined in this study are given in Table 1. Embryos were raised from eggs of a White Leghorn chicken strain.

Windows were ground in the shell to expose embryos for surgery. Embryos were spinalized by removing a segment of neural tube between 4–6 somatic segments in length using a vibrating needle (Wenger, 1968). Thoracic-gap embryos were prepared by removing segments of the neural tube in the region of the most caudal somites at stages 14–16 (50–56 hr) (stage series of Hamburger and Hamilton, 1951). Cervical-gap embryos were prepared by removing segments of the neural tube between somites 1–5 of stage 10–11 (45–48 hr) embryos. After surgery, the shell windows were covered with a glass cover slip, sealed with paraffin and incubated. Control embryos were incubated until needed for recording.

Techniques used in preparing embryos for recording are similar to those previously described (Provine, 1971, 1972). A single incision was always made in the lumbosacral region over the glycogen body and occasionally a second incision was made in the brachial region between the wings. The glycogen body was removed by aspiration with a small pipette. Only those embryos which showed vigorous movements after the above operations were used in the experiments. Nine-day and older embryos were immobilized by injecting d-tubocurarine (2.5 μg/g embryo wet weight) into the leg

TABLE 1
Spinal Cord Recordings

	6d	7d	8d	13d	15d	17d	19d
Control	5	2	4	11	10	17	10
Chronic Cervical Gap	4	4	6	10	13	10	7
Chronic Thoracic Gap	4(2*)	—	3(2[a])	2	5(2*)	3(3[a])	—

* Simultaneous recordings obtained from both brachial and lumbar regions.

muscles with a 30-gauge syringe needle. Younger embryos were usually immobilized by placing several drops of a 1 mg/ml curare solution on the chorioallantoic membrane. After preparation, embryos were allowed to acclimate for approximately 30 min in a temperature and humidity controlled chamber before an experiment was initiated.

Glass insulated tungsten recording electrodes with 25-μm tips were used in all experiments. These electrodes record multiunit burst discharges and also clearly show single unit activity. "Floating" electrodes modified from the standard electrode permitted recording from motile embryos without producing injury artifacts (Ripley and Provine, 1972). The reference electrode was a stainless steel wire which was placed in either the amniotic fluid or threaded through the skin. The recording electrodes were lowered into the middle or ventral portion of the cord until prominent bursting was encountered. Burst discharges are widely distributed along the rostro-caudal extent of the ventral cord (Provine, Sharma, Sandel, and Hamburger, 1970; Provine, 1971). Such transregional coupling of burst activity is not observed between the dorsal and ventral columns of the cord. Once suitable electrode placement was obtained, the embryo was allowed to acclimate at least 10 min before an experiment was begun.

A single 15-min period of cord electrical activity was recorded from each embryo. Activity was amplified using an AC preamplifier with a gain of 10,000 and a bandpass of 10–10,000 Hz. and stored on magnetic tape with an FM tape recorder. Data analyses were made from paper records written out from recorded activity by a Bruel and Kjaer Level Recorder (see Provine, 1972, for details). Input to the Level Recorder was filtered (200–10,000 Hz bandpass). The Level Recorder was used as an integrator with rise and decay time constants of approximately 200 msec which was set to yield a write-out which approximates log unit firing density. The baseline threshold level of the Level Recorder was adjusted independently for each embryo so that only multiunit discharges matching the experimenters' subjective impression of a "burst" gave an above baseline response. The amount of burst activity present in a given record is stated as the percentage of the 15 min recording interval during which any above baseline event was present.

The stages (Hamburger and Hamilton, 1951) of all embryos were appropriate for the 24-hr chronological periods described in the results. Each spinal embryo was dissected to confirm the presence of a spinal gap. In eight questionable cases, embryos were fixed, sectioned, and stained for histological analysis to determine if a gap was present. If no gap, or an incomplete gap was found, the record from the embryo was not considered in the data analysis.

RESULTS

The comparison of spontaneous spinal cord bioelectric activity from curarized intact control embryos with that from embryos with chronic spinal gaps is facilitated because multiunit burst discharges in intact cords undergo reliable stage-specific shifts during development in regard to: a) the shape of the burst envelope; b) the time ordering or periodicity of discharges; and c) the amount of burst activity (Provine, 1972). Chronic cervical and thoracic spinal gap em-

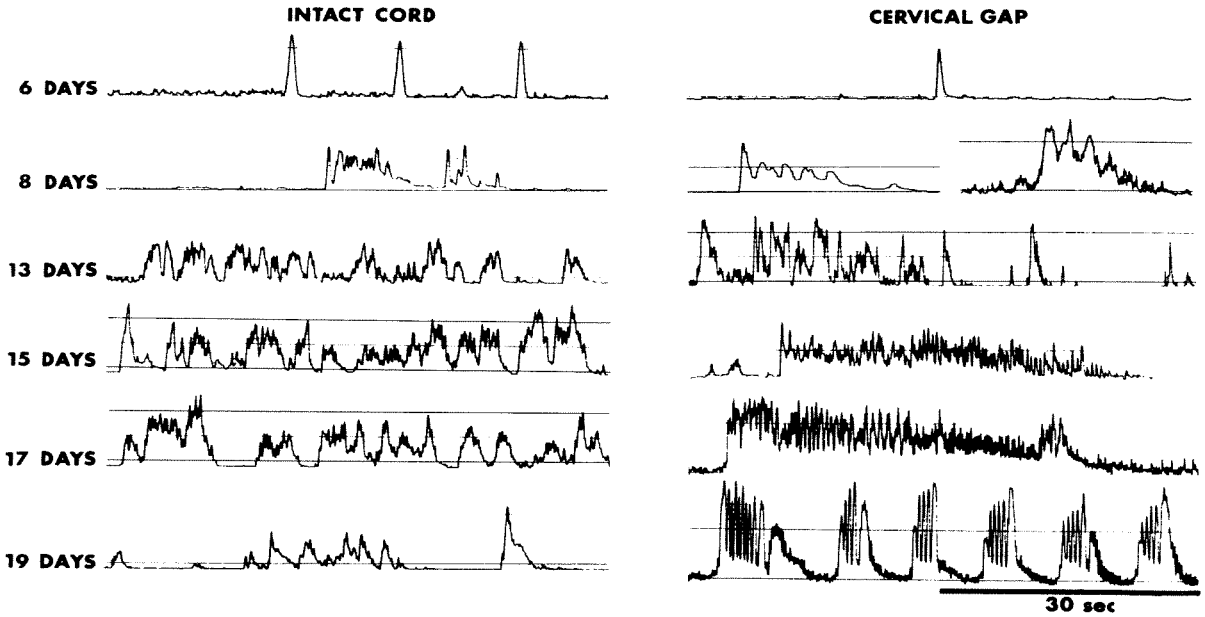

Fig. 1. Records of lumbosacral burst discharges from intact and chronic cervical-gap embryos from 6 to 19 days. Between 6 and 8 days, there is an increase in burst duration and complexity due to the appearance of the burst afterdischarge. Several examples of abnormal repetitive afterdischarges are shown in the 15-, 17- and 19-day records from cervical-gap embryos. The repetitive afterdischarges are interspersed among episodes of otherwise normal discharges.

bryos are similar to intact control embryos in regard to many of these characteristics.

Ongoing unit activity and multiunit burst discharges were present in both intact control and cervical and thoracic spinal gap embryos by 6 days, the earliest stage observed. The envelope of burst discharges undergoes a marked change in duration, shape and complexity between 6 and 7 days of incubation in intact and spinal gap embryos due to the appearance of afterdischarges following some, but not all, bursts. [Afterdischarges are the trailing-off of unit activity after an "initiating" discharge (Provine, 1972)]. Afterdischarges were *never* observed at 6 days in the lumbosacral records of the 5 control, 4 cervical-gap and 4 thoracic-gap embryos which were examined. At 6 days, *all* bursts were relatively simple, short duration (approximately 2 sec) accelerations in unit firing density. In contrast all intact and spinal embryos 7 days or older had at least one afterdischarge during a 15 min recording interval. The transition in the burst envelope between 6 and 8 days is shown in the intact cord (IC) and cervical-gap (CG) records of Figures 1 and 2. Burst afterdischarges in the lumbosacral (LS) region of an 8-day thoracic-gap embryo are shown in Figure 3.

The regular alternating intervals of burst activity and "quiescence" (ongoing unit activity is present between bursts) with a cycle length of approximately 1–2 min typical of early stages is lost in intact, cervical- and thoracic-gap embryos by day 13 when discharges occur in an irregular and almost continuous sequence (Figs. 2 and 3). The 6-, 8-, and 13-day intact cord (IC) and cervical-gap (CG) records of Figure 2 depict the transformation from regular to irregular periodicity

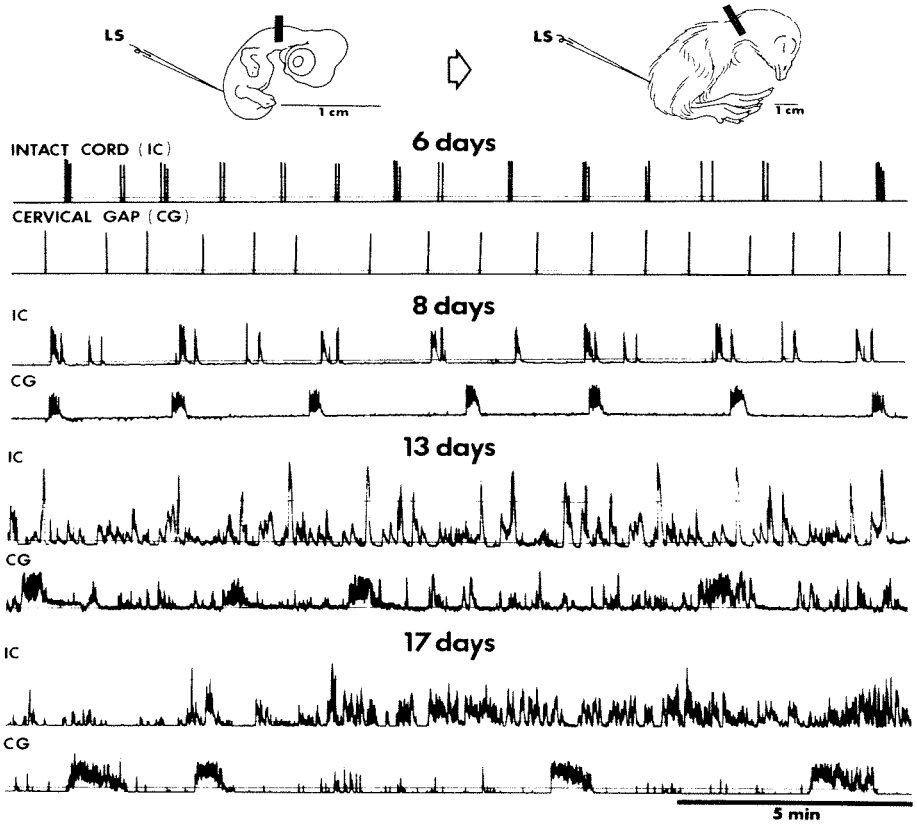

Fig. 2. Spontaneous lumbosacral spinal cord bioelectric activity from intact cord (IC) and chronic cervical-gap (CG) embryos. Embryos pictured at the top show the general appearance, site of recording, level of spinal transection and scale of embryos at 6 and 17 days, the range of ages whose records are shown. Note the increase in burst duration and complexity between 6 and 8 days in both intact and cervical-gap preparations and the loss of regular burst periodicity between 8 and 13 days. The prominent clusters of burst activity in the cervical-gap records at 13 and 17 days are the repetitive afterdischarges described in the text. All records are of integrated unit activity.

as do the brachial (B) and lumbosacral (LS) records from the intact and thoracic-gap cord preparations of the 8- and 15-day embryos of Figure 3. Periodicity is lost as activity periods lengthen and inactivity periods shorten until the cord is almost continually active at 13 days. The occasional periodically occurring clusters of burst discharges (repetitive afterdischarges) which appear among otherwise irregular bursting in the 13-day and 17-day CG records of Figure 2 and the 15-day thoracic LS record of Figure 3 are not an exception to the loss of regular periodicity at 13 days; they are a repetitive burst pattern which appears in some spinal gap embryos during the last week of incubation (quantitative data are provided below).

Chronic spinal and intact control embryos show similar trends in the amount of burst activity present at a given developmental stage (Fig. 4). The average percentage of time that above baseline burst discharges were present in 15 min records of integrated bioelectric activity obtained from chronic cervical-gap embryos at a given stage increased from 6 days (9% ($\bar{x}$) ± 3% (SD) spinal, 5% ±

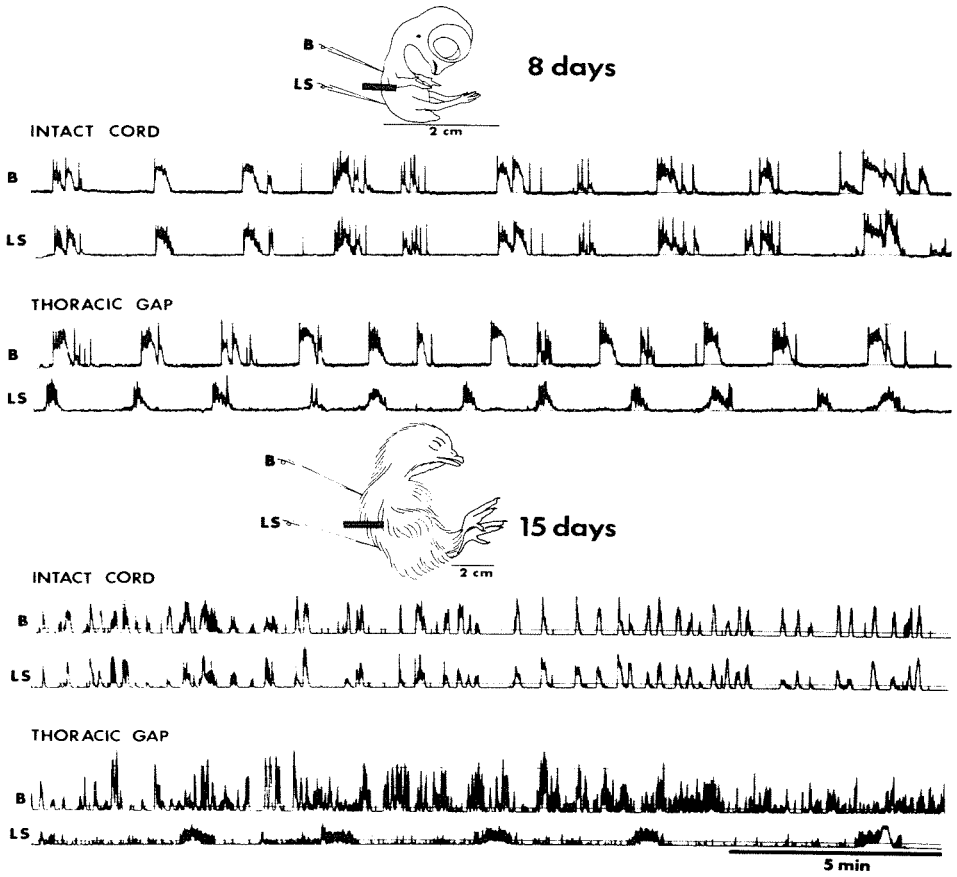

Fig. 3. Simultaneous recordings of burst activity from brachial (B) and lumbosacral (LS) regions of intact and chronic mid-thoracic-gap embryos. Synchronous discharges are shown in both channels of activity from intact cord embryos. In contrast, burst activity is asynchronous rostral and caudal to the gap in the thoracic-gap embryos. The few isolated instances of apparently synchronous burst discharges in the two channels of the thoracic-gap embryos are due only to the very compressed time scale of the records. Note the absence of the short-duration bursts in the LS record of the 8-day thoracic-gap embryos and the presence of the repetitive afterdischarges in the LS channel of the 15-day thoracic- gap embryo shown at the bottom of the figure. At 8 days, bursts from both B and LS channels of the thoracic-gap embryos are periodic and have afterdischarges.

4% intact) and 8 days (14% ± 6% spinal, 18% ± 5% intact) up to 13 days (32% ± 13% spinal, 42% ± 10% intact) and declined at 15 (19% ± 8% spinal, 36% ± 13% intact), 17 (13% ± 10% spinal, 26% ± 14% intact) and 19 days (4% ± 7% spinal, 19% ± 13% intact). However, a two-tailed t-test indicated that spinal embryos are significantly less active than intact controls at 15 ($t = 3.86$, $df = 15$, $p < 0.02$), 17 ($t = 2.40$, $df = 25$, $p < 0.05$) and 19 days ($t = 2.66$, $df = 15$, $p < 0.02$). The difference between means at 13 days was not significant ($t = 2.00$, $df = 19$, $p < 0.1$). At 6 and 8 days, the sample sizes were too small to permit meaningful tests of significance.

Simultaneous recordings from ipsilateral ventral brachial (B) and lumbosacral (LS) spinal loci of two 6-day and two 13-day embryos with chronic cervical

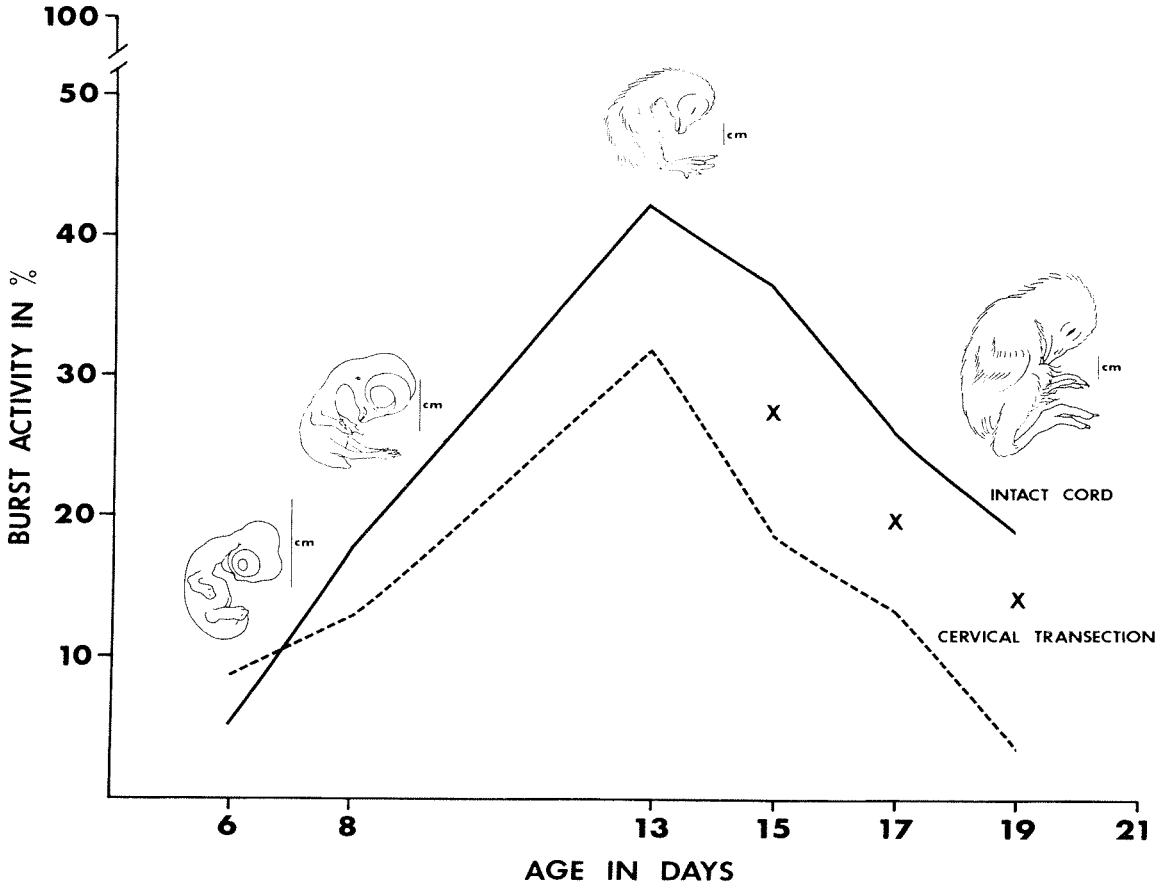

Fig. 4. Percentage of total recording time during which burst discharges are present in intact cord and chronic cervical gap embryos between 6 and 19 days. Percent burst activity is the percentage of a 15 min observation interval during which above-baseline burst discharges are observed in the integrated records of unit electrical activity. The x's indicate stages at which significant differences ($p < 0.05$) were found between cervical-gap and intact embryos. All recordings are from the lumbosacral region.

gaps show that bursts at the two loci are nearly synchronous as in the intact control embryos shown in Figure 3 (see also Provine, 1971). As in control embryos, synchronization between bursts at two sites is good but not perfect; while a burst at one site is usually accompanied by a burst at a second, bursts recorded at different loci differ in unit composition, onset times (0–200 msec or more onset differences), complexity, and duration. Double electrode recordings from the ipsilateral brachial and lumbosacral regions of embryos with thoracic-gaps show independent burst patterns rostral and caudal to the gap (Fig. 3). Compare this result with the synchronized activity shown in the intact cord preparations of Figure 3. The finding that brachial and lumbosacral activity is asynchronous in mid-thoracic gap embryos eliminates the possibility that nonspinal processes may be responsible for the trans-regional synchronization of bursts and correlated movement in intact and cervical-gap embryos.

Recordings with "floating" electrodes from uncurarized embryos with cervi-

cal-gaps (two 6-day and two 15-day embryos) indicate that burst discharges occur synchronously with embryonic movements. Spinal cord burst events heard by means of an audio monitor were highly correlated with the visually observed movement of some embryonic body parts although not always that part nearest the recording electrode. The synchronization between embryonic movements and spinal cord bursts was not due to movement-produced cord injury discharges or other movement artifact; typical cord bursting patterns continued unchanged after curarization. Furthermore, passive movement of the embryo by the experimenter using forceps evoked no cord bursts.

Several differences are noted between spinal cord bioelectric activity in intact and chronic spinal gap embryos. The significantly lower amount of burst activity found in chronic cervical-gap as compared to intact control embryos at 15, 17, and 19 days has already been noted (Fig. 4). Another difference involves long-lasting repetitive afterdischarges which were identified amongst otherwise normal bursts in cervical- and thoracic-gap records at 13 days and afterward. These discharges consist of long series of short repetitive bursts of progressively lower amplitude which may last 30 sec or more before fading out (Fig. 1, cervical-gap records at 15, 17 and 18 days; Fig. 2, CG records at 13 and 17 days; Fig. 3, LS thoracic-gap record at 15 days). Whereas repetitive afterdischarges were *never* observed in intact embryos at any stage, they became increasingly prominent in spinal embryos at later stages, at least one example being observed in 2 of 9 (22%) cervical-gap records at 13 days, 8 of 13 (62%) at 15 days, 6 of 10 (60%) at 17 days and in all 19-day records. The unusual pattern of burst discharges shown in the 19-day cervical-gap record of Figure 1 was observed in only one embryo and is probably a special case of the repetitive afterdischarge where the repetitive episodes are grouped into clusters.

DISCUSSION

A detailed comparison of the burst activity of chronic cervical and thoracic spinal embryos with that of intact control embryos between 6 and 19 days of incubation reveals similarities and differences.

The similarities are summarized as follows: a) the appearance of spike discharges; b) the organization of unit discharges into synchronized multiunit bursts; c) the establishment of intracord synchronization of burst discharges over wide expanses of the spinal cord; d) an increase in burst duration and complexity at 7 days due to the appearance of the burst afterdischarge; e) an increase in the amount of burst activity from 6 to 13 days and its subsequent decline until hatching at 21 days; f) a shift from periodically occurring to irregularly occurring episodes of burst activity at 13 days; and g) the existence of the spinal cord burst discharge as a neural correlate of embryonic movement. The general conclusion is that many aspects of spinal cord bioelectric activity develop independently of supraspinal input. Electrophysiological results from chronic spinal gap embryos show further that the site or sites involved in burst production are widely distributed within the cord. This is indicated by the finding that bursting survives regardless of the level (cervical or thoracic) of spinal transection, as sug-

gested by earlier behavioral experiments (Hamburger and Balaban, 1963; Hamburger, Balaban, Oppenheim, and Wenger, 1965; Hamburger, Wenger, and Oppenheim, 1966; Oppenheim, 1975). The general finding that the immature spinal cord is capable of highly autonomous function is supported by numerous studies of a variety of vertebrates *in vivo* (Tracy, 1926; Hooker and Nicholas, 1930; Barcroft and Barron, 1937; Wang and Lu, 1940, 1941; Barron, 1941; Rhines, 1943; Holtzer and Kamrin, 1956; Corner, 1964; Hughes and Prestige, 1967; Sharma, Provine, Hamburger, and Sandel, 1970; Armstrong and Higgins, 1971; Stelzner, 1975; Provine, 1976) and *in vitro* (Crain, 1974). The *in vitro* studies by Crain and others effectively demonstrate that spontaneous unit electrical activity develops and increases in complexity in isolated portions of the vertebrate spinal cord maintained in long-term cultures (reviews in Crain, 1974; 1976).

What, then, are the consequences of chronically depriving the embryonic spinal cord of supraspinal influences? The amount of burst activity was significantly lower in chronic cervical spinal embryos than in intact control embryos at days 15 through 19 although as already noted, the trend in both groups was toward decreased activity at these later stages. A second effect of chronic spinal transection was the appearance of sequences of long-duration, repetitive burst discharges during the last week of incubation in chronic spinal preparations. It is of interest that similar repetitive-type burst discharges occur in cultures of fetal rodent spinal cord tissues which have also been deprived of supraspinal influences (Corner and Crain, 1972; Crain, 1974; 1976). Thus, the afterdischarges of the *in vitro* system resemble a distinctive feature of the decentralized spinal cord *in situ;* the repetitive burst pattern is probably not an artifact of tissue culture.

It is not possible at this time to specify the mechanism through which the above effects were brought about. The subtractive procedure of spinal transection provides a much clearer picture of the function of residual spinal cord structures caudal to the gap than of possible brain influences. For example, experimental effects in spinal embryos may arise from degenerative changes or aberrant synapses in the cord which result from the absence of descending or ascending fiber tracts, rather than from the withdrawal of physiological influences of supraspinal centers. We must, therefore, be cautious in interpreting the significance of modified burst patterns which were observed in chronic spinal embryos. That gross morphological or cytoarchitectonic alterations are not involved is suggested by the normal appearance of the cords of chronic spinal gap embryos (observations of the authors; see also Hamburger, 1946). However, only careful EM study of the type and quantity of synapses and vesicles is capable of ultimately assessing the degree of structural integrity possessed by the chronically decentralized spinal cord.

The present study established that the cord polyneuronal burst discharge is an electrophysiological correlate of overt motility in chronic cervical-gap embryos. Both movements and bursts occur synchronously and undergo parallel changes at various developmental stages as they do in control embryos (Ripley and Provine, 1972; review in Provine, 1973). Therefore, it is not surprising that the

present electrophysiological findings are compatible with earlier behavioral results (Hamburger and Balaban, 1963; Hamburger, Balaban, Oppenheim, and Wegner, 1965; Corner and Bot, 1967; Boethius, 1968; Oppenheim, 1975). All studies which have examined the *amount* of activity (cord bursts or motility) of spinal and intact embryos agree that the activity levels of both increase up to a peak around 13 days and then decline until hatching. However, the method used to quantify activity influences the amount of activity reported at a given stage and determines whether differences between spinal and control embryos are detected (Oppenheim, 1970 and review in Oppenheim, 1975).

The behavioral results of Oppenheim (1975) based upon observations of leg movements are in good agreement with the present electrophysiological study concerning the relative amount of activity (percent burst activity versus number of leg movements) of chronic spinal gap embryos present at a given stage. However, the finding by Oppenheim (1975) that spinal embryos are as active as intact embryos if the total number of leg movements is examined, appears to be inconsistent with the finding that significantly lowered amounts of correlated burst activity are found in the cords of spinal embryos at 15 through 19 days. There are several possible explanations for this discrepancy. Bursts reflect the interval during which movements occur (Ripley and Provine, 1972), but not necessarily the absolute number of movements which are performed during that interval. Thus, spinal embryos may be producing more movements than controls during a burst of a given duration. We must also consider that the present study used curarized (paralyzed) embryos which are deprived of both movement-produced feedback (Provine, 1973) and brain input. Under these conditions, the loss of supraspinal input may have a relatively greater impact than upon the freely moving embryos of Oppenheim. At 15–16 days, Oppenheim notes a clonic-like repetitive leg movement which is probably a motility correlate of the repetitive afterdischarge described as early as 13 days in the present report. In addition, Oppenheim (1975) detected a lengthening of the period of chronic spinal activity as compared to that of controls at 7 days in thoracic gap embryos and at 10 days in cervical gap embryos. Although not quantitatively evaluated in the present study, a similar effect was observed in several records of cord bioelectric activity (8-day CG record of Fig. 2). At 7 days, Oppenheim attributed the periodicity shift in thoracic spinals to the loss of propriospinal input, with the first supraspinal effect coming at 10 days when the effect was observed in cervical spinal embryos. On the basis of these results, Oppenheim concludes, "What the brain appears to be doing after 10 days is modulating the temporal aspects of spinal activity" (p. 47).

Whereas the influence of supraspinal input on embryonic behavior during most of development is generally subtle and requires further analyses, there is good agreement that supraspinal input has a profound effect on *late* embryonic behavior and correlated spinal cord processes. After 16 days of incubation, spinal embryos fail to initiate the coordinated pre-hatching and hatching movements which are necessary for hatching and survival (personal observations; see also Oppenheim and Narayanan, 1968; Oppenheim, 1972, 1973). During this period, intrinsic spinal cord activity probably undergoes profound transformations in

respect to its contribution to behavior. The challenge remains of describing these transformations and determining the specific mechanism through which the brain modulates the spinal cord activity described in this report.

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