THE ONTOGENY OF COORDINATED LINB MOVEMENTS IN PRE-HATCHED CHICKS: EFFECTS OF CENTRAL NERVOUS SYSTEM MANIPULATIONS ON THE FREQUENCY AND PATTERNS OF MOVEMENTS

Theses for the Dogree of Ph. D. MICHIGAN STATE UNIVERSITY Alfeo M. Nikundiwe 1972





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thesis entitled

THE ONTOGENY OF COORDINATED LINE MOVEMENTS IN PRE-HATCHED CHICKS: EFFECTS OF CENTRAL NERVOUS SYSTEM MANIPULATIONS ON THE FREQUENCY AND PATTERNS OF MOVEMENTS.

presented by

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has been accepted towards fulfillment of the requirements for

Ph.D. degree in Zoology

Major professor

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ABSTRACT

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By

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This investigation is a two-part study designed to examine the development and control of coordinated limb movements in pre-hatched chicks, between day 19 of incubation and hatching. In the first part of the study, two developmental variables were examined: the temporal pattern of activity and the magnitude of limb coordination. It has been shown by other workers that the alternation of legs (stepping), among other integrated movements, does not occur at all until shortly after day 17. This study, therefore, attempted to ascertain whether some features in the development of leg coordination could still be shown during the last two days of incubation.

The second part of the study dealt with various manipulations of the central nervous system, the purpose of which was to specify those (CNS) areas which function to subserve the alternate pattern of leg movements. To this end, different types of lesions were performed on different groups of embryos. The mid-thoracic transverse section (MTTS) aimed at excluding the possible influence of the brain from reaching and acting on the lumbosacral cord. The lumbosacral mid-saggital section (LSMSS) served to isolate that segment of the spinal cord into left and right halves. This type of lesion not only was designed to uncover subtle reciprocal influence between each half of the cord, but it also enabled the experimenter to determine whether or not limb coordination could occur in the absence of cross fibers. To answer the question as to whether peripheral feedback is involved in the alternate pattern of coordination, deafferentation of a group of embryos was accomplished by extirpation of the dorsal half of the lumbosacral cord (LSDF). In addition to these "basic" lesions, mid-saggital sections and deafferentation were carried out in conjunction with mid-thoracic transections on two other groups of embryos in order to further determine the brain/lumbosacral cord interaction. These "double" lesions were termed mid-thoracic transverse section plus lumbosacral mid-saggital section (MTTS + LSMSS) and mid-thoracic transverse section plus lumbosacral deafferentation (MTTS + LSDF) respectively.

All lesions were performed directly on the neural tube when the embryos were $2\frac{1}{2}$ -3 days old (stages 15-18, Hamburger and Hamilton, 1951). The eggs were left to incubate until day 19, at which time each embryo had part of its shell removed from the blunt end so as to expose the legs. No regeneration was observed in any of the lesions. Leg movements were recorded by use of a polygraph recorder coupled with force-displacement transducers.

Results demonstrated that neither the degree of limb coordination nor the absolute rate of movements manifested clear-cut changes during the two-day period. One conclusion drawn from these findings is that a high degree of limb coordination is already well established by day 19.

Animals with mid-thoracic spinal gaps were able to perform the alternate movements which were indistinguishable from those of the control group. However, activity level of the spinal animals was significantly

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lower than that of the controls. It was nonetheless concluded that the lumbosacral cord possesses the specific apparatus that is competent to bring about consistent alternation of legs. Embryos with mid-saggital sections exhibited a higher rate of activity than did the controls, but the pattern of movements was of the type in which the two legs moved simultaneously. From this experiment two conclusions were made: there exists reciprocal inhibition between the two halves of the cord segment; and that cross fibers are necessary in the execution of the alternate movements. Similarly, deafferentation caused an increase in the rate of coordinated movements, while the resulting pattern of movements was of the simultaneous type. The results suggest that sensory neurons not only act to attenuate motor output, but they are also necessary for consistent alternation of leg movements.

Embryos with the double lesions perform the simultaneous coordinated movements at much higher rates than the control groups. Since activity level in the double-lesioned animal is also greater than that of the corresponding single lesion (i.e. MTTS + LSMSS > LSMSS; MTTS + LSDF> LSDF), it was concluded that the brain inhibits neuronal activity occurring in the lumbosacral cord, but that this inhibition is best detected when the lumbosacral cord is disrupted (saggital section or deafferentation). The results also further suggest that the brain and lumbosacral influences are additive. An alternative explanation which accounts for the tremendous increase in activity in the double-lesioned groups is to postulate that the brain does not directly inhibit the motor neurons involved in leg movements, but rather inhibits some other cells in the lumbosacral cord, whose action, in turn, is to inhibit leg motor neurons. In essence, such a mechanism would be inhibition of inhibition. Were such a mechanism to operate, it would also account for the observed lowering of activity in embryos with only mid-thoracic transections. Possible mechanisms subserving the simultaneous pattern of coordination are discussed. THE ONTOGENY OF COORDINATED LIMB MOVEMENTS IN PRE-HATCHED CHICKS: EFFECTS OF CENTRAL NERVOUS SYSTEM MANIPULATIONS ON THE FREQUENCY AND PATTERNS OF MOVEMENTS

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A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology



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INTRODUCTION AND REVIEW OF LITERATURE

A number of approaches have been employed in the study of patterns of coordinated movements. The type of strategy each investigator selects to use is determined by, among other things, the tools and expertise available to him, the nature of the problem under investigation, and often by the type of specimen with which he works. The array of tactics used and the questions raised all share a common goal; that of attempting to elucidate on the structuro-functional organization of the organism or of its parts.

At the present time, the different approaches can be classified into four broad categories: the electrophysiological method, focal electrical stimulation, gross lesion, and limb grafting and cord transplantation. Despite the superficial distinction among them, these tools overlap quite widely in application. The relative merits and demerits of each approach are all too obvious to the investigator. Lesion studies, for instance, are hardly the tools with which to study the integrative properties of the nervous system, but neither can the "unit" approach be said to tell much about the behavior of neurons at the population level. Nevertheless, discrete use of any one of these methods or a combination thereof, has enabled investigators to compile invaluable information about systems and subsystems and how they interact to produce whole behaviors.

The insect neurophysiologist records the discharge patterns of leg motor neurons during normal walking in unrestrained animals (Ewing and Manning, 1966; Usherwood and Runion, 1970). The results of these investigations define fairly accurately, the normal patterns of motor neuronal

activities which, in turn, must be described in terms of the properties and connections of the cells within the central nervous system and other neural activity in the various reflex pathways. Motor neuronal activity can also be investigated in dissected preparations by selective stimulation of the various constellations of peripheral receptors (Usherwood, Runion, and Campbell, 1968). Furthermore, it is possible to record spontaneously generated activity of the motor neurons before and after deafferentation (Pearson and Iles, 1970). By comparing these records and those obtained from freely walking animals, conclusions can be drawn about the existence of central programs and how they are modified by peripheral feedback.

Recently, the technique of focal electrical stimulation of the brain with chronically implanted electrodes has been used to study simple integrative functions of the nervous system (Fraser-Rowell, 1963 a, b; von Holst and Saint Paul, 1962, 1963; Vowles, 1961; Huber, 1965). The behavioral patterns which have been investigated mostly are locomotion, breathing, stradulation and flight (Wilson and Gettrup, 1963; Huber, 1962). These kinds of studies were not so much concerned with finding which brain centers or regions control what behavioral activities, as they were concerned with delineating the role played by the brain and other areas in influencing those areas directly involved with the execution of movement patterns themselves.

The lesion method, by which a portion of neural tissue is surgically removed or the continuity of the nervous system disrupted, has served to complement both electrophysiological and focal electrical stimulation studies and vice versa (Bethe, 1930; Roeder, 1963; Hughes, 1957; Hamburger and Narayanan, 1969; Ten Cate, 1961; to mention only a few).

During the last decade or so, a sophisticated method of lesioning has been developed in which small areas of neural tissue are destroyed by use of electrocoagulation (Ballintijn, 1961; Vowles, 1958). Included in the lesion category is selective irradiation of neural tissue with gamma rays (Oppenheim et al., 1970). The majority of these studies dealt with varicus attempts to reconstruct the structural basis for control of behavior from what is already known about the properties of the nervous system. One of the primary objectives as well as consequences of this approach has been to give the investigator an insight into, and a first approximation of, the system with which he is working and on which to build further refinements.

The pursuit to study development of motor patterns has led investigators to use embryonic preparations exclusively. The method which makes it possible to manipulate the embryo has been cord grafting and limb transplantation (Coghill, 1929; Carmichael, 1926, 1927; Detwiler, 1936; Weiss, 1941 a, b; Szekely, 1963; and Hamburger, 1972). The results obtained from the experiments carried out by these workers suggest that in vertebrates, at least, the spinal cord segment at the limb level alone possesses the specific apparatus which enables the limb to move in a given manner. Furthermore, Szekely (1967) and Hamburger (1972) were able to demonstrate that the type of spinal cord segment, whether brachial, thoracic, or lumbosacral, determines the type of limb coordination to be exhibited. Thus, in a 19-day chick embryo, only the brachial segment will permit simultaneous flapping of the wings, while the lumbosacral segment alone will allow the legs to kick in alternation. Limbs which are grafted in the thoracic region of the spinal cord either become motionless or merely exhibit slight twitches. The inability of the thoracic segment to coordinate limb movements is attributable to "faulty" neural connections

within itself, since the muscles of the grafted limbs receive adequate innervation and normal nerve distribution from it (Detwiler, 1922; Piatt, 1956).

Armed with this kind of data, Straznicky (1963) took advantage of the fact that the avian pattern of locomotion is different from that of the much researched land tetrapod. By replacing the brachial segments with thoracic and lumbosacral segments in chick embryos, he was able to confirm the results obtained by workers previously mentioned. Basically, he demonstrated that whereas wings innervated by the grafted thoracic segments remained perfectly motionless, those innervated by the lumbosacral segments exhibited functional establishment. Such chicks, when walking, raised and lowered their wings at the shoulder joint in parallel synchrony with the stepping of the ipsilateral leg. From all these results, one unequivocal conclusion has been drawn: that clear differences prevail in the functional capacity of different spinal cord segments, and that these differences are determined already in early embryonic life (Straznicky and Szekely, 1967).

In the study of coordination of limb movements, one of the inevitable questions is to determine whether a particular pattern is centrally programmed. The converse is to show if and how peripheral factors modify centrally occurring events. One of the most controversial studies has been the work of Weiss (1941 a, b) in which the left and right forelimb rudiments of salamander embryos were interchanged at a stage when the antero-posterior axes of the limb had already been determined. When normal limbs developed, they were backwards instead of forewards. After nerve connections with periphery had been established, the grafted limbs moved just as they would have done if they had been left in their original

positions, working to move the animal backwards when the movements of the rest of the body were working to move the animal forewards. Experience of up to one year did not appear to modify the motor pattern. Because such motor patterns develop in the absence of sensory innervation, their manifestation has been considered to be the consequences of existing central programs. In a more recent paper, Szekely et al. (1969) have recorded muscle potentials from eight muscles of the forelimb in freely moving, normal and deafferented newts. The myograms revealed delicate interaction of the antagonistic muscle groups. Despite a few irregularities in the placing of a limb, deafferentation of one or both limbs did not appear to alter normal activity patterns of the muscles. These investigators, like Weiss, concluded that the brachial segment is capable of securing timed activity of agonistic and antagonistic limb muscles without the necessity of receiving afferent feedback from the moving limb. Moreover. by employing Weiss's (1950) "deplantation" technique. Szekely (1967) was able to demonstrate that spinal segments from the limb level have the competence to coordinate limb movements even if they are isolated from the rest of the nervous system, and receive non-rhythmic excitation.

The existence of central programs which determine the output pattern of motor neurons exclusive of sensory feedback in vertebrates is supported by many electrophysiological findings in invertebrate systems. In the locust flight system, the generation of normal output requires sensory input from peripheral receptors, but phasic information in these afferent signals is irrelevant (Wilson and Gettrup, 1963). The crucial point in this study lies in the fact that feedback, although present, does not determine the wing beat cycle; it simply serves to excite the central nervous system in a nonspecific way so that it operates faster. A similar

conclusion has been drawn for the sound-producing mechanism in the cicada (Hagiwara and Watanabe, 1956).

Just as there are systems that establish the existence of central programs, so can the peripheral patterning of motor output be shown. In the toad, Gray and Lissmann (1946 a, b) demonstrated that the rhythmical sequence of limb movements can only occur if at least one intact spinal nerve is present. Furthermore, peripheral influence is not simply a matter of local reflexes, but rather sensory input from each limb has some influence on the posture of all limbs (Gray, 1950). In mammals, deafferentation of even a single limb usually results in the failure of that limb to show locomotory activity (Lassek and Mayer, 1953). However, because severance of the sensory nerves in the distal portions of the cat's limbs does not prevent walking, it seems probable that the essential feedback comes from the proprioceptors in the proximal sections (Sherrington, 1910).

In insects, too, removal of tibial and tarsal receptors from the femoral chordonotal organ in the locust results in changes in motor activity and non-coordination of leg movements (Usherwood et al., 1968; Usherwood and Runion, 1970). Similarly, removal of coxal hair plates results in overstepping (Wendler, 1966). In the cockroach, there is evidence to suggest that both central programming and reflex control are important in producing rhythmic movements of single legs and coordinating the movements of different legs (Pearson and Iles, 1970; Delcomyn, 1971).

The present study represents a departure from the observations of Hamburger and Oppenheim (1967) and Hamburger (1968), who reported that before day 17 of incubation, each limb in the chick embryo moves independently of the other. After day 17, the two wings flap in unison, while the legs kick alternately. The questions I asked include:

- 1) From the earliest time in which an embryo can be safely opened and movements recorded, to the time of hatching, what is the temporal relationship between coordinated leg movements and total leg activity? In other words, does the degree of coordination increase over time?
- 2) If, in fact, there is a shift from relatively non-coordination to coordination, how does the change occur developmentally? Does it represent a gradual and sequential change or is it a sudden turn-on event? The studies carried out by Balaban and Hill (1969) describe behaviors appearing around hatching time as being sudden changes in levels of performance.
- 3) In specific, does the change in the pattern of leg movements from relatively non-coordination to a high degree of coordination represent changes in local events occurring at the lumbosacral region alone, or do other parts of the central nervous system, especially the brain, contribute in the development as well as in the actual performance of these coordinated movements?
- 4) Do sensory inputs modify the level and/or pattern of these movements?

Table 1 illustrates the experimental design, dealing with the following aspects:

- 1) The possible influence of the brain on the rates and pattern of coordinated movements of the leg.
- 2) The possible existence of a reciprocal influence between each half (right vs left) of the lumbosacral segment, and how this relationship affects both rate and pattern of coordinated leg movements.

4) The possible existence of suprasegmental (brain) and segmental * (lumbosacral) interaction, and the degree to which this relationship influences rates and pattern of coordinated movements.

In order to put these different aspects of the experimental design under test, experiments involving surgical manipulations of the central nervous system were carried out. The description of each manipulation is incorporated in the design, which also allows the following comparisons to be made:

Table 1. Experimental Design.





Figure A. Schema of Lesions.

MATERIALS AND METHODS

All embryonated eggs were obtained from a flock of White Leghorn chickens, which were raised by a commercial hatchery (Richard Hutting, Lansing). They were then stored in a refrigerator at 6 Celsius for a period of 24-48 hours in order to decrease the variability in the stages of development among individual embryos (Gottlieb, 1963). They were incubated on their sides (lateral position) at a temperature of 37.5 Celsius and relative humidity of 70-80 % in commercial incubators (Sears Roebuck and Co., Model No. 288.735). The incubators were not provided with contraptions for turning the eggs, and for reasons stated in the surgical section of this study, no attempt was made to rotate the eggs manually. Preparation of Embryos

To prepare embryos for surgical operations, eggs were removed after they had incubated for $2^{l}z$ -3 days. Each egg was then candled to mark the position of the embryo inside the shell. A sterilized probe was used to puncture the egg at the center of its blunt end over the air space in order to equalize the pressure inside the egg prior to carving a lateral window through which surgery is performed. Equalization of pressure prevents the embryo from adhering rigidly to the overlying shell membranes.

The window itself was made by placing the egg on its side into a carved-out rubber egg-holder, whose receptacle is in turn egg-shaped. The portion of the shell to be removed was sterilized with 70 per cent ethyl alcohol. With the egg securely placed in the egg-holder, a dental drill (Emesco Model No. 10), using round bits (Busch No. 4 and 5), was used to

dig an oval-shaped furrow on the shell. The circumscribed shell was then lifted with a pair of sterilized forceps. The shell membranes were carefully peeled from the underlying embryonic membranes, thus creating a window, which exposes the embryo for manipulation. The drilling method offers two distinct advantages over other methods: it allows the experimenter to accurately control the shape and size of the window; and it produces minimal disturbance to the integrity of the embryo even after extensive vascularization has occurred.

In order to resolve the structures of the developing embryo in greater detail, i.e. neural from somites, vital stains were used. For all the experiments, neutral red embedded in an agar carrier was the preferred stain. Staining itself consisted of dropping a small cake of the stain material onto the area to be operated upon, care being taken not to overstain. After adequate staining had been achieved, the cake was removed with a pair of forceps. Those embryos which were not sufficiently developed for operation had their windows temporarily closed with Scotch tape and were then returned to the incubator to be attended to later.

Surgical Operations

Operations on embryos involving the creation of spinal "gaps" were performed by removing small segments of neural tube in the mid-thoracic region during stages 15-16 (Hamburger and Hamilton, 1951). The length of each gap was equivalent to the length of 2-3 somites. All other operations involving the sectioning of the lumbosacral cord in one way or another (mid-saggital section, and deafferentation) were performed during stages 17 and 18. A mid-saggital section of the lumbosacral cord was favored by these later stages because of three main factors: during these stages, the neural tube has just closed along the dorsal posterior portion, an event which enables the experimenter to make an accurate median section,

which divides the neural tube into equal left and right halves; the size and growth of the neural tube is of substantial magnitude so as to endow the tissue with inertia, against which the surgical knife acts to make a clean cut without maceration; and lastly, these stages are immediately followed by growth and development of limb buds, whose increasing mass have a pulling effect on tissue surrounding each side of the cord, thus ensuring that the separated halves do not come together. This strategy obviated the placing of neural blocks, such as tantalum foil, along the mid-line of the neural tube. Operations which involved the removal of the dorsal half of the neural tube (deafferentation) were best performed if the neural tissue were first separated from the adhering pia and dura mater prior to the shaving of the entire lumbosacral segment.

The operations described above made use of microsurgical technique whose basic elements and virtues have been described (Hamburger, 1960; Wenger, 1968). The vibrating needles used in these operations were driven by a phonograph crystal cartridge (Astatic Model N 4-2), a regulated power amplifier (Elin RA-1100), and a wide range oscillator (Hewlett-Packard 200 CD). The exact shape and size of each needle were determined by the type of lesion to be performed. The basic hook-up equipment is diagrammed below:



Figure B. Block diagram of the microsurgical apparatus.

The variable frequency oscillator was particularly suitable because at any given voltage, the maximum amplitude of the vibrating needle tip depended on, among other things, the output frequency, the resonance characteristics of the crystal cartridge, as well as the material strength of the needle itself. It was generally found that for this combination of equipment components, a frequency of 2300 Hz., and an output voltage of 150-200 V were very optimal for most of the operations.

When each operation was completed, the window on the egg was sealed with a cover glass, which was glued to the shell with a thin film of melted paraffin. The egg was then returned to the incubator and left there to incubate in the lateral position, with the cover glass uppermost. The eggs were not rotated during the remaining 16_{2} -17 days, even though rotating eggs enhances hatchibility. The reason for not rotating the eggs is based on the fact that, whenever young embryos are allowed to come into close contact with the cover glasses, they get stuck there, and invariably, death ensues.

Recording

Embryos were removed from the incubator on day 19 (at 456 hours) of incubation. Only those embryos whose beaks had penetrated the choricallontoic membrane and were exhibiting strong pulmonary respiration were used. Each embryo had part of its shell removed from the blunt end so as to expose the head and wing areas. The choricallontoic membrane was carefully peeled in the direction of the blood flow in order to avoid excessive bleeding in the still existing choricallontoic blood vessels. The head, which is normally tucked under the right wing, was pulled out and the coiled neck straightened. The two wings were placed in such a way that they drooped over the shell on each side. Similarly, the neck and head were allowed to droop forward over the shell in a position tangential to the wings. This symmetry was crucial to the proper recording of leg movements in that it kept the movements of the other parts of the embryo's body (wings, head) from pulling on or interfering with the strings attached between the feet of the preparation and the transducers. With the aid of a blunt pair of forceps, each leg was pulled up and out of the shell; a piece of thin thread was attached to the web of each foot between the second and third digits. The legs were then gently pushed back to their normal position within the shell. Each embryo was then placed in a glass fish-bowl which, in turn, was partially submerged in a water bath

(Precision Scientific Co., Thelco Model No. 83), where temperature and relative humidity were similar to those of the incubator. The loose ends of the thread were attached to force-displacement transducers (Grass type FT .03). Care was taken to ensure that tension on each thread was neither too taut so as to hinder leg movements, nor too loose, such that some movements went unrecorded. The transducers were connected by means of cables to a polygraph (Grass Model &P) using D.C. pre-amplifiers. Recording of leg movements was commenced after the animal had adapted to the new situation for a period of two hours. During this time also, the machine was turned on and the amplifiers calibrated.

Measurements

From the polygraph print-out, two basic measures were selected and analyzed to account for the ways in which leg movements occur in the prehatched chick: frequency and pattern. Needless to say, frequency in this study refers to the number of coordinated leg movements per unit time. Two basic patterns of coordination were found in the course of experimentation: the simultaneous pattern in which the two legs move in unison, and the alternate pattern in which the two legs move within a determined time unit of each other. A criterion established to distinguish one pattern from the other was that, in order for any movements by the two legs to be considered simultaneous, they had to occur within one second of each other. For the alternate pattern, the movements had to occur between 1-2 seconds of each other. By this definition, then, whenever movements by the two legs were separated by an interval of more than 2 seconds, they would be considered uncoordinated. In reality, however, non-coordination (regardless of the pattern of movements displayed) consisted of movement(s) made by one leg, unaccompanied by the corresponding movement(s) of the other leg.

RESULTS

In order to determine whether or not coordination of leg movements incorporates developmental features during the last two days of incubation (from day 19 to hatching), two linear scales have been used: the rate of coordinated movements as percentage of total activity (coordinated plus uncoordinated movements), and absolute rate.

Figures 1-5 illustrate that coordinated leg movements, regardless of the pattern displayed, constitute about 90 per cent or more of total activity. This ratio does not alter in any significant degree during the two-day period. These results also hold true for the absolute rate; the quasi increase in frequency of movements observed in the two groups (figures 4 and 5) is not significantly different from the zero slope (regression analysis).

Figures 1-5: the top line represents coordinated movements and its scale is the left vertical axis. The bottom line represents frequency and its scale is the right vertical axis. The legend for each group is provided below each figure.







Figure 2. Mid-thoracic transverse section.



Figure 3. Lumbosacral deafferentation.



Figure 4. Lumbosacral mid-saggital section.



Figure 3. Lumbosacral deafferentation.



Figure 4. Lumbosacral mid-saggital section.



Figure 5. Mid-thoracic transverse + Lumbosacral mid-saggital sections.

Activity Peak as a Basis for Analysis

When one draws a curve which describes an activity of an animal recorded over a period of time, it is observed that the temporal pattern is such that each animal builds its activity to a peak, after which activity decreases. Both the build-up and decline phases may be gradual or precipitous. According to this study, there occurs only one peak per day, although the time of the appearance of the peak seems to be related neither to a strict chronologically-determined developmental stage, nor to the time of the day. Nonetheless, this build-up of activity to a peak is one characteristic that is shared by all animals. Therefore, activity peak has been selected as a reference point at which the axis of one curve is superimposed on another in order to compute average activity level. It has also been selected as a single point at which the level of activity of one group is compared to another. In doing so, developmental time in terms of days and hours of incubation has been collapsed into a single curve, with two temporal components: time before and time after activity peak.

To make comparisons on the rates of coordinated movements among the various groups, the Mann-Whitney U tests were chosen. The experimental design (Table 1) was adopted in order to make comparisons (activity peak curves) which provide answers to the following questions:

- 1) What kind of influence, if any, does the brain have on the frequency and pattern of coordinated leg movements?
- 2) Are there influences within the lumbosacral spinal cord which affect both frequency and pattern of leg movements?
- 3) Does sensory feedback play a role in the degree to which the frequency and pattern of coordinated movements occur?
- 4-7) Is it possible to show the existence and magnitude of the brain/ lumbosacral interaction, insofar as it affects both frequency and pattern of coordinated leg movements?

I. Effects of mid-thoracic transverse section on the frequency and pattern of coordinated movements.

When the frequency of coordinated movements in animals with midthoracic spinal gaps is compared with that of the controls, a significant difference is obtained (0.05 > P > 0.02, two-tailed; Fig. 6). In conformity with the rest of the groups, comparisons are made at the activity peak of each group.



The pattern of coordinated movements in animals with spinal gaps of the thoracic segment is basically one of alternation. In this regard, the spinal animals were no different from the controls. Upon hatching, the chicks were able to tuck their legs under the venter and perform the righting response when placed on their backs as well. These spinal animals, however, unlike the controls, could neither stand nor walk. The only locomotory movements they were capable of were those akin to crawling, which was achieved by rapid kicking of the legs braced against a rough surface.

II. Effects of lumbosacral mid-saggital section on the frequency and pattern of coordinated movements.

Animals with, essentially, separate left and right halves of the lumbosacral spinal segment have significantly higher rates of coordinated activity than the controls (.01 > P > .005, two-tailed; Fig. 7).

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The pattern is different from that of the two previous groups in that simultaneous kicking of the legs, rather than the alternate configuration, is observed. Yet the rapid flexions and extensions of the legs in unison are so precise that they must be reckoned as coordinated movements. As with all other groups, uncoordinated activity consisted of single or multiple movements by one leg, unaccompanied by corresponding movements of the other leg. This type of lesion imposes a severe posture on the animal after hatching. The newly-hatched chick lies prostrated on its venter, while both legs are rigidly extended parallel to each other behind the rest of the body. Any attempt to forcibly flex and tuck the legs under the animal's venter is met with persistent resistance, followed by a resumption of the extended-legs posture. These animals cannot right themselves, or do so with extreme difficulty.
III. Effects of deafferentation of the lumbosacral segment on the frequency and pattern of coordinated movements.

Removal of the dorsal half of the spinal cord in the lumbosacral segment constitutes a form of deafferentation. Animals with such lesions (ISDF) manifest a high level of coordinated activity that is significantly different from that of the control group (0.05 > P > 0.025, two-tailed; Fig. 8).

Deafferented animals perform simultaneous movements of paired limbs in a fashion that is indistinguishable from that of mid-saggitally dissected preparations. Similarly, the posture assumed immediately after hatching is one in which the chicks prostrate on their venters, with both legs extended behind the body.



IV. Effects of mid-thoracic transverse and lumbosacral mid-saggital sections (MTTS + LSMSS) on the frequency and pattern of coordinated movements.

Preparations with both transverse and longitudinal sections not only exhibited rates of activity which were significantly higher than those of the controls (P < 0.002, two-tailed; Fig. 9), but manifested patterns of activity together with postures which parallel those of the mid-saggitally lesioned animals as well.



A description of coordinated movements accompanied by changes in posture after hatching in the double-lesioned animals fits quite well, with minor variations, that of the animals with longitudinal sections alone. Thus, simultaneous kicking of the legs and extension of the legs behind the body are consistently observed. One of the postural differences that exists between a double-lesioned animal and one with a mid-saggital section is that the degree to which the legs are rigidly extended behind the body subjectively appears to be less in the former than in the latter. The reduction in rigidity (measured by flexing the legs ventrad) is, perhaps, attributable to the thoracic transection.

V. Effects of deafferentation and mid-thoracic transverse section on the frequency and pattern of coordinated movements.

If in addition to deafferentation, embryos are subjected to midthoracic transections, a higher rate of activity is observed in the double lesioned animals as compared to the controls (P < 0.002, two-tailed; Fig. 10). The purpose of the transverse section is, as it was for group IV (MTTS + LSMSS), to prevent the influence of the brain from reaching and acting upon the lumbosacral segment of the spinal cord.



The pattern of movements in the double lesioned animals conforms to that of groups II, III, and IV, i.e. the simultaneous kicking of the legs. On the other hand, unlike group IV (MTTS + LSMSS), in which the post-hatch posture exhibits a marked reduction in the degree to which the two legs are extended behind the body, presumably because of the transverse section, the same transection bears no effect on the posture of the deafferented embryo. That is to say, the rigidity of the legs in the deafferented embryo is subjectively equivalent to that of the deafferented and transected embryo.

VI. <u>Lumbosacral mid-saggital section vs. mid-thoracic transverse + lumbo-</u> sacral mid-saggital sections (LSMSS vs MTTS + LSMSS).

The purpose in comparing these two groups is to determine if the level of activity of the legs is influenced in any significant degree by the interaction of the brain and the lumbosacral spinal cord. Suffice it to mention that the two groups share two other characteristics which make this comparison even more valid: they exhibit the same pattern of coordination; and they both share the mid-saggital section. Figure 11 illustrates that animals with both mid-thoracic transections and longitudinal sections exhibit higher levels of leg activity than those with longitudinal sections alone (0.05 > P > 0.025, one-tailed).



VII. Deafferentation vs deafferentation + mid-thoracic transverse section.

These two groups are compared in order to assess the relative magnitude of the influence of the brain on the lumbosacral region. Figure 12 shows that the double lesioned embryos show a greater level of activity than those with simple deafferentation (0.025 > P > 0.01, one-tailed).



Figure 12.

Table 2. Summary of Results.

Type of Lesion

	MITS	SSMSI	LSDF	NTTS + LSMSS	MITS + LSDF
ACTIVITY					
Control	lower	higher	higher	higher	higher
SSAST	1	1	8 8 8	higher	8
LSDF	•	:	:	1	higher
PATTERN	alternate	simultaneous	simultaneous	simultaneous	simultaneous
POSTURE	a) righting b) legs tucked	 a) no righting b) legs extended c) legs rigid 	 a) ? b) legs extended c) legs rigid 	 a) no righting b) legs extended c) legs less rigid 	a) no righting b) legs extended c) legs rigid

DISCUSSION

As re-statement of the problem to which the present study addressed itself, a search was made for the development of coordinated leg movements in the pre-hatched chick. The temporal relationship between coordinated activity and total leg activity envisaged changes in pattern as well as in magnitude. Finally, through the various manipulations of the central nervous system, we sought to relate behavioral patterns and their magnitude to their underlying neurological substrates.

Temporal Pattern of Coordinated Movements.

While this study failed to establish the existence and mode of development for coordinated movements, there were two fundamental rationales for attempting to do so. First, since coordination of limbs does not set in until day 17 of incubation (Hamburger and Oppenheim, 1967), it was reasonable to assume that some residual component of this development could still be shown between day 19 of incubation and hatching. The second rationale has been proposed or alluded to for telecological reasons (Hamburger and Oppenheim, 1967; Oppenheim and Narayanan, 1968). That is to say, a high degree of coordination, in terms of absolute rate and types of movements, is thought to be necessary in order to enable the animal to escape from the mechanical confines of the shell. That the rate and pattern of coordinated movements do not alter during the last two days of incubation has been adequately shown in this study (figures 1-5).

Another aspect of the ontogeny of coordinated movements, the existence for which could not be established in this study, is the mode or pattern of development. By pattern of development it is meant, whether a

high degree of limb coordination such as is observed in a 19-day-old embryo results from gradual and sequential changes in central and/or peripheral factors, or whether it is simply a function of sudden, turn-on events. Kuo (1967) has argued in favor of the former as the mechanism underlying development of most behaviors. Yet it is known that certain behaviors such as vocalization, head-lifting, and eye-opening in chick embryos appear very suddenly, and are thereafter maintained at high levels (Balaban and Hill, 1969). Similarly, the back thrusts of the head in duck embryos suddenly increase in frequency about 16 hours prior to hatching (Oppenheim, 1970). The righting response and, to a large degree, hatching belong to the latter category. It is evident from this study that any further attempts to study the ontogenetic facets of limb coordination must be directed to embryos which are at least younger than 19 days, a task which imposes severe limitations on the ability to expose the embryo well enough for observations or recording and still maintain it alive.

A temporal pattern in the form of a slight increase in the rate of activity was exhibited by two experimental groups (ISMSS, MTTS + ISMSS; figures 4 and 5). The trend toward an increase is attributable to the mid-saggital section since other groups with other types of lesions, together with the control group, did not show such a pattern. It is believed that a larger sample size in each of the two groups might have rendered the increase significant. Were that to occur, such an increase would be in contradiction to the normal activity pattern in which there is a precipitous decline in overall activity after day 17 of incubation (Hamburger et al., 1965; Hamburger and Oppenheim, 1967). This decline has been shown in this study to have reached an asymptote by day 19 and remains in that level until about the time of hatching. At any rate, further questions

could be asked about the nature of the increase; whether it is the product of a change localized in the lumbosacral region, or whether an early mid-saggital section somehow activates the organism to perform a higher level of overall activity, and not just the legs. It would also suggest that the general decline in overall activity observed in normal embryos could be partially reversed by certain specific lesions. That in turn would lead one to ask the question as to whether or not the onset of integration of movements and the decline in overall activity seen shortly after day 17 have a cause-effect relationship.

By continuous recording of leg movements, it has been possible to formulate a description of the temporal pattern of activity, which culminates in a peak, wherein activity level often represents a three-fold increase from a "base-line" level. To my knowledge, such a phenomenon has not been described before for chick or duck embryos, either as a dailyoccurring event or as an occurrence specific to animals in their last days of incubation. Activity peak of this nature has eluded observance and, therefore, description primarily because most investigators use a standard 15-minute observation period or a variation thereof (Hamburger and Balaban, 1963; Hamburger et al., 1966; Decker and Hamburger, 1967). This sampling approach makes it difficult to reconstruct a relatively shortlived event such as peaking, especially since under standard incubation conditions, the time of appearance of activity peak seems to bear no relationship either to a strictly developmental stage or to the time of the day.

Effects of Mid-thoracic Transverse Section on the Frequency and Pattern of Coordinated Movements.

The rate of coordinated movements was significantly lower in animals with thoracic gaps than in the controls ($P \ge .05$). These results are in

agreement with the early work of Hamburger et al. (1965), who found that spinal embryos between the age of 8-17 days exhibited significantly lower amounts of leg motility than controls. In disagreement with both of these findings is the study made by Oppenheim and Narayanan (1968), who failed to show such a difference in 19-20-day embryos. What the present study points out, contrary to Oppemheim's and Narayanan's implicit suggestion, is that the lowering of leg activity in spinal animals is not consequential to the 8-17-day embryos only, but attends to older embryos as well,

Hamburger and co-workers have attributed the low level of leg motility in spinal animals to the onset of degeneration of the spinal cord motor neurons. Two arguments are presented here to suggest that additional factors other than degeneration might be responsible. First, if low level of leg motility were the result of degeneration, then, assuming equal or equivalent activity, older embryos which would be attended by extensive amount of degeneration should perform movements at significantly lower levels than younger embryos (i.e. comparing rates in 19 and 20-day embryos or Hamburger's 16 and 17-day embryos). This is not what is observed. Second, in this study, where extensive lesioning was employed (multiple lesions, figures 9 and 10), a corresponding lowering of activity representing extensive degeneration should be expected. In general, however, the results have shown just the opposite.

The capacity of the lumbosacral segment of the spinal cord to perform alternate coordinated movements of the legs without the aid of the other parts of the central nervous system has been borne out in this study as well as in other studies (Hamburger and Balaban, 1963; Oppenheim and Narayanan, 1968; Detwiler, 1936; Szekely, 1963, 1967). Similarly, locomotion in mantids (Roeder, 1963) and in crickets (Huber, 1959) appears to

be highly organized in the thoracic ganglia. The role of the brain is seen as one of providing stimulus for excitation and inhibition to the otherwise competent thoracic ganglia. Furthermore, even highly complex behaviors such as flight and jumping in locusts can be accomplished after complete decapitation (removing the subesophageal ganglia) (Wilson, 1961).

A few studies, however, emphasize the importance of the higher centers in the actual execution of movement patterns. Thus, Decker and Hamburger (1967), Hamburger and Narayanan (1969) suggested that the performance of coordinated movements such as wing flapping and tucking of the head under the right wing in chick embryos is correlated with the maturation of higher centers. Hughes and Prestige (1967) found that tadpoles with spinal transections of the cervical region fail to exhibit swimming movements of the legs.

Effects of Lumbosacral Mid-saggital Section on the Frequency and Pattern of Coordinated Movements.

Animals with longitudinal cuts performed simultaneous coordinated movements at higher rates than the controls (P < .01). These results point out that the two halves of the lumbosacral segment, in addition to providing reciprocal inhibition for the execution of some reflexes, as is commonly found in vertebrates, there is also mutual inhibition of endogenous activity. An analogous situation has been reported for the metathoracic ganglion of the cockroach, in which it was found that activity of many motor neurons was partially suppressed by neural connections with the opposite side of the same ganglion (Weiant, 1956).

That the two legs should perform simultaneous movements after neural connections between the two segments of the lumbosacral region have

been severed is an unexpected finding. The type of information which the isolated segments use to signal the movement of their respective legs in synchrony is not yet understood. Since further isolation of the segments from the possible influence of the brain (mid-thoracic transection) results in the same basic pattern, the brain could not be operative in providing the necessary information.

The postural reflexes shown immediately after hatching indicate that the severing of cross-fibers between the two segments so strongly activates the extensor muscles that the opposing action of the flexors is overcome. The net result of this action is the rigid extension of the legs behind the body, a phenomenon somewhat akin to mid-collicular decerebration (Sherrington, 1906).

Effects of Mid-thoracic Transverse and Lumbosacral Mid-saggital Sections on the Frequency and Pattern of Coordinated Movements.

The two limbs in the double-lesioned preparation move in perfect synchrony (simultaneous) at a much higher rate than that of the control (P < .002). Furthermore, the frequency of movements in animals with both the transverse and longitudinal cuts is greater than that of animals with the longitudinal alone. From these results it can be inferred that the nervous system anterior to the thoracic region normally exerts an inhibitory influence on the lumbosacral region, but that this influence is best demonstrated after the integrity of the lumbosacral segment has been disrupted (mid-saggital section). The literature is abundant with studies showing inhibition of the spinal cord or its analogue by the brain (Roeder, 1963; Huber, 1959; Decker, 1968; Weiant, 1956). Roeder has reported on the effect of severing the connections between the brain and the subesophageal ganglion in the mantid as being continuous forward locomotion.

A similar situation obtains for the cricket except that the locomotor mechanisms in the thoracic ganglia are activated by the central body, in addition to the subesophageal ganglion, and that both are in turn regulated through inhibition arising from the mushroom bodies (Huber, 1965). In the chick embryo, Decker has shown that unilateral or bilateral extirpation of the otocysts results in increased total body motility, suggesting that one of the normal functions of the vestibular centers is to check the spontaneous activity of the spinal cord motor neurons. Weiant's study, which has already been cited in the previous section, revealed that activity in the efferent fibers of the metathoracic ganglion was greatly enhanced as soon as the ganglion was further isolated from other nerve connections. The greater the isolation, the higher the frequency of firing.

An alternative explanation which accounts for the tremendous increase in the rate of activity in the double lesioned embryos is to postulate the existence of a mechanism by which the brain inhibits some neurons in the lumbosacral cord. These neurons, in turn, inhibit the motor neurons involved in the performance of leg movements. Such a mechanism could be said to operate through inhibition of inhibition. Such a mechanism would also account for the lowering of activity observed in embryos with mid-thoracic transections. The validity of the mechanism is even more apparent if it is assumed that the lumbosacral inhibition alone produces greater effects than that which is projected from the brain. Figure C illustrates how this mechanism might operate:



Figure C. Block diagram of the inhibitory mechanism.

Similarities between animals with saggital sections and those with saggital plus transverse sections have been mentioned already; similarities in elevated rates of activity, of posture, pattern of movements, and of the temporal pattern of activity during the last two days of incubation. Of these, perhaps the most intriguing is the pattern of movements. The very reason for carrying out double lesions was to test the hypothesis as to whether or not the animal could and might be using information from the brain to coordinate its movements. That that is not the case has been shown already. A few speculative explanations are offered to account for the simultaneous pattern of movements. The first is one of mechanical factors; that is, because recordings of the leg movements are made while the animal'is still partly propped up in the shell, the tarsals and tarsal joints are frequently braced against the shell. The mechanical force resulting from the two legs braced against the shell, and from the friction arising from the movement as the tarsals scrape the inside of the shell--- these factors could simultaneously excite the afferent fibers in the two segments, and the afferents, in turn, send volleys to their respective motor neuronal pools, and thus enable the legs to move in phase. Second, proprioception as the source which signals the next sequence of movements cannot be ruled out, especially if severing of the connections between the two segments favors the development of biological oscillators in each segment. These oscillators could be synchronized by the position of the limbs, or by the velocity of the limb movements themselves.

A third and remote possibility is for the accumulation of a large resevoir of transmitter substance in between the two half-segments. Were the substance to possess the property of exciting the neurons embedded along the inside borders of the segments, it is conceivable that tonic stimulation of the motor neurons might take place.

The simultaneous pattern of movements makes it possible for a testable statement to be made about the normal ontogeny of coordination. Namely, that the onset of <u>alternate</u> coordinated movements does not simply originate because of the establishment of competent structural and functional connections between the two halves of the lumbosacral segment, but also because of a host of other changes which take place in other areas of the lumbosacral region and even beyond. To put it another way, if one were to observe the movements of a mid-saggitally sectioned preparation before day 17 of incubation, it is predicted that the animal will exhibit the random and unccordinated movements of the two limbs to the same degree that one sees in the unoperated control. Furthermore, if it is assumed or shown that the development of the alternate pattern i.e. both types of patterns begin to operate around day 17, then it can be further postulated that both of these patterns are subserved by common features which

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undergo changes to trigger, or allow, coordination to take place. The difference between the two patterns, therefore, would be reduced to the mere presence or absence of cross fibers.

Effects of Deafferentation of the Lumbosacral Region on the Frequency and Pattern of Coordinated Movements.

The rate of coordinated movements in the deafferented animals was significantly higher than in the controls (P < .05). One characteristic which is unique to the deafferented animal is uniformity of activity. Even though it shows activity peak as such, the element of dynamic range (high activity interspersed with low activity), which is so prevalent in all other groups, is at a minimum or lacking. It is as if the interneurons and the motor neurons associated with the initiation and maintenance of spontaneous activity are firing at a fairly constant rate, which in itself would suggest that one of the functions of the sensory cells is to check endogenous activity. That sensory input determines motor output, particularly in terms of reflex activity control mechanism, is common knowledge; but data are scanty to support the notion of long-term suppression of free-running motor activity by sensory input. Ruck (1961) succeeded in demonstrating that endogenous activity originating in the terminal processes of large axons that form the ocellar nerve in the insect is inhibited by light impinging on the photoreceptor cells. The neural signal transmitted to the brain, therefore, represents a compromise between endogenous activity of the fibers and the inhibition imposed by the photoreceptors. Decker's (1968) experiments in which the otocysts were removed, led him to conclude that vestibular mechanisms inhibit activity in the neurons of the spinal cord. Although sensory organs and their central connections have not been implicated in this study, it is worth mentioning that experiments in which the influence of the brain was excluded from reaching the

deafferented lumbosacral cord showed increased activity of the legs over those in which only the lumbosacral cord is deafferented (P < 0.025).

In conflict with these results is the study carried out by Hamburger et al. (1966), who reported that extensive deafferentation of the chick embryo neural tube from the lumbosacral region all the way to the thoracic area does not produce a marked effect on the motility of the legs in embryos that were between $8\frac{1}{2}$ and 15 days of incubation. They reported, however, a sharp decline in leg motility in 17-day embryos, an event attributed to numerical depletion and partial degeneration of the lateral motor columns. In yet another set of experiments, Hamburger and Narayanan (1969) deafferented the trigeminal area of the head, and then proceeded to record total body motility in the usual manner. They found no significant differences between normal and deafferented embryos up to 15 days of incubation. It is difficult to reconcile the differences between their results and those reported in this thesis, except to mention that their studies were carried out during the early and middle life of the embryos, while those reported here were conducted during the terminal portion of the incubation period.

The pattern of movements observed in the deafferented animals is the simultaneous type. The loss of the alternate pattern, or rather lack of its development following deafferentation, can be attributed to two things: the sensory cells and their connections in the central nervous system are instrumental to or mandatory in the development and execution of the alternate pattern, much as they are important in the performance of crossed extensor reflexes; and that shaving the dorsal half of the spinal cord removes cells which normally tonically facilitate the inhibitory coupling between motor centers on the two sides of the cord. The

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latter, of course, would also serve to explain why there was increased activity in the deafferented group. Which of the two mechanisms is operating remains to be worked out.

The importance of sensory feedback in the control of rhythmic movements in many systems has been emphasized. Huber (1960) reported that sound production by crickets is a fairly complex behavior, involving feedback from the musculature. In the locust, removal of tibial and tarsal receptors results in changes in motor activity and uncoordinated leg movements (Usherwood, Runion, and Campbell, 1968). Overstepping in the stickbug occurs after the removal of coxal hair plates (Wendler, 1966). The cockroach displays such diverse locomotory patterns that investigators were led to conclude that such a repertoire could only exist if the nervous system possessed sufficient plasticity so as to meet various contingencies (Bethe, 1931). Despite the existence of demonstrably rigid central programs which form the basis of cockroach locomotion (Hughes, 1957), proprioceptive reflexes with both tonic and phasic components are superimposed upon these central programs (Wilson, 1966; Pearson, 1972).

In this study, the simultaneous pattern of movements, which persists after complete deafferentation, indicates that for the two legs to move in unison, they must receive simultaneous commands. There exists ample evidence to suggest that central factors alone can account for some patterns of movements. Neither the overt rhythmic leg movements in the milkweed bug nor the underlying activity burst in leg motor neurons is affected by deafferentation (Hoy and Wilson, 1969). It has also been established that the rhythmic discharge of the swimmeret movements of the crayfish does not require sensory feedback (Ikeda and Wiersma, 1964). The abdominal ganglia are quite sufficient in initiating and perpetuating the rhythmic

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output. Even when the abdominal nerve was completely isolated, rhythmical bursts of electrical activity in the motor roots leading to the swimmerets could still be recorded. Another example of very rigid central patterning is the sequence of motor impulses from the cerebral ganglion of the bivalve mollusc Mya, which normally brings about the retraction of the mantle and closure of the shell (Horridge, 1961). Single stimuli applied to the preganglionic (sensory) axon is capable of eliciting a sequence of motor impulses from the ganglion in which up to 10 motor axons can be individually identified; the patterning of the impulses being similar to successive repetitions. The sequence does not change when all other nerves to the cerebral ganglion are severed and when the motor nerve is cut beyond the recording electrode. The sequence does not depend on proprioceptive feedback from the movement it causes either. Web-building movements in the spider are not abolished by amputating one or two legs (Szlep, 1952). Von Holst (1939) described the "superposition" effect in the teleost, whereby the amplitude of one of two fins beating out of phase with each other is determined (may be increased or decreased) by its phase relationship with the beat of the other fin. This superposition effect persists in the deafferented spinal fish. The scratch reflex of the dog consists of a fairly complex movement, requiring the participation of 19 muscles (Sherrington, 1931); yet it can be elicited by a simple stimulation from the deafferented hindlimb. It has also been shown that deafferentation of both hindlimbs in the dog does not abolish rhythmic movements during normal walking (Tomita, 1967). Engberg and Lundberg (1962) recorded EMG from the extensor of the knee (Vastus lateralis) in the cat; they then correlated the EMG with limb movements photographed at high speed. They discovered that a burst of activity of the muscle appeared even

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before the foot had had a chance to touch the ground; that is to say, before the muscle was stretched by the weight of the body. In most mammals, swallowing is a complex behavior in which activity in the motor neurons scattered all the way from the mesencephalic level to the third cervical level is integrated to produce a coordinated pattern of contraction in about twenty muscles (Doty and Bosma, 1956). Once swallowing has been initiated, the temporal pattern, duration, and amplitude of contractions are independent of sensory feedback. Finally, the work of Szekely (1969) demonstrated that deafferentation of one or both forelimbs in the newt does not alter normal activity patterns in the agonistic and antagonistic muscle groups in unrestrained animals during normal walking. Generally, a system which continues to maintain its basic output pattern in the absence of sensory feedback is thought to be under the control of pacemaker mechanisms.

SUMMARY OF AND CONCLUSIONS FROM LESION EXPERIMENTS

The results obtained from the various manipulations of the central nervous system make it possible to outline ways in which various parts of the nervous system interact to influence both activity level and patterns of leg movements. From the outset, it ought to be pointed out that the only form of influence demonstrated by the methods used in this study is one of inhibition.

Frequency of Coordinated Movements.

Even though a decline in activity was observed in embryos following mid-thoracic transections (Fig. 6), the influence of the brain on the lumbosacral cord is considered to be one of inhibition rather than excitation. Evidence for this conclusion is derived from the demonstration that embryos with double lesions (MITS + LSMSS; MITS + LSDF, Figs. 11, 12) perform movements at rates higher than those of the corresponding single lesions. The apparent disparity between the two opposed findings lies in the belief that the inhibitory effect of the brain markedly manifests itself only when the integrity of the lumbosacral region is disrupted (LSMSS, LSDF). In other words, an integral lumbosacral cord is capable of absorbing or masking the inhibition from the higher centers.

The two left and right halves of the lumbosacral segment are engaged in reciprocal inhibition by virtue of increased activity following a midlongitudinal cut. The increase in activity may be due to an inherent property of the cells after being released from inhibition, or it may simply be the result of an increase in the responsiveness of neurons in each half of the cord to the external environment. In either case, it is argued that

the presence of cross fibers between the two half-segments is implied in the attenuation of activity.

Similarly, one of the curious and unexpected findings is the increase in activity which accompanies deafferentation, an observation which suggests that peripheral feedback acts to damp spontaneous and long-term motor output. It is not possible to predict from this study where in the sensory system this inhibition originates---- whether at the level of the sensory cells themselves or at the level of the receptor sites. The former would indicate inhibition through spontaneous activity of the sensory cells, while the latter would suggest responses to changes in the external environment.

From these findings it is concluded that not only are the inhibitory effects from the different parts of the nervous system additive, but also that if it were possible to get an accurate measurement of the relative magnitude of inhibition, it is suggested that the inhibition which originates from within a segment produces greater effects than that which is projected extra-segmentally.

Pattern of Movements.

Whereas the brain has been shown to have an influence on the activity of the legs, it has virtually no effect on the pattern of movements. An integral lumbosacral cord is all that is required for the performance of alternate movements. When the integrity of the lumbosacral cord is interfered with, either through a mid-saggital section or by deafferentation, the alternate pattern is altered to that of simultaneous kicking of the legs. The one conclusion drawn from these observations is that sensory cells including their central terminals and, naturally, cross fibers

between the left and right half-segments are very essential for alternation of legs to occur, much as they appear to be at the base of crossed extension reflexes.

The simultaneous pattern is mediated by the lumbosacral segment alone since the exclusion of brain influence (MTTS + LSMSS and MTTS + LSDF) does not in any way alter the pattern. Because deafferented embryos exhibit the same (simultaneous) pattern as embryos with longitudinal cuts, it is tempting to look for a common mechanism, or common feature within a mechanism, through which this form of coordination is achieved. Yet in reality, the same pattern may be subserved by two entirely different mechanisms. Since coordination occurs in the absence of sensory feedback, the information to propel the legs synchronously must consist of a central command. The command, moreover, makes use of the existing cross fibers to set the rhythm of the moving legs. However, in embryos with complete midlongitudinal cuts, the sensory system is still connected separately to each side. The information which determines the synchrony, it is suggested, is one of mechanical factors by which afferents on each side are stimulated simultaneously. The position of the embryo within the remaining portion of the shell is such that the toes and tarsal joints are braced against the inside of the shell, thus creating a mechanical friction. The resulting friction, then, comprises the simultaneous stimulus. If this should be the mechanism which operates in the mid-saggitally-sectioned embryos, then one can predict that prior to hatching, complete peeling of the shell and membranes, together with allowing the legs no access to mechanical surface, would result in asynchronous coordination. Similarly, one predicts that non-coordination will result from subjecting an embryo to mid-saggital section plus deafferentation. Consequently, the difference

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between simultaneous pattern of coordination in deafferented and mid-saggitally-sectioned embryos may be that central factors are prepotent in the former, while the latter is subserved by peripheral factors.

As was pointed out in the discussion section, another mechanism which might be operative in the deafferented embryos is the suggestion that peripheral feedback is not essential for the alternate pattern of coordination. The simultaneous pattern observed after deafferentation is simply the consequence of the central lesion, whereby shaving of the dorsal portion of the lumbosacral cord removes, along with the target primordial sensory cells, a pool of other cells, the normal function of which is to facilitate inhibitory coupling between motor centers on the two sides of the cord. One way to resolve the question concerning these two possible mechanisms is to develop a method of deafferentation which removes only the dorsal roots, while leaving the cord essentially intact.

It is not possible at this juncture to reconstruct events in development which lead to the commencement of coordination. It cannot be ascertained whether the final features are either central or peripheral or both, but this study reiterates the notion that the beginning of coordinated alternate movements does not originate simply in the establishment of functional connections between the two half-segments, but that other factors distributed in the entire lumbosacral cord are involved also. Given this supposition, the simultaneous pattern of coordination can be viewed as having undergone a development of itself, rather than merely a consequence of neural disruption. To put it in another way, it is quite conceivable that different kinds of results, in terms of rates of activity or even pattern, may be obtained if the same lesions (i.e. LSMSS or LSDF) were performed in chick embryos just prior to hatching. Differences

in results may arise from the fact that in the developed chick, most features in the central nervous system are already "set" in and neural plasticity is all the more reduced.

Despite the possible existence of a multitude of factors, it is very tempting to suggest that the final feature which chronicles the alternation of legs in normal embryos is the establishment of functional connections between sensory terminals and central neurons. This idea gains support from the established fact that in most developing neural systems in vertebrates, sensory mechanisms become functional long after motor systems have been in operation.

GENERAL SUMMARY

Since it has been established that general as well as specific integrated movements in the chick embryo do not appear until shortly after day 17 of incubation, it was proposed that the development of coordination of leg movements could still be shown during a two-day interval, between day 19 and hatching. Complementary to this inquiry, were the various manipulations of the central nervous system, which sought to provide an understanding of the neural mechanisms subserving coordination. The results from these two areas of investigation were deemed necessary before strong statements or predictions could be made about the type of features (structural and/or functional) which alter to mediate development of coordination.

During the two-day period, the degree of limb coordination (measured in terms of a ratio between coordinated movements and total activity) did not appear to change. However, a trend toward an increase in the rate of activity was observed in two experimental groups (figures 4 and 5). The increase was attributed to a lesion common to both groups. No other <u>pattern of development</u> relevant to limb coordination could be shown from day 19 onwards.

Animals with mid-thoracic gaps, which serve to exclude the influence of the brain from reaching the lumbosacral cord, are able to perform alternate coordinated movements which are quite indistinguishable from those of the controls. However, the frequency of the movements of these spinal animals is significantly lower than that of the control group.

Embryos in which the lumbosacral segment has been sectioned midsaggitally showed an increased rate of activity over that of the controls. It was concluded that the two halves of the cord segment reciprocate in inhibiting endogenous activity. The pattern of movements displayed by this group is that of the simultaneous kicking of the legs.

Deafferentation of the lumbosacral area results in increased activity over that of the controls, suggesting that the normal action of sensory cells is to inhibit endogenous motor output. An alternative interpretation is to propose that the form of deafferentation used in this study has the effect of removing cells (interneurons) which normally facilitate the inhibitory coupling between motor centers on the two sides of the cord. The pattern of movements in the deafferented embryos is of the simultaneous type. It is preliminarily concluded that sensory cells are necessary in mediating the alternate pattern of coordinated movements.

Embryos with both transverse sections in the mid-thoracic level and the mid-saggital sections of the lumbosacral cord perform coordinated movements at a much higher rate than the controls. Similarly, embryos with both mid-thoracic sections and deafferented lumbosacrals perform coordinated movements at a rate much higher than that of the controls. Since the rate of leg activity in the double lesioned animals is also greater than that of the corresponding single lesion (i.e. MTTS + LSMSS LSMSS; MTTS + LSDF LSDF), it was concluded that the brain acts to inhibit the lumbosacral cord; but that this inhibition is best detected after the disruption of the lumbosacral cord. Furthermore, it was also concluded that segmental (lumbosacral) and extrasegmental (brain) influences were additive. Like animals with longitudinal sections, or deafferented lumbosacrals, the double lesioned animals exhibit the simultaneous pattern of leg

movements. Mechanisms which subserve the simultaneous pattern are suggest-

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APPENDIX

APPENDIX

This appendix consists of a compilation of raw data from which the various values presented in the different experiments were derived. These raw data have been arranged to follow, more or less, the same order in which the transformed data were presented in the body of the thesis. That is to say, there are three sets of data: the one set deals with the temporal pattern of activity in terms of absolute rates; another set concerns the temporal pattern of activity with respect to the magnitude of limb coordination, measured as a ratio (percentage) between coordinated limb movements and total leg activity; and the last set consists of activity peak values, out of which the various groups of embryos were compared. Except for the last set of data (Tables 13-18), the successive values in the tables represent the temporal performance of each embryo at that particular stage of development. Tables 3-7 contain the raw data for the frequency of leg activity as a function of developmental time. The actual numbers represent the number of coordinated movements per minute. As with all tables, the legend for each group is provided at the bottom of the table.

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	20	۲.03
	16	1.56 3.20 2.70 6.93
	12	2.92 2.92 5.59 1.05 3.40 3.40
	8	0.54 3.89 2.37 2.37 2.37 2.37 2.37 2.37 2.37 2.37
	4	7.80 80 80 7.90 80 7.90 80 80 7.80 7.80 7.80 7.80 7.80 7.80 7.
20		2.30 0.64 1.47 2.39 2.18 2.18
	20	22.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20.00 20
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	12	6.07 3.24 1.10 1.29 1.22 1.22
	8	6.1.2 2.6 2.6 2.8 2.8 2.8 2.8 2.8 2.8
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Table 3.

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Table 3.

Control.

Table 4.

21		2.30 2.98 3.14 3.14
	20	2.30 2.37 2.37 3.0 2.50 2.50
	16	0.90 3.19 5.07 2.26 2.13 7.22 5.22
	12	0.97 2.93 1.30 1.14 1.12 1.12 1.12 1.12 1.12 1.12 1.12
	8	1.15 2.75 2.12 2.12 2.12 2.13 2.13 2.13 2.18 2.18 2.18 2.18 5.16
	4	1.52 1.52 1.57 1.57 1.57 1.57 1.57 1.57 1.57 1.91
20		1.17 2.10 5.16 3.62 5.32 5.32
	20	1.70 2.93 2.96 2.96 2.18
	16	3.57 2.11 1.77
	12	3.55 2.14 2.93 1.63
_	8	4.08 2.49 3.45
19	4	2.57 1.08 2.12
CHICK #	·	£285,888885,2525,54545,588

Mid-thoracic transverse section.

Table 5.

DAYS AND HOURS OF INCUBATION

2.62 5 5.62 4.95 3.38 6.04 20 6.78 11.80 4.78 4.42 2.60 5.52 16 13.82 3.51 3.67 7.95 12 7.05 5.04 5.66 4.30 4.77 ω 4.77 2.76 3.09 5.11 3.23 7 20 7.42 4.52 6.73 8.82 4.22 4.55 11.70 2.82 4.05 3.65 7.61 20 16 0.72 6.02 6.59 6.59 9.27 7.89 2.91 3.92 3.33 12 6.87 0.65 4.40 ω 2.75 2.75 2.49 19 CHICK #

Lumbosacral mid-saggital section.

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Table 6.

Lumbosacral deafferentation.

21	6.93 2.65
C	6.42 5.38 1.33 4.15
16	8.25 4.81 6.82 3.83
- - -	6.03 1.93 1.93 1.93 1.93 1.93 3.20 3.20
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.88 3.86 3.86 3.70 3.70 3.70 3.38 3.70 3.38 3.70 3.38 3.70 3.38 3.38
-	3.36 3.36 3.38 3.38 3.38 3.38 3.38 3.38
50	3.43 3.43 7.75 5.27 5.27 3.39 3.39 2.11 1.32 11.32 11.32 11.32 11.40
0	6.93 6.57 6.57 7.009 4.10 7.400 4.43 6.443 4.000
16	2.73 2.73 2.71 2.78 2.78 2.78 2.78
101	3.76 3.18 6.45 4.47 4.47
8	6.30 5.78 6.76 4.41
19	5.75 2.75 2.75
CHICK #	273 273 273 273 273 273 273 273 273 273

Table 7.

5	J	
	20	3.0 3.0 3.0 3.0
	16	5.28 3.28 3.28 5.65 6.65
	12	6.35 3.86 6.23 6.23 4.10
	8	5.73 5.73 5.73 5.73 5.73 5.73 5.73 5.73
	4	6.27 2.143 7.284 5.30 2.52 2.52 2.52 2.52
00		5.76 4.21 15.16 15.16 11.03 11.03
	20	7.66 6.18 5.24 6.83 7.10 7.10
	16	7.61 6.19 4.555 4.555
	12	7.64 1.19 3.03 3.03 3.03
	8	6.24 2.0 3.6 3.6
19	14	3.33
CHICK #		621 621 622 623 622 622 622 622 622 622 622 622

Mid-thoracic transverse section + Lumbosacral mid-saggital section.

2		
	20	3.14 8.16 3.0
•	16	5.28 3.28 3.28 5.65 6.65
•	12	6.35 6.35 6.23 6.23 4.10
	8	5.55 5.73 5.73 5.73 5.73 5.73 5.73 5.73
	4	6.27 9.27 5.302 1.91 1.91
20	2	5.76 4.21 15.16 15.16 1.00 1.03
	20	7.66 6.18 5.24 6.83 7.10 7.10
	16	7.61 6.19 4.55 4.55
	12	7.64 4.49 3.03 0.88
	8	6.24 3.6 3.6
19	4	3.33
CHICK #	<u> </u>	625 625 625 625 625 625 625 625 625 625

Table 7.

Mid-thoracic transverse section + Lumbosacral mid-saggital section.

Tables 8-12 contain the raw data showing the degree of limb coordination. The percentages are ratios between coordinated limb movements and total leg activity.

	21		96 <b>.</b> 2
		20	98.6
		16	97.0 99.5 87.3 95.9
		12	99.3 97.6 92.9 92.0
N		8	90.7 97.9 97.9 98.1 85.9 100.0 100.0 100.0 88.3
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α	82.7 92.2 92.6 92.6 92.6 92.6 92.6 92.6 92.6	
-	97.4 97.4 97.4 988.8 99.4 99.4 93.1 95.2	
20	76.3 97.8 95.4 93.4 93.9 93.9	
 C	86.7 93.79 93.79 93.79	
1 2 6	96.7 97.2 90.7	
	96.0 91.2 89.6 82.7	
α	97.2 988.4 888.4	
19	96.2 99.0 92.3	
CHICK #	5002 2005 2005 2005 2005 2005 2005 2005	

Mid-thoracic transverse section.

67

98.7

100.0 92.9 84.7 97.9 90.0

	2	L2	100.0
		00	98.0 99.0 85.0
		1 2	100.0 99.0 100.0 100.0 99.0
		10	99.0 97.0 97.0 97.0
Z		œ	96.0 97.0 97.8 97.8 90.5 100.0
NCUBATIO			4 100.0 97.6 98.0 99.0
URS OF I		50	100.0 99.0 97.9 99.0
S AND HO		00	96.5 96.4 96.4 98.6 99.6
DAY		16	98.0 96.1 96.0 96.0 96.0
		10	99.5 90.0 99.0 98.0
		ď	98.6 99.0 98.0
10.		ور ا ر	99.0 98.9 99.0
Table	CHICK #		467 2222 273 277 277 277 277 277 277 277 2

Lumbosacral mid-saggital section.

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DAYS AND HOURS OF INCUBATION

2													98.7									
	20												98.9			98.7				93.0		
	16												97.9			99.0		99.2		97.0	89.0	
	12					1	66°	82.0			99.0		97.6	95.5		98.8		97.8	2.66	94.6	90.1	
	8					89.0	99 <b>.</b> 0	94.0			100.0		99.6	91.0	100.0	98.5	0.66	97.7	99.6	95.0	85.0	
	7	99 <b>.</b> L				82.0	99.0	94.0			99.4		98.9	94.5	100.0	0.99	98.0	100.0	97.5	97.0		
20		98.8		98.0		93.0	100.0	95.0	92.0	89.0	98.6		99.5	95.0								
	20	99.4		0.66	1	30.5	100.0	97.0	91.0	93.0	98.7	100.0	98.0									
	16	98.0 99.6	97.5	99.5	0.66	90.06																
	12	99•0 99•0	97.0	100.0	90.2								<u>d</u> = 2									
	ω	98.0 100.0	98 <b>.</b> 5	20 20	9.76																	
19	7	99.0 100.0																				
CHICK #		279 305	267	ξ	007	2.2	269	309	500	276	301	306	308	307	11	281	278	313	303	277	310	ل

Lumbosacral deafferentation.

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<del>...</del>

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DAYS AND HOURS OF INCUBATION

Table 11.

2	- J	
	20	90.0 98.0 97.0
	16	100.0 96.5 99.0 98.0
	12	100.0 99.0 94.0 100.0
	8	99.0 96.0 99.0 98.0 98.0
	4	98.0 91.5 98.0 97.5 99.0 98.0 98.0
00	2	92.6 92.0 98.0 98.5 98.5 98.5
	20	98.0 95.0 99.0 97.0 91.0
	16	95.0 99.7 95.0 97.0
	12	97.0 97.0 98.0 100.0
	8	98.0 96.0 97.0
19	1, 1,	0.92
CHICK #		622 627 627 627 627 627 627 627 627 627

Mid-thoracic transverse section + Lumbosacral mid-saggital section.

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DAYS AND HOURS OF INCUBATION

Table 12.

Tables 13-18 contain frequency data for all the embryos used in the experiments. The performance of each embryo is represented by an activity peak curve, consisting of two temporal components: time before and time after the peak. Note that activity peak values were the basis for all the comparisons for the different groups.

PEAK	2	- 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2	0000 00	2.2.4 2.2.4 0 2.2.4 0	
RS FROM	Р		- ⁻ ⁻ ⁻ ⁻ ⁻ ⁻ ⁻ ⁻	2000 2000 2000	
E IN HOU	2	۰۰۰۰ ۵ ۵ گه ۵ گر ۵	00000 00000	າຜູູູດູ ທູ່ຕູ່ທູ່ທ	
TIM	4	0-1-0 0.5 1-0 0.5 7 0 0.5 7 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0.09 0.09 0.09 0.09 0.09 0.09 0.09 0.09	2.6 1.3 6.1	
	9	1.2	1.3 1.1	1.9 1.1 3.4	
	8	0.8	0•0	1.2	
13.	10				
Table	CHICK #	110 162 163 165 130 130	111 111 111 111 111 111 111 111 111 11	113 1402	

0.8 0.9

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2.3

TIME IN HOURS FROM PEAK

			_						
	10			0.8		1.2			1.9
	8		0 0 0 0	0.6	1.0	1.9			ی ۵
	6	1.3	د د يرد	0.9	2.4 2.4	2.7	Э <b>.</b> 2	2.9	1.0
	4	1.0	 9	 	0 0 0 0	1.0 1.0	Э.7 2.0	0 0 0 V	2. 2
PEAK	2	1.3	1.8	2.1 1.8	0 0 M N	0 5 M M	۳. م.۲	2°0	7.2
RS FROM I	Р	1.4 1.8	ທ ທ ຕຳ	2.9	00	۳. ۳. ۳.	4 1	<del>ლ</del> რ აა	8.2
INOH NI E	2	1.0	 0, 1,	۲. ۳. ۳.	0 0 - 7	1.7 2.4	3.6 9.6	- m	l4.1
INCLL	4	0.7 1.4	 	- 9 -	5 <b>0</b>	1.1	ດ ດ ທີ່ທີ່	0.t 2.3	2.9
	6	1.3 1.4	د د بُ ہ	1.3	1.8	6.0	2.1	- 2° - 8°	0.9
	8	0.3 0.3	0.8	0.5	1.2		0.6 1.1	2•0	
14.	10								
Table	CHICK #	70	37 82 82	71 98	1415 83	203 200	187 101	179 3	66

Mid-thoracic transverse section.

10	
8	0.6 1.6 1.6
9	1.0 1.0 1.1 1.1 1.0 1.0 1.0 1.0 1.0 1.0
14	400,400 400 4000 404,000 400 400
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7	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
9	2.0 3.4 3.4 2.0 2.0 2.0
8	10 .0 0.8 0.9 7.9 9.0
10	
CHICK #	451 95 157 158 280 280 280 280 280 280 280 280 280 28

Lumbosacral mid-saggital section.

TIME IN HOURS FROM PEAK

Table 15.

Table 16.

TIME IN HOURS FROM PEAK

											_				
	Ч	ע מ	i v v	4.0	4.1	<del>ل</del> ر	2.4 2	\$ \$	о Л	6.8	5.2	7.8	9.6	6.6	17.1
	2	06	2.6 2	2.9	<b>З.</b> 8	2.9	4.8	ۍ 8	ື້	6.2	ۍ ۲۰	2.0	2•0	ی ک	2.0
	4	0 6	1.7	1.9	3 <b>.</b> 2	3.9	4.0	4.4	1.9	4.4	5.4	ر م		8 <b>.</b> 2	7.4
	6	8			2.4	2.1	4.3	4.4	2.3	4.9	6.1	5.L		<b>6.</b> 3	о У
	8				1.4	1.1	4.7		_			4.9		4.0	7.7
	10					0.7	2.7								2.4
CHICK #		<b>27</b> C	201	200	310	309	281	266	311	31 J	279	215	267	205	269

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Lumbosacral deafferentation.

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TIME IN HOURS FROM PEAK

10	2.4 3.1 2.8
8	4.5 2.2 3.5
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4	
2	8.000000000000000000000000000000000000
Ч	<i>7.00000000000000000000000000000000000</i>
2	
4	です。 で、 し、 し、 し、 し、 し、 し、 し、 し、 し、 し
6	4.27 4.27 4.27 4.27 4.27 4.27 4.27 4.27
8	2.2 2.0 3.6 7.5
10	2.6 2.6 7.7
CHICK #	624 624 625 623 623 623 623 623 623 623 623 623 623

Mid-thoracic transverse section + Lumbosacral mid-saggital section.

18.	
Table	

TIME IN HOURS FROM PEAK

CHICK #

10	
8	3.0 3.40 3.20
6	6.0 3.36 5.40 7.93 1.00 1.00
4	4.10 1.72 5.39 3.80 1.70 6.20 6.20
5	7.30 8.43 8.43 8.43 8.43 7.43 8.40 8.40 8.40
<b>P</b> .	8.70 6.93 6.08 6.12 6.12 71.30 7.58 11.30
~	6.21 6.22 7.20 7.20 7.20 7.20 7.20 7.20 7.20 7
ן ד	3.70 3.18 8.27 6.90 3.18 3.18
9	4.20 1.68 6.27 3.60 4.0
8	3.90 0.70 4.0
10	
	709 707 705 705 705 700 703

Mid-thoracic transverse section + Lumbosacral deafferentation.

