NEURAL CORRELATES OF EMBRYONIC MOTILITY IN THE CHICK

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INTRODUCTION

Indirect evidence suggests that the motility of the chick embryo is neurogenic from the start. By *neurogenic* we mean that behavior results from neural discharges which drive the muscles, as opposed to *myogenic* activity which results from spontaneous muscular contractions. A role for the nervous system in embryonic motility is suggested by the findings that electrical stimulation of the nervous system evokes movement and that curare, a neuromuscular blocking agent, immobilizes early embryos[2,6,14]. That embryonic behavior is neurogenic is indicated further by Alconero[1], who found that somites of 3-day chick embryos explanted on the chorio-allantoic membrane failed to develop spontaneous movement except when innervated by accompanying spinal cord fragments.

More evidence for the involvement of the nervous system in embryonic motility has been provided by developmental studies of spinal cord electrical activity[10,11,13]. On the basis of these studies, polyneuronal burst discharges localized within the ventral portion of the spinal cord were proposed as neural correlates of embryonic motility. The amount and periodicity of these burst discharges observed at a given stage of development were highly correlated with data reported for embryonic motility[5,8]. In the present study, the polyneuronal burst discharge is established as a neural correlate of motility by simultaneously recording spinal cord electrical discharges and either body movements or motor nerve discharges. These data provide the first unequivocal evidence of the neurogenic nature of embryonic motility in the chick.

MATERIALS AND METHODS

Forty-two chick embryos between the ages of 4 and 21 days of incubation were used in various parts of this study. Eggs were incubated in a large forced draft incubator at 37.5 °C and approximately 70% humidity. The eggs were turned several times daily.

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Embryos were prepared by opening a window in the shell and making an incision in the vertebrae of the lumbosacral, or in one case, the brachial region, in order to expose the spinal cord. Embryos were maintained in a position beneath the shell window by two fine stainless steel wires which were threaded through the skin and muscle anterior and posterior to the spinal incision and taped to the shell. This technique did not prevent frequent or vigorous movement. The wires also served as ground electrodes for electrical recordings.

Spinal cord electrical activity was observed in freely moving embryos by means of 'floating' electrodes. These electrodes were made by placing unsharpened 25 μm tungsten wires in glass capillary tubes and pulling the glass around the wires with a conventional electrode puller. After pulling, the shaft of the pipette was broken off above the taper, leaving a glass insulated tip that was several mm in length.

At early stages, the high surface tension of the fluids in the spinal incision helped hold the electrode in position in the cord. For older embryos, a ring of Oxycel was built up around the spinal incision and a small disk of thin plastic sheet several mm in diameter was seated on top of the Oxycel ring. This disk had a small hole in its center through which the electrode tip could be passed into the cord. This device enabled the electrode to move with the embryos and helped to eliminate movement artifacts. The electrode was inserted into the cord with forceps. Advance of the electrode ceased when a region having a high level of polyneuronal burst activity was encountered. This activity was usually found in the ventral part of the cord[13]. The position of the electrode is not critical because of the wide distribution of burst activity along the dorsoventral and rostrocaudal axes of the spinal cord[9,13]. If records of electrical activity remained stable for 5–10 min after placement of the electrode, an experiment was begun. Activity was amplified × 10,000 and recorded on magnetic tape.

In order to correlate neural activity with behavior, 15 min of motility and spinal cord burst discharges were simultaneously recorded from the same embryo and stored on magnetic tape. Behavior was observed visually by K.R., who activated an event marker in response to all discrete movements of *any body part* of the embryo.

TABLE I

NUMBERS OF EMBRYOS USED IN VARIOUS CONDITIONS

	Age (days)													
	4	6	7	8	11	13	15	16	17	18	19	20	21	N
Movement/cord	1	2(3)*	1(2)	1	2(3)	1	2	1	2(3)	1	1	2	1(2)	18(23)
Cord/sciatic							2		5			3		10
Cord/radial/sciatic							5		2					7
Sciatic/radial							2		5					7
														42(47)

* Total number of 15 min observation periods in brackets if more than one from each embryo.
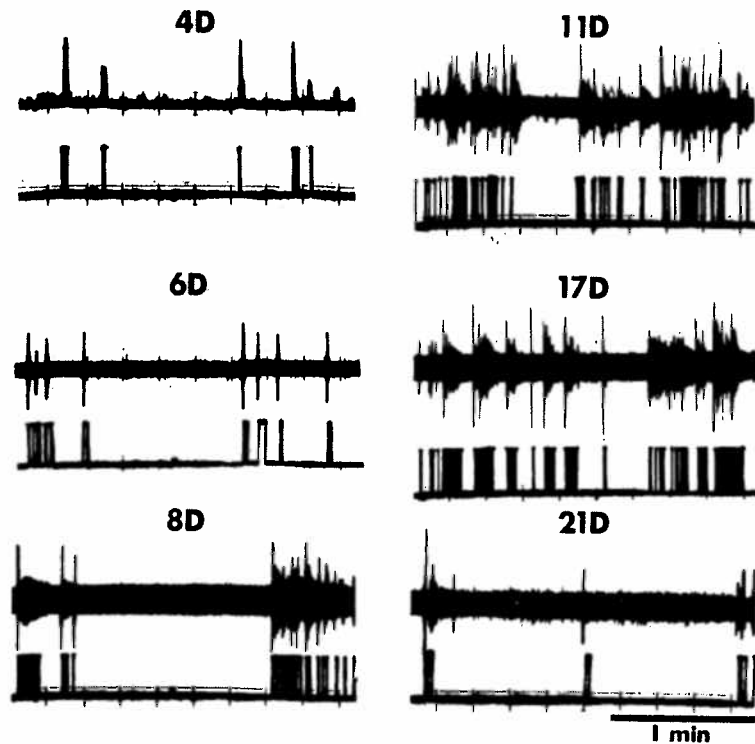
Fig. 1. Comparison of cord burst discharges (upper trace) with visually observed body movements (lower trace). Four-day cord activity was integrated to emphasize the low amplitude activity. Cord discharges were recorded from the lumbosacral region except at 4 days when the brachial cord was monitored. D = days.

The observer did not receive feedback concerning cord electrical activity.

A variation of the above experiment involved the use of curarized embryos (2.5 μg D-tubocurarine/g embryo wet wt.) and the substitution of the sciatic and/or radial nerve discharges for motility as an index of cord motor output. In these cases, cord and sciatic nerve, cord and radial nerve or cord plus sciatic and radial nerve were concurrently monitored. The spinal cord electrode was always at the lumbosacral level. In a few cases, only the sciatic and radial nerves were recorded. The numbers of embryos used in various experimental conditions are given in Table I. In order to facilitate the comparison of electrical events recorded from the cord and the sciatic nerve, activity was integrated by specially built circuits having rise and decay time constants of 2 msec and 300 msec, respectively. Integration allowed bursts to be treated as clearly defined events rather than as aggregates of spikes. The integrated signal recorded from the sciatic nerve was inverted and placed base-to-base with the integrated signal from the lumbosacral cord, forming a 'cross envelope'[9]. In order to compare 3 channels of activity, records were written out by a B and K Level Recorder and superimposed. The properties of this integrator are described elsewhere[10].
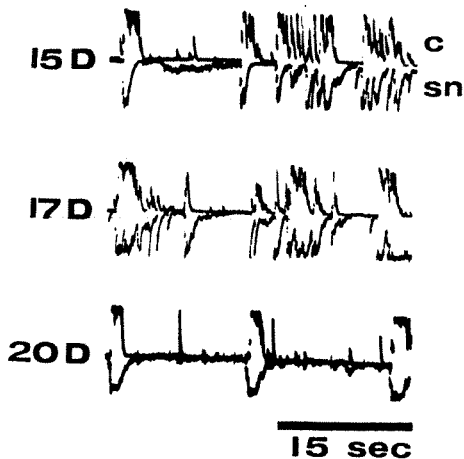
Fig. 2. Cross-envelopes comparing integrated burst activity simultaneously recorded from lumbo-sacral cord (c) and sciatic nerve (sn) of 15-, 17-, and 20-day embryos.

RESULTS

Examples of cord burst discharges and concurrently recorded movements of *any body part* are given in Fig. 1. All records of cord activity are from the lumbo-sacral region at approximately the level of spinal segment 27, except at 4 days when activity could be identified only in the brachial region. A very high correlation was found between visually observed movements and spinal cord burst discharges in embryos ranging in age from 4 days, near the onset of motility, until 21 days, the day of hatching. The correlation between movements and bursts would be slightly lower if only the movements of a specific body part were considered. Most impressive was the almost complete absence of movement when no burst activity was present. The correlation between movements and spinal cord burst discharges was most clearly depicted in those embryos in which comparison was simplified by low levels of movements and bursts. No special effort was made to relate burst discharges to specific types of movements. However, we should note that bursts were correlated with the slow curling movements of early embryos as well as the jerky uncoordinated (type I and II) and coordinated (type III) movements which are observed at later stages.

A second series of experiments was conducted using curarized embryos in which discharges were recorded in the sciatic nerve, the distal end of which had been cut. The efferent discharges in this nerve were compared with concurrently recorded discharges in the ipsilateral half of the lumbosacral cord. Fig. 2 illustrates the cross-envelope (see Method section) of electrical activity recorded at the two sites. The *symmetry* of the cross-envelopes reveals that sciatic nerve discharges were correlated with cord burst discharges in the 15-, 17-, and 20-day embryos which were examined. These results indicate that cord burst discharges are a reliable index of cord motor output in curarized preparations. The results indicate further that cord burst discharges were not a result of movement artifacts in the floating electrode preparations.
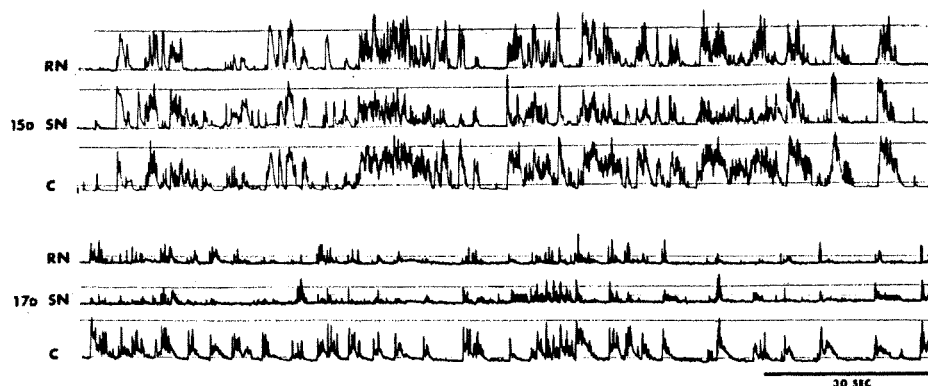
Fig. 3. Comparison of records of integrated electrical activity simultaneously recorded from the lumbosacral spinal cord (C) and the ipsilateral radial (RN) and sciatic (SN) nerves. The nerves have been cut distal to the recording electrodes.

In another experiment, the lumbosacral spinal cord activity was compared with the activity of the ipsilateral radial and sciatic nerves. In a few cases only radial and sciatic nerve activity were recorded. The activity of the nerves provides a rough index of wing and leg movements. As before, the embryos were curarized and the distal ends of the nerves were cut. The results for one 15-day and one 17-day embryo are summarized in Fig. 3. The records have been written out with a B and K Level Recorder. Spinal cord burst discharges show a good but not perfect correlation with discharges in the two nerves. In most cases, discrepancies between cord and nerve activity involved differences in the amplitude, duration, and envelope of discharges in the two structures (Fig. 3). However, one or both of the nerves would occasionally fail to respond to certain spinal cord discharges. The 17-day case showed a number of such differences (Fig. 3). A crucial point to be made concerning these observations of cord and nerve is that only rarely did a discharge occur in a nerve without a correlated spinal cord burst.

The comparison of radial and sciatic nerve activity gives us an approximation of the degree of correspondence between the wing and the leg. In general, we found a rough correspondence between the radial and sciatic nerve activity (Fig. 3). While discharges in the two nerves were by no means completely synchronous, a high level of activity in one nerve was usually accompanied by some related activity in the other. However, great variation was observed in the amplitude, duration, and envelope of discharge in the nerves. High amplitude discharges were usually correlated at both sites; most regional differences involved low amplitude activity. Also, instances were frequently encountered in which a discharge in one nerve was not accompanied by a discharge in the other. Such cases are common in the 17-day record of Fig. 3.

DISCUSSION

The present study offers the first direct electrophysiological evidence that embryonic motility in the chick is *neurogenic*. In embryos ranging in age from 4 to 21 days,

polyneuronal burst discharges within the spinal cord accompany movements of the embryo. The results substantiate the proposal that bursts are motility correlates which was made by Provine[10,13]. The finding of a close relationship between bursts and movements throughout development is a rather surprising result when one considers that the earliest motor end-plates containing cholinesterase do not appear until 10 days in the paraspinal musculature and 12–13 days in the muscles of the distal leg[2]. This localized cholinesterase activity represents an early response of the embryo's muscle membrane to innervation[15]. Before the establishment of the motor end-plates, only provisional neuromuscular contacts are present.[14] Our present results suggest a continuity in the ability of the embryonic muscle to respond to motor nerve input despite the occurrence of profound morphological transformations in the neuro-muscular junction during development[2,7]. The continuity of the pharmacological response of the neuromuscular junction in developing embryos has been previously established[2].

Our investigations have shown that the burst discharges recorded in the lumbo-sacral cord are correlated with the visually observed movements of many widely distributed body parts. The records of activity in the radial and sciatic nerves also demonstrate that cord burst discharges are correlated with activity of at least two widely separated channels of spinal cord motor output. For this reason, a burst discharge recorded from any spinal cord region should be considered as a *general* motility correlate, rather than as a correlate of only those body regions immediately proximal to the recording electrode. This finding is not surprising if one considers that almost simultaneous burst discharges are observed along the entire length of the cord of 6–19-day embryos[9]. However, careful comparison of the simultaneously recorded activity of the radial and sciatic nerves reveals many differences, some of which are great. This is of considerable interest, because the differences between the radial and sciatic nerve activity are usually greater than between the brachial and lumbosacral cord which gives rise to the nerves[9]. This suggests that some kind of selective process may be taking place within the cord. This sorting, if present, may reflect the ontogeny of region specific circuitry within the spinal cord which accompanies behavioral differentiation.

This and other related electrophysiological studies of the embryonic nervous system and behavior[10–12] have several methodological advantages over previous studies which are primarily behavioral in nature. Most earlier behavioral studies relied upon visual observation as a means of data collection, a technique which is fraught with the many limitations associated with subjective decision making. In contrast, spinal cord or peripheral nerve electrical activity provide an objective and easily quantified index of embryonic motility. The measurement of electromyographic activity would also be an excellent technique. One of the attractions of the present methods is that they allow the testing of many hypotheses concerning neural or behavior development which would be difficult or impossible to perform behaviorally. For example, the importance of self-stimulation in initiating and maintaining embryonic movement may be tested by comparing the amount of spinal cord burst activity or motor nerve discharges recorded before and after the immobilization of an embryo

with curare. The results of such an experiment will be reported elsewhere[12]. Also, the effect of periods of chronic immobilization or blockage of bioelectric activity on the development of subsequent behavior can be evaluated with minimal concern for the effects of ankylosis and myopathy which would accompany such procedures and confound the interpretation of purely behavioral results[3,4]. Investigations based on spinal cord electrical activity and related peripheral nerve activity should be relatively resistant to those problems which would interfere with that very labile dependent variable, spontaneous embryonic motility.

SUMMARY

The neurogenic nature of spontaneous embryonic motility in the chick was established in embryos ranging in age from 4 to 21 days. This was done by demonstrating that polyneuronal burst discharges within the spinal cord were correlated with either simultaneously observed body movements or motor nerve discharges. The spinal cord burst discharge was found to be a good neural correlate of general body motility.

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