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Anatomical and Physiological Development of the *Xenopus* Embryonic Motor System in the Absence of Neural Activity

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Embryos of *Xenopus laevis* were continually immobilized by immersion in solutions of either chloretone or lidocaine, from the late neural-fold stage to the approximate time of hatching. Such treatment has been shown to result in only transient quantitative effects on swimming behavior. Chronic immobilization was without either immediate or long-term effect on the ventral root output exhibited during "fictive" swimming episodes. Development under these conditions of diminished or absent neural activity similarly had no effects on a number of measures of the size and complexity of motoneuron dendritic arborizations. These results support the premise that the early development of specific neuronal morphology and connectivity may be largely independent of functional activity.

In 1904, Ross Harrison first performed a classic deprivation experiment in which amphibian embryos were immersed in solutions of an anesthetic drug before the onset of motility. After prolonged periods of immobility, animals were reported to behave normally after removal from the drug solutions. This work and its replications by Carmichael (1926, 1927, 1928) and Matthews and Detwiler (1926) remain the most widely cited *in vivo* examples of the indifference of the earliest stages of neurobehavioral development to functional activity.

In an extended replication of Harrison's (1904) paradigm, in which quantitative measures were applied to the swimming behavior of *Xenopus* embryos (Haverkamp and Oppenheim, 1986), the basic conclusion of these classic studies was supported: Such treatment results in no permanent effect on behavioral development. This later study did reveal, however, a transient period immediately following drug removal, during which the previously immobilized animals swam significantly more slowly and for shorter distances, while appearing normal in the pattern and form of their swimming (cf. Fromme, 1941). This period may have reflected either the time needed for metabolism and excretion of residual drug, the subsiding of immobilization effects on non-neural development, or, perhaps most interestingly, may have been the result of subsequently accommodated abnormal neural development.

Although the nervous system was never directly examined in any of the above studies, important inferences regarding neural development have been drawn from these behavioral data. The early works of Harrison and the others have, in fact, been largely instrumental in establishing the premise that, during the earliest stages of ontogeny, the nervous system develops in "forward reference" to, and without benefit from, functional activity. A direct examination of neural development in immobilized amphibian embryos should give an indication of the validity of such an inference, as well as helping to localize effects of immobilization that result in transient, quantitative behavioral deficiencies. The functional output of spinal motoneurons, as well as the dendritic arborizations of these cells, was therefore analyzed in control and pharmacologically immobilized embryos.

The rhythmic activity bursts within the segmentally arranged ventral roots are the immediate neural bases of *Xenopus* swimming, with the two sides tightly coupled to result in alternating contraction of the myotomes on either side of the embryo. The source of such cyclic output in this relatively simple system is a central pattern generator, largely characterized in a thorough set of studies by Kahn and Roberts (1982a; Kahn et al., 1982; Roberts and Kahn, 1982; Roberts et al., 1981). The motoneurons that are the source of this activity have extensive dendritic arborizations, entirely restricted to the presumptive white matter of the cord. At stage 35, these dendritic trees are predominantly oriented in a plane orthogonal to the body's long axis, and the dendrites of a single neuron may occupy up to one-half of the cross-sectional white matter of the available cord.

Analysis of ventral root activity patterns during "fictive swimming" episodes demonstrated that prior neural blockade did not alter any measure of this functional output. Quantifications of the dendritic arborizations of the motoneurons also indicated no statistically significant effects of prolonged interference with impulse activity. These data support the hypothesis that certain aspects of early neural differentiation and connectivity are largely independent of functional activity.

Materials and Methods

The conditions for obtaining and rearing *Xenopus* embryos, as well as techniques for inducing neural blockade, are described in detail elsewhere (Haverkamp, 1983; Haverkamp and Oppenheim, 1986). To summarize, embryos at the late neural-fold stage (stage 17—Nieuwkoop and Faber, 1967), obtained from single breedings, were randomly assigned to control or experimental groups. Experimental embryos were reared through stage 35 (approximate time of hatching, 50–55 hr post-fertilization) in solutions containing either 1.7 mM chloretone or 0.64 mM lidocaine.

Physiology

Recordings of embryonic ventral root activity were obtained using minor modifications of the techniques described by Kahn (1980) and Kahn and Roberts (1982a). A single animal was placed in a solution of 10^{-4} M curare (*d*-tubocurarine chloride; Sigma) and pinned through the an-

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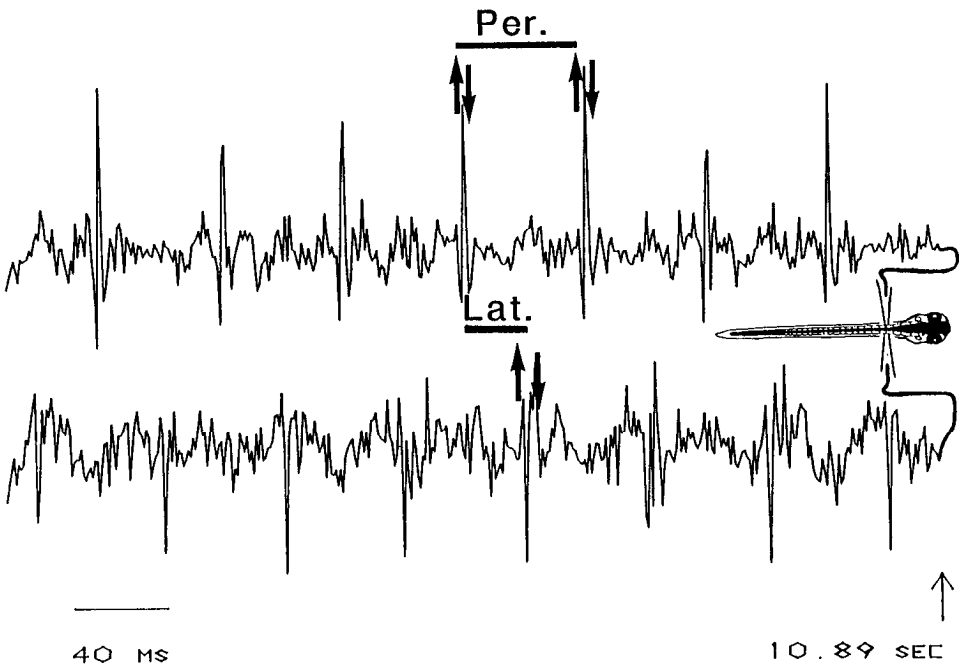

Figure 1. Typical ventral root recording from a stage 35 *Xenopus* embryo. Upward and downward pointing *arrows*, Times of onset and offset of activity burst. *Per.*, Duration of trace defined as the cycle period length. *Lat.*, Duration of trace defined as the latency of firing between the two sides. Measures of phase relation between the two sides were derived by dividing each latency measure by the left-side period measure during which the burst on the right side occurred.

terior and posterior notochord to a small Sylgard block, dorsal side up. A small section of skin was removed one-third caudad along the trunk (approximately at the level of the 6th postotic myotome), and suction electrodes were placed on either side of the body between the myotomes. Electrical activity was monitored until all indications of EMG in the recording had disappeared. The electrodes were then repositioned to obtain optimal amplitude of motor nerve activity. This ventral root activity occurred spontaneously, or it was induced through light stroking with a pressure-calibrated hair or by passing a shadow over the embryo (Roberts, 1978). The amplified signals from each side were simultaneously displayed on an oscilloscope and recorded on either channel of a stereo audio cassette tape.

Recordings from normal embryos were made at stages 35, 40, and 45. Recordings from chloretone-reared animals were obtained at stage 35 (2 hr after removal from the drug solution) and at stages 40 and 45. Recordings from lidocaine-reared embryos were first made at stage 40, owing to a high mortality of these stage 35 embryos under recording conditions, and again at stage 45. Recordings were also obtained from normal stage 35 animals immediately before, during, and after the addition of chloretone or lidocaine to the bathing solution.

These records of ventral root activity were digitized and analyzed using a computer-assisted graphics display system. The traces recorded on audio cassettes were digitized by a 16-channel A/D converter at a rate of 1.034 points/msec. The points from each channel (representing the left and right sides of the embryo) were sequentially stored in the upper and lower halves of the 96K word Hicore memory of a PDP-11 computer. At the sampling rate used, 42.55 sec of continuous activity (containing 800–1400 individual ventral root bursts) were therefore available from each recording episode. Through the use of an NDP2 computer graphics display system (Capowski, 1978), the points representing the activity trace were displayed on a CRT as they were retrieved from computer memory. By employing a matrix translation option of the NDP2 system, a moving trace, which was an exact representation of the recorded activity, was displayed. The traces were variously amplified along the x or y axes, moved at differing speeds, filtered for coherent signal (noise) of any frequency (Kelly and Calvert, 1970), reversed in direction, and advanced or reversed to any point in time. To quantify the recordings, the digitized trace from one side of an animal was displayed traversing the CRT. An observer conveyed to the computer the moments of onset and offset of activity bursts for the entire 42 sec recording period for the left side and then for the right side. In this manner, 34 bilateral tracings, representing 20 animals and approximately 34,000 individual activity bursts, were analyzed. Methods for deriving burst length, period between bursts, latency of firing between the two sides, and phase relation of activity on the two sides of the embryo are described in Figure 1. The means and variances of these measures, from each of the tracings from a single animal, were analyzed both as functions of time from onset of the bout and of period length. Group measures were statistically compared by Student's t test and the Mann-Whitney U tests.

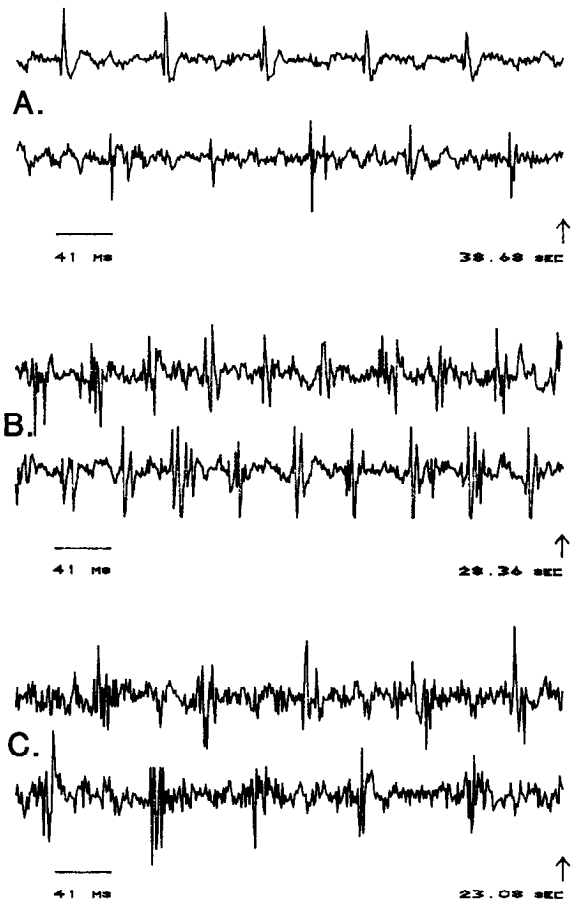

Figure 2. Representative traces of ventral root activities recorded from normally reared *Xenopus* embryos. *A*, Stage 35; *B*, stage 40; *C*, stage 45.

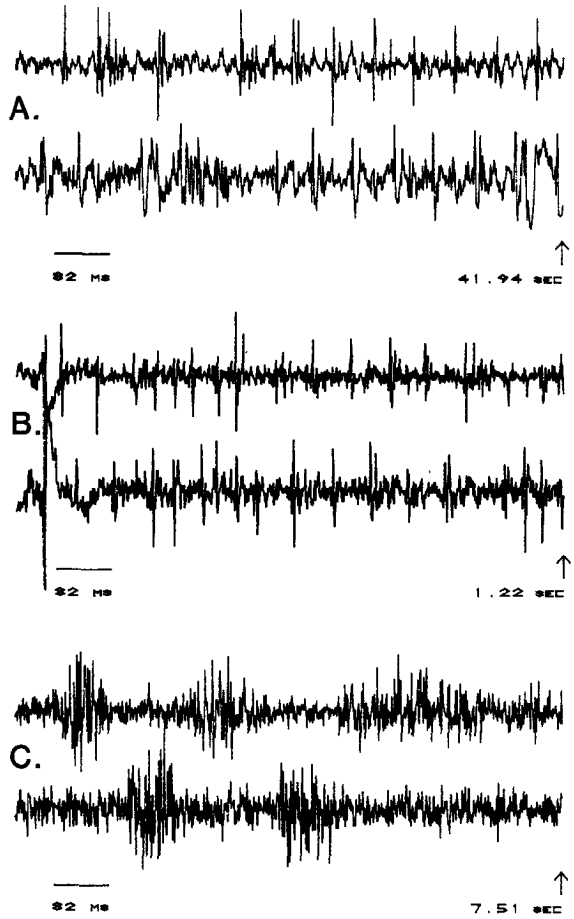

Figure 3. Traces of ventral root activities recorded from normally reared *Xenopus* embryos, demonstrating atypical firing patterns. Traces *A, B,* and *C* were taken, respectively, from stage 35, 40, and 45 embryos. *A,* "Coil," in which one side of the animal showed a complete lack of bursting for two to five cycle periods (*top trace* in this example), during which time the opposite side showed continuous activity of increased amplitude. *B,* "Synchrony," during which the bursts on the two sides occur coincidentally, rather than alternately. *C,* "Struggling," in which the duration and amplitude of individual bursts are increased and the interburst interval is reduced.

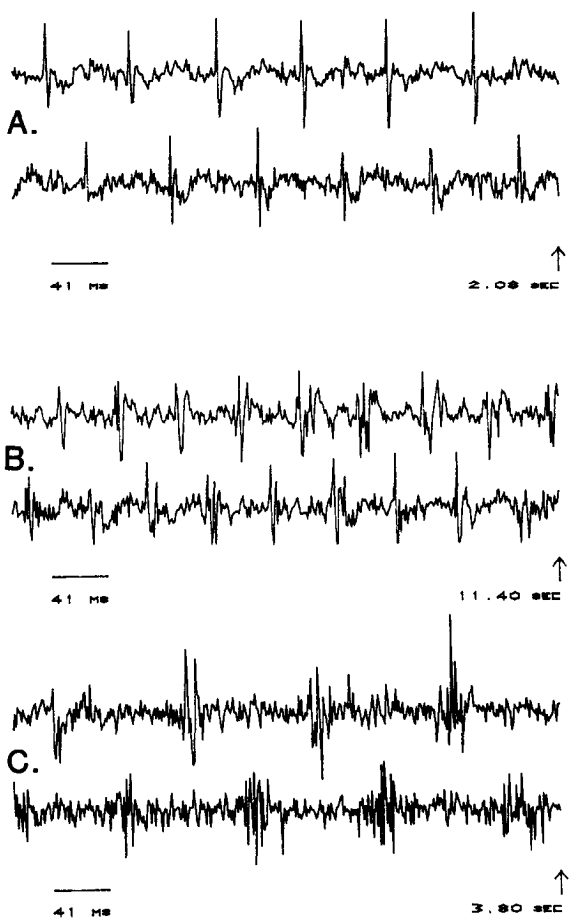

Figure 4. Representative traces of ventral root activities recorded from chloretone-reared *Xenopus* embryos. *A,* Stage 35 (2 hr after removal from the drug); *B,* stage 40; *C,* stage 45.

Anatomy

Visualization of motoneuron dendrites was accomplished through retrograde labeling with HRP. Under specific conditions, this dendritic labeling is comparable to that obtained with Golgi and intracellular HRP injection methods (e.g., Christman and Povlishock, 1980; Keefer et al., 1976; Roberts and Clarke, 1982; A. Roberts, personal communication; Fig. 11) and is amenable to morphometric study.

Stage 35 embryos were anesthetized in a 0.05% solution of Finquel (MS-222, tricaine methanesulfonate; Ayerst) in rearing solution. Chloretone- and lidocaine-reared animals were transferred directly from the drug solution to the Finquel. A small amount (approximately 0.1 μg) of HRP (Sigma type VI or Boehringer grade 1) was recrystallized near the tip of a minuten insect pin (see Farel and Bemelmans, 1980). The pin was inserted into the embryo at approximately the level of the 6th myotome, in the vicinity of the notochord, such that the HRP was contained within the myotomal musculature. The pins remained in place 30 min, after which time the animals were removed to 100% normal rearing solution or that containing the appropriate immobilizing drug. Six to eight hours after removal of the HRP pin, the embryos were again placed in Finquel solution. The spinal cord was removed and fixed in 5% glutaraldehyde for 90 min at room temperature and stored in 0.1 M phosphate buffer, pH 7.4, at 4°C. Embryonic cords were reacted for HRP *en bloc* (see Lamb, 1976) with 3,3'-diaminobenzidine (DAB; Sigma). The reacted cords were embedded in glycol methacrylate (JB-4 kit; Polysciences) and sectioned at 10 μm by means of a rotary microtome with a heated blade.

The dendritic arborizations of motoneurons were quantified using the computer reconstruction and quantification system described by Capowski and Sedivec (1981). In this system, an operator interacts with a PDP-11 computer to generate a three-dimensional, digitized representation of each dendritic tree of single neurons. These mathematical representations were quantified in terms of branch number, total fiber length, and fiber length as function of branch order; Sholl analyses (Eayrs, 1955; Sholl, 1953) in three dimensions; area Sholl analyses (for which cross-sectional dendritic areas are summed, rather than dendritic intercepts tallied, at radial increments of 0.5 μm); fiber Sholl analyses (for which intercept distances for tallying are defined as the real measured distance following each fiber's course, rather than at set radii in space); and area fiber Sholl analyses. The number of neurons analyzed from each animal (averaging 2.5) and the number of animals in each group (averaging 4.7) were limited by the time required for complete reconstruction and analysis. For statistical comparisons, the derived values for all neurons from a single animal were averaged and these average values applied both to Mann-Whitney *U* and Student's *t* test comparisons of groups.

Results

Functional development

Examples of typical bilateral ventral root recordings of patterned activity in normally reared stage 35, 40, and 45 embryos are presented in Figure 2. The activity consists of bursts of single or multiple fibers, alternating in a regular manner between the two sides. Although the use of suction electrodes makes mean-

ingless any indication of the absolute amplitude of the bursts, they have been plotted to represent relative amplitudes obtained with optimal electrode placement at each stage.

Observation of the digitized tracings revealed three types of variations on this characteristic pattern. The most commonly observed atypical pattern (Fig. 3*A*) occurred most frequently in stage 35 animals and may be the motor nerve activity correlate of "coiling" behavior (see Coghill, 1929). The pattern of activity termed "synchrony" by Kahn and Roberts (1982a) was seen most often in stage 40 animals and almost always occurred within the first 1–2 sec after stimulation (Fig. 3*B*). The "struggling" pattern of activity (Fig. 3*C*), also observed by Kahn and Roberts (1982b), was so common in the curarized and restrained stage 45 embryos that recordings of normal fictive swimming episodes were exceptional.

Animals reared in chloretone and lidocaine solutions showed no abnormalities in any qualitative aspects of the ventral root recordings (Figs. 4 and 5). The shape, duration, and general appearance of the tracings did not differ in any noticeable manner from those of normal animals. Furthermore, all three types of atypical patterns were seen in the drug groups. Tallies of their appearance within the recordings showed that their frequencies of occurrence did not differ markedly from those of normal animals.

Plots of cycle period versus time (Fig. 6) illustrate the constancy of this measure over short intervals. Individual strategies of period variation during fictive swimming episodes ranged from exhibiting uniform period lengths over the entire 45 sec trace, to showing an entirely unpredictable variability. Each embryo, however, tended to exhibit the same type of period

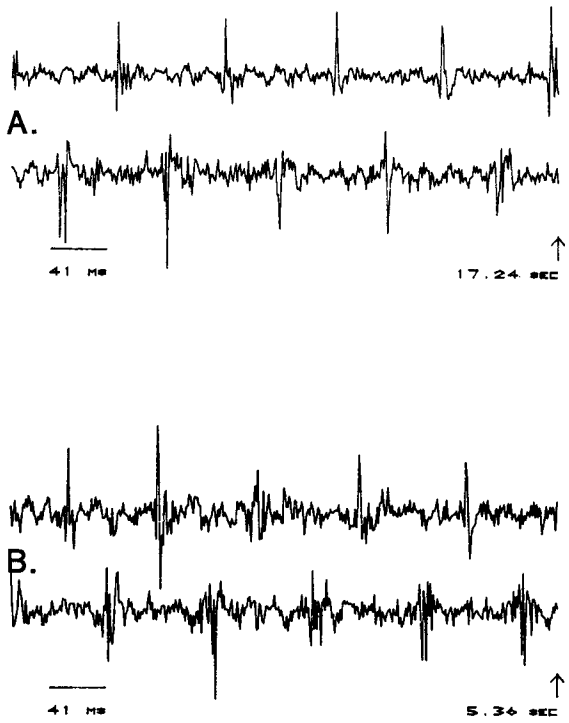

Figure 5. Representative traces of ventral root activities recorded from lidocaine-reared *Xenopus* embryos. *A*, Stage 40; *B*, stage 45.f

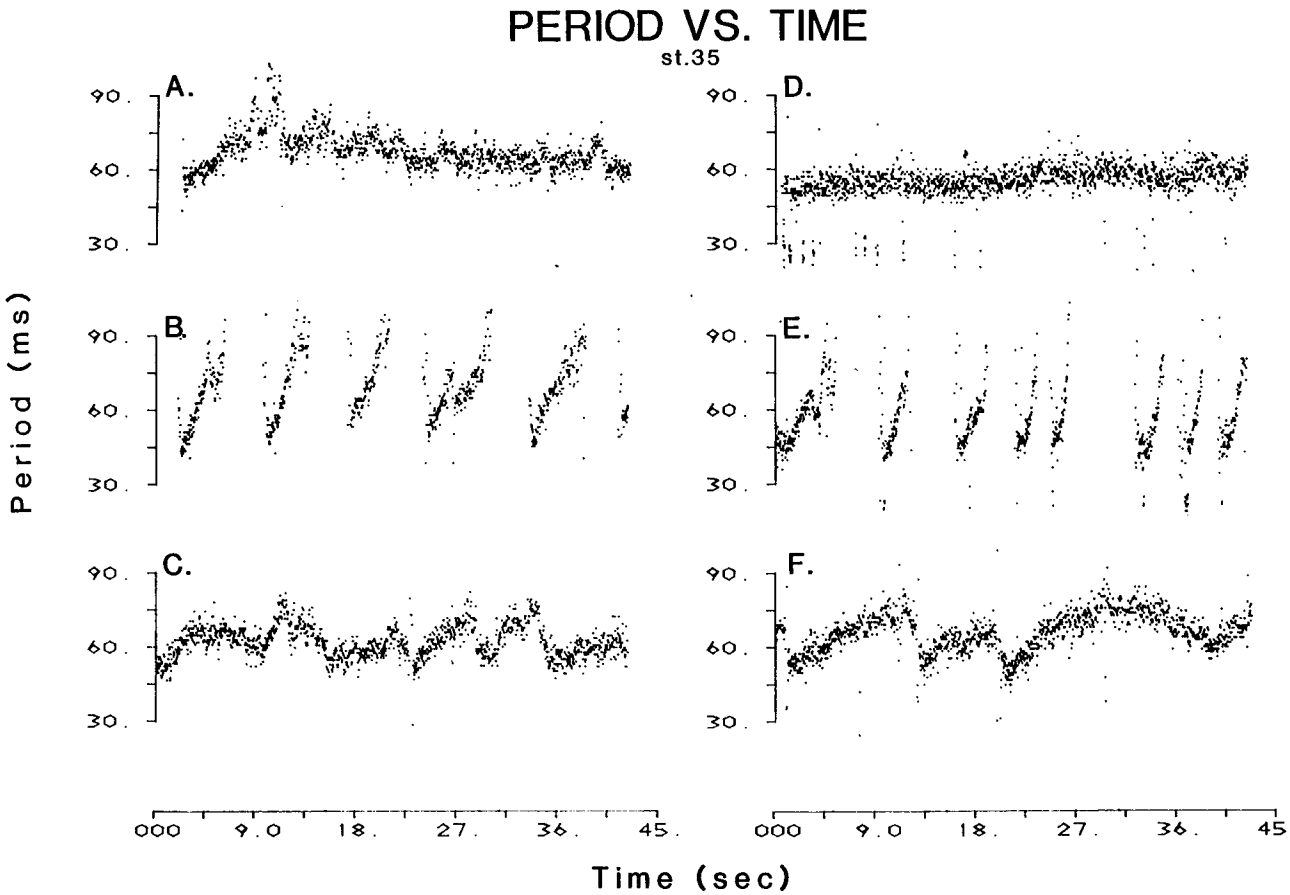

Figure 6. Representative graphs of ventral root cycle periods versus time for normally reared (*A–C*) and chloretone-reared (*D–F*) stage 35 embryos.

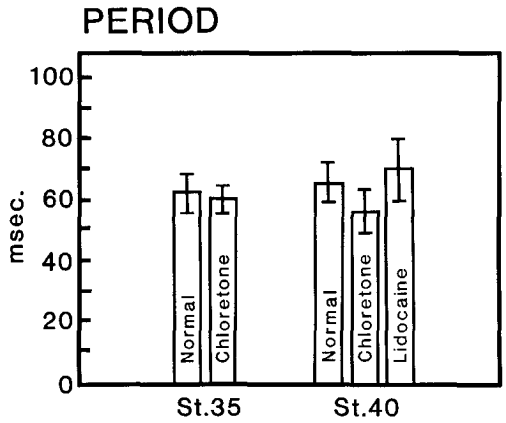

Figure 7. Histogram of mean ventral root cycle periods for stage 35 and 40 normally reared and immobilized embryos. *Error bars* represent SD.

variations in all fictive swimming episodes, even when recordings were separated by as much as 2 hr.

The type of variability in period length exhibited was not related in any way to the presence or absence of neural impulse activity during motor system development. For example, in Figure 6, plots *A–C* are from normally reared stage 35 animals, whereas plots *D–F* were obtained from embryos that had been removed from the chloretone solution only 2 hr prior to the recording.

Derivation of mean period lengths exhibited by the embryos in control and experimental groups showed the rearing environment to have had no effect on this measure (Fig. 7). There were no differences between the period lengths either of normally reared and chloretone-reared stage 35 animals or between any of the three groups at stage 40. Note also that the period lengths of normally reared stage 35 animals also did not significantly differ from those of stage 40 animals.

Figure 8 contains representative graphs of the phase coupling of activity from the left and right sides of stage 35 control and experimental animals. As is apparent from the plots, the data points cluster tightly around a value of 0.5, and the strength of this relationship does not change over time. Statistical comparisons of the mean values and of variability of this measure revealed no differences between control and experimental groups.

Prolonged immobilization also had no effects on other measures of ventral root activity. There were no differences among the groups in either duration of individual bursts, duration of bursts as a function of period length, or in the relation of phase coupling to period length.

The effectiveness and extent of neural blockade under the immobilizing rearing conditions were demonstrated by substitution during recording sessions of chloretone or lidocaine solutions for the curare-containing bathing solution. Such replacement of media resulted in a complete cessation of motor nerve impulse activity in normally reared stage 35 embryos. Repeated applications of tissue damaging stimuli failed to elicit any response, with concentrations of the drugs approximately equal to 75% of those concentrations used during rearing. When the chloretone or lidocaine solutions were in turn replaced by "normal" curare medium, ventral root activity returned. Recovery from this acute chloretone exposure appeared complete within minutes of removing the drug. Recovery from lidocaine exposure was occasionally prolonged, taking as much as an hour after replacement of the bathing media. Motor nerve responses to stimulation were foreshortened immediately after acute lidocaine exposure, usually consisting of only 10 to 20 individual impulse bursts.

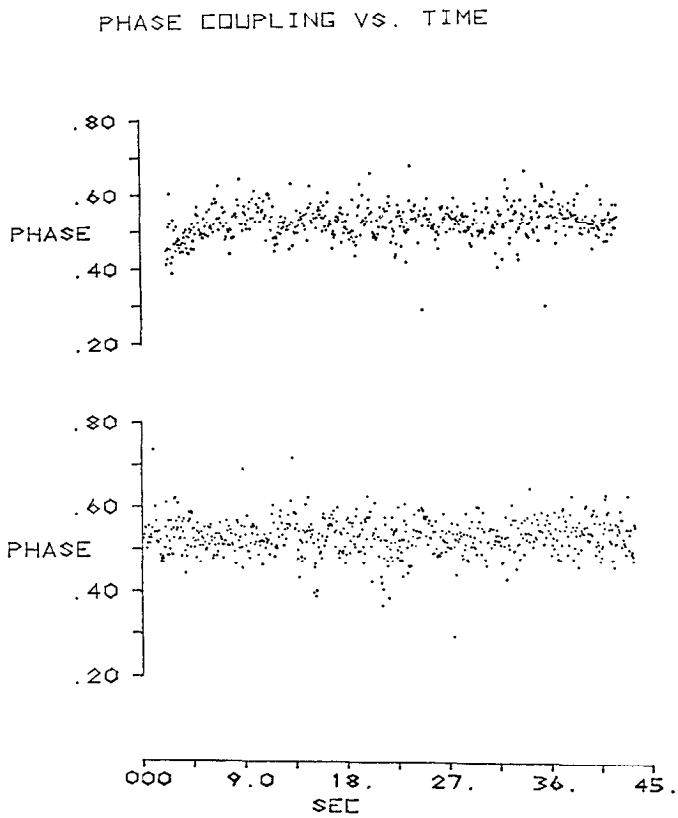

Figure 8. Representative graphs of phase relation of ventral root activities on the left and right sides versus time. *Upper graph,* From data obtained from a normally reared stage 35 embryo. *Lower graph,* from data obtained from a chloretone-reared stage 35 embryo, 2 hr after removal from the drug.

Morphological development

When reconstructions of the injection site revealed that applied HRP was confined to the musculature, three cell types within the cord were labeled: (1) Extramedullary cells were only rarely observed, perhaps owing to an abundance of melanocytes in the same general region (Fig. 9*A*). (2) An abundance of Rohon-Beard cells were labeled throughout large extents of the cord (Figs. 9 and 10). The great majority of these cells were large, spherical, and located in the most dorsal portion of cord (see Dushane, 1940; Hughes, 1957; Roberts and Clarke, 1982). It was not unusual, however, to observe presumed Rohon-Beard cells in the more rostral portions of the cord and caudal hindbrain, which were more ventrally located and were flattened along the dorso-ventral axis. Although processes from the rounded, dorsal Rohon-Beard cells generally exited the cord dorsally (Fig. 9*A*, 10*C*; though see Fig. 9*B*), the flattened type of Rohon-Beard neurons had processes that traversed directly lateral to exit the cord. (3) Labeled motoneurons typically exhibited a very large soma (see, though, Figs. 9*B*, 10*C*) set in the most lateral aspect of the ventral half of the cord, with dendritic arborizations entirely confined to the white matter and generally contained within the ventral half of the cord at stage 35. At stages 40 and 45, the arborizations greatly increased in complexity, often extending quite far towards the dorsal aspect of the marginal layer (Fig. 10, *A–C*).[1]

[1] These experiments were initially conceived to include studies of naturally occurring cell death of Rohon-Beard cells and motoneurons. The physical dispersal and ontogenetic transience of these cell populations (Hughes, 1957; Roberts and Clarke, 1982; Spitzer and Lamborghini, 1981) make them unique in comparison with the neurons of other systems for which cell death has been characterized (see Oppenheim, 1981). The stages during which embryos were immobilized in this work include the developmental period analogous to the time frame in which

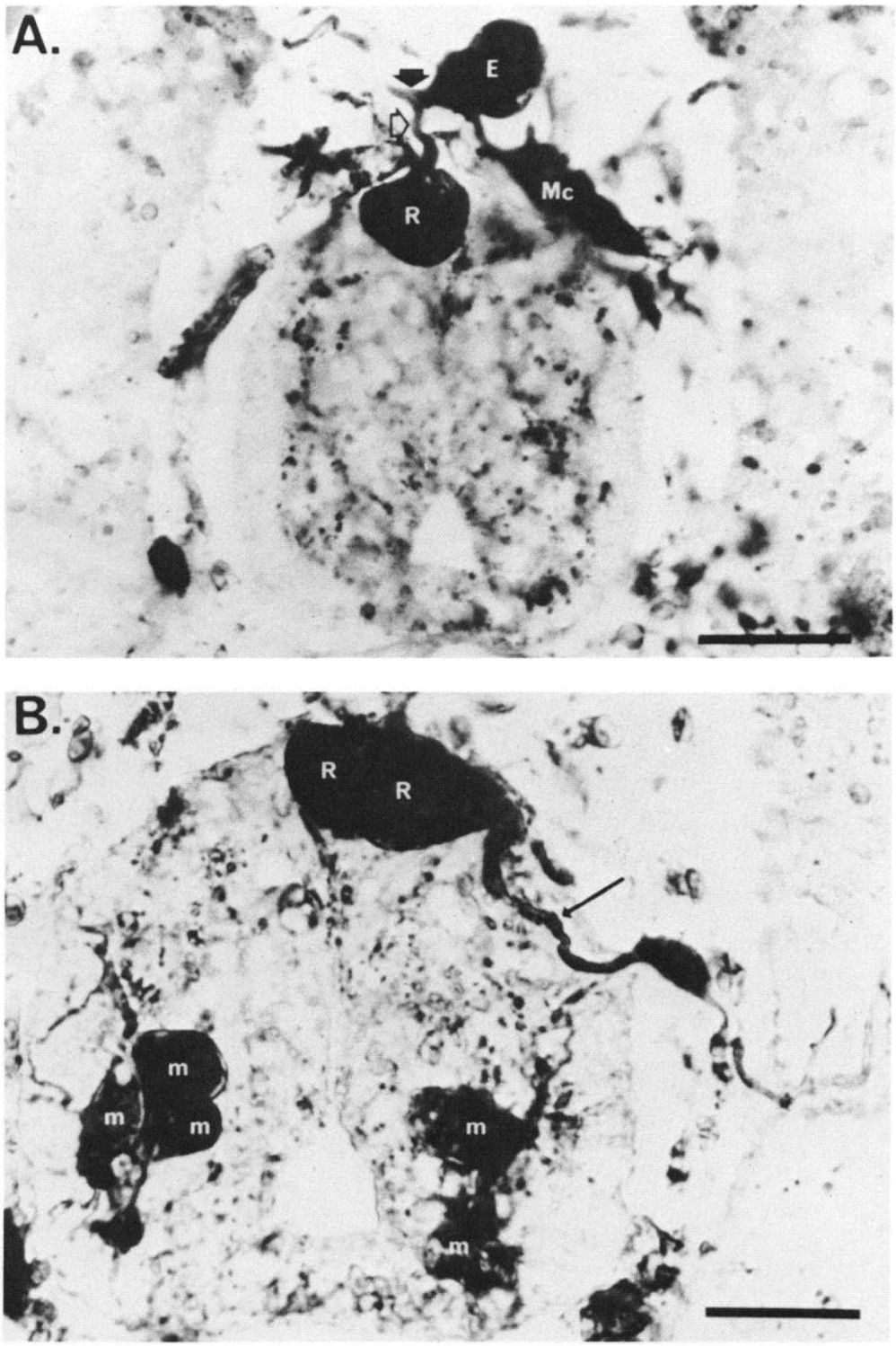

Figure 9. Transverse sections through the spinal cord of normally reared stage 35 *Xenopus* embryos, showing neurons labeled through peripheral application of HRP. *E,* Extramedullary neuron; *R,* Rohon-Beard cell; *Mc,* melanocyte; *m,* motoneuron. Open and filled *arrowheads* in *A,* Processes of Rohon-Beard cell and extramedullary neuron, respectively. *Arrow* in *B,* Rohon-Beard cell projection. Scale bar, 25 μm.

A great degree of variability of dendritic branching and fiber lengths was noted, both between motoneurons of a single animal, as well as between those of different animals (Fig. 11). The mean values for normally reared and immobilized animals—of dendritic trees/neuron, total fiber length/neuron, and number of branch points/neuron—are given in Table 1. Figure 12, *A* and *B,* shows graphs of number of branches versus branch order, and fiber length versus branch order, respectively. Graphic representations of Sholl, area Sholl, fiber Sholl, and area fiber Sholl analyses (see Materials and Methods) for stage 35 normal, chloretone-reared, and lidocaine-reared embryos are presented in Figure 13, *A–D.* Statistical comparisons among the immobilized and control groups revealed no significant differences for any of these measures.

manipulations of synaptic transmission can either prevent or accelerate cell death in other systems (e.g., Olek and Edwards, 1980; Oppenheim and Chu-Wang, 1983). However, owing to the lack of discrete distribution of these cells and the proportion of both Rohon-Beard cells and motoneurons with atypical somal size or placement noted above, complete cell counts are not possible. The specific reaction of these two cell types for acetylcholinesterase, seen in older amphibian larvae (Farel and McIlwain, 1983), yielded inconsistent and unreliable results in *Xenopus* embryos.

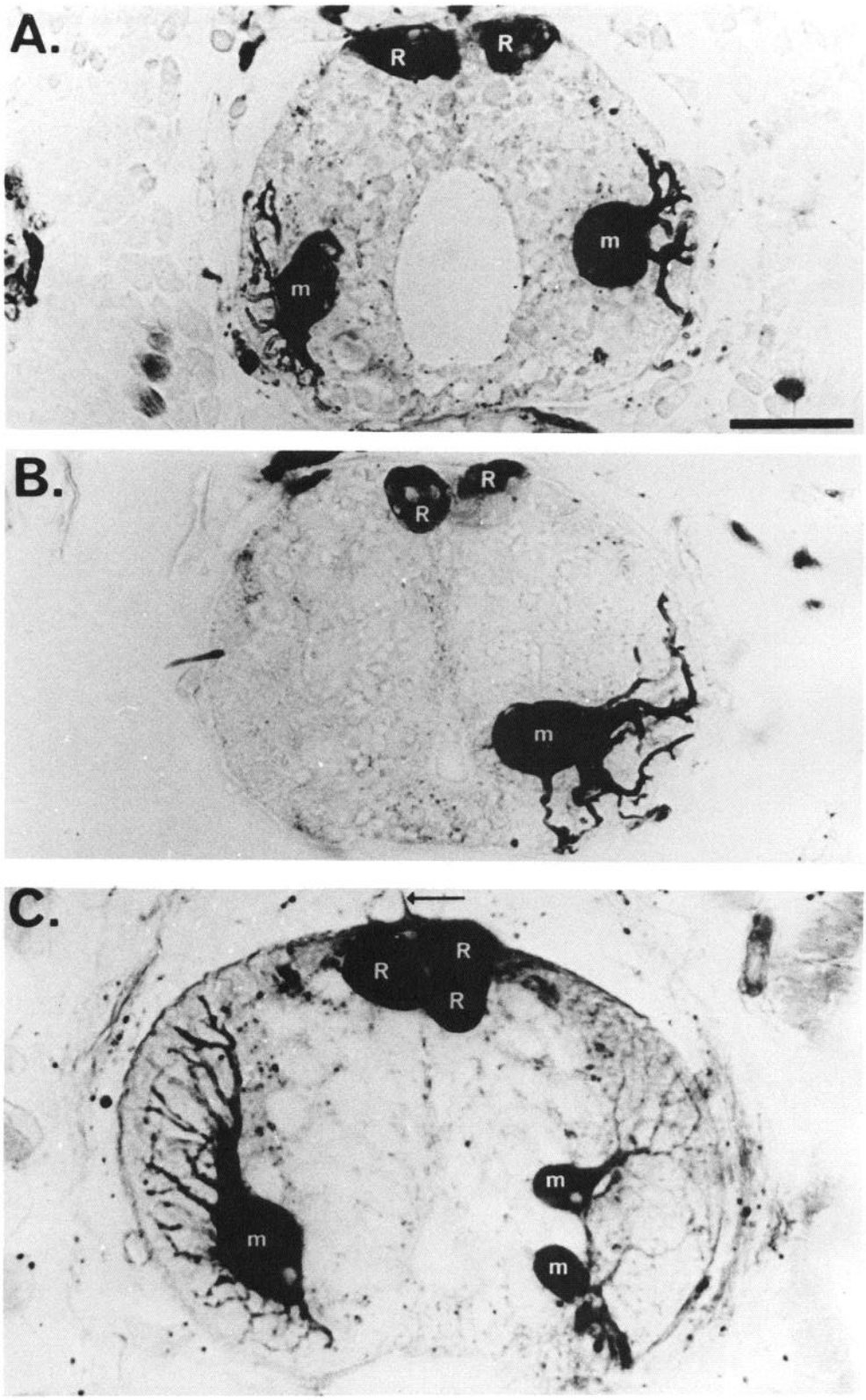

Figure 10. Motoneurons labeled in normally reared embryos. Abbreviations as in Figure 9. *Arrow* in *C*, Rohon-Beard cell process. Magnifications of all three sections are identical. Scale bar, 25 μm. *A*, Stage 35; *B*, stage 40; *C*, stage 45.

Discussion

These studies have investigated the effects of neural activity blockade on the earliest stages of *Xenopus* motor system development. They have demonstrated the lack of significant effect of such treatment on the development of a number of components and measures of that system. Two hr after removal from a chloretone solution (and approximately 24 hr after removal from lidocaine—the earliest stage tested for that group), the physiological output of the central pattern generator governing swimming was qualitatively and quantitatively indistinguishable from that of control embryos. The dendritic arborizations of motoneurons in anesthetized embryos were statistically

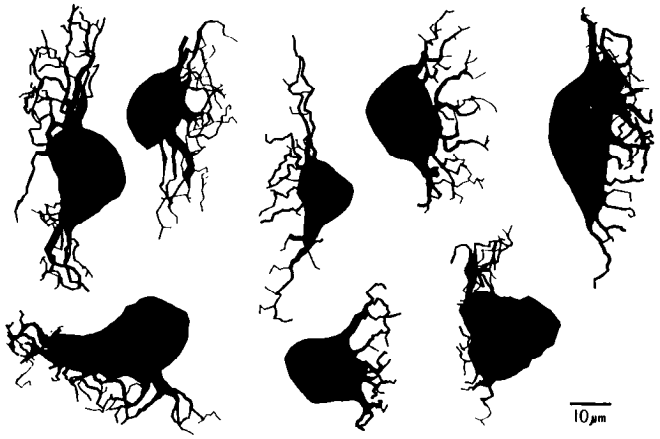

Figure 11. Computer-generated tracings of dendritic arborizations of motoncurons from stage 35 embryos, illustrating the variability among the cells' dendritic complements. The four tracings nearest the *left side* of the figure are of neurons from normally reared embryos, and the two furthest to the *right* are from chloretone-reared embryos; the remaining two are from lidocaine-reared embryos.

equivalent to those of normal animals. The companion work (Haverkamp and Oppenheim, 1986) to these studies shows that chronic immobilization during this developmental period also has no permanent effect on the behavior of these amphibian embryos.

The observations reported here on ventral root activity during fictive swimming are in essential agreement with those made by Kahn and Roberts (1982a). These authors have proposed a model of the central pattern generator for swimming in *Xenopus* embryos (Kahn and Roberts, 1982a; Kahn et al., 1982; Roberts and Kahn, 1982; Roberts et al., 1981; Soffe and Roberts, 1982a, b) that envisions the reciprocal connections of at least six neurons at each somitic level, as well as descending input to these assemblies. Since the measures of ventral root activity of the previously immobilized embryos in this study were indistinguishable from those of normal embryos, the circuitry responsible for this pattern generation must have developed to a largely normal degree in the absence of synaptic and impulse activity. [Since the conditions employed for immobilization were shown, in this preparation, only to arrest completely the motoneuron output, a similar effect of the drugs on purely central neurons cannot be immediately assumed. Furthermore, it is not possible to know whether the applied concentrations of these drugs affected synaptic and/or impulse activity arising from spontaneous as well as stimulated neural activity at all stages of development under consideration. Therefore, while it is probable that the conditions under which the experimental animals were immobilized resulted in nearly complete cessation of neural activity, it is not possible to state this with certainty. For additional discussion of the effects of the immobilizing drugs, see Haverkamp and Oppenheim (1986).]

In behavioral studies performed on identically treated embryos (Haverkamp and Oppenheim, 1986), there was an initial period of several hours after removal from the drug solution when the animals appeared to behave normally, yet swam more slowly and for shorter distances. Since these behavioral differences were not reflected in recordings of ventral root activity, it is likely that the physiologic correlates of these behavioral effects lie more peripherally than the ventral roots.

Among sites for such peripheral effects are the intra- and intermyotomal gap junctions by which the embryonic axial muscle cells are electrotonically coupled (Blackshaw and Warner, 1976; Hayes, 1975). Armstrong et al. (1983) have demonstrated that immobilization of *Xenopus* embryos prior to stage 26 prevents the normal regression of this coupling, with return of activity resulting in the belated disappearance of junctions over a 36 hr period. Another potential locus for immobilization effects is at the neuromuscular junction, Kullberg et al. (1977) having demonstrated the regression of the initially polyneuronally innervated *Xenopus* somitic musculature to a mononeuronal state. In mammalian (Benoit and Changeux, 1978) and avian (Srihari and Vrbova, 1980) systems, decrements of muscular activity result in a delay in withdrawal of polyneuronal innervation. A third possibility is an effect of immobilization on the development of the musculoskeletal system, as seen in paralyzed chick embryos (Drachman and Sokoloff, 1966; Oppenheim et al., 1978), which may also occur in immobilized axolotl embryos (Haverkamp and Oppenheim, 1986).

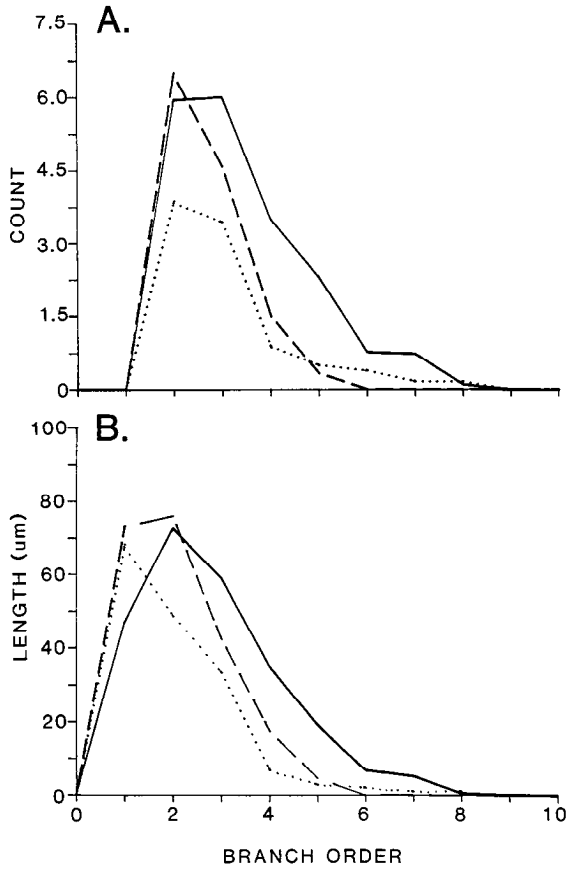

Figure 12. Relationships between measures of motoneuron dendrites of stage 35 embryos. No statistically significant differences occur among the groups at any branch order value. *Solid lines,* Normally reared; *dashed lines,* chloretone-immobilized; *dotted lines,* lidocaine-immobilized. *A,* number of dendritic branches versus branch order. *B,* dendritic fiber length versus branch order.

Table 1. Measures of *Xenopus* motoneuron dendritic arborizations

	Normally reared	Chloretone-immobilized	Lidocaine-immobilized
Trees/neuron	7.60 (1.75)	9.51 (3.00)	7.56 (1.39)
Branches/neuron	19.33 (8.19)	12.88 (4.01)	9.39 (0.98)
Fiber length/neuron	244.7 (92.2)	214.0 (79.5)	163.9 (17.2)
Fiber length/tree	32.21 (6.74)	23.56 (1.99)	22.85 (2.35)

Values given are group means, with standard deviations of each measure in parentheses. None of the measures significantly differs among the groups.

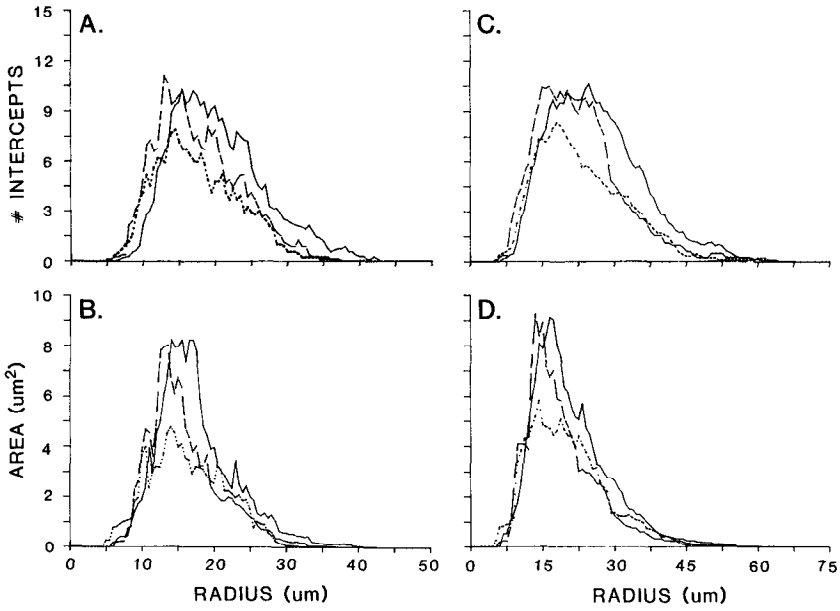

Figure 13. Graphic displays of analyses of primary motoneurons of stage 35 *Xenopus* embryos. *Solid lines,* Normally reared; *dashed lines,* chloretone-immobilized; *dotted lines,* lidocaine-immobilized. Sampling increments, 0.5 μm for all analyses. No statistically significant differences exist among the groups at any radial distance. See Materials and Methods for explanations of different analyses. *A,* Sholl analysis; *B,* area Sholl analysis; *C,* fiber Sholl analysis; *D,* area fiber Sholl analysis.

There is a final possibility, that the prolonged neural blockade may, indeed, have had developmental effects on the CNS that were not reflected in, or were below the resolution of, the physiological measures used here. For example, although the timing of activity in individual motoneurons might be correct, the absolute number of motoneurons contributing to a particular ventral root activity burst may be decreased as a result of prior neural blockade. While arguments can be made against this particular scenario, the possibility of normal ventral root output in the presence of some type of abnormal neural development remains. One likely locus for such central effect of neural blockade is the growth and differentiation of neuronal dendritic arborizations.

A widely accepted theory of dendritic growth proposes that in the absence of afferent synapses, dendritic filopodia will be reabsorbed, while those dendritic growth cones receiving synaptic contact will stabilize to become dendrites (Berry and Bradley, 1976; Morest, 1969; Vaughn et al., 1974). The many studies which have shown the influence of afferent fibers on the growth and development of dendrites (e.g., Altman, 1973; Bradley and Berry, 1976, 1978; Kimmel et al., 1977; Smith, 1974) have demonstrated these effects through lesions of afferents and cannot, therefore, differentiate synaptic activity effects from non-impulse-regulated trophic effects. Effects on dendritic development have also been demonstrated with defined sensory manipulations and afferent stimulation (e.g., Borges and Berry, 1978; Coleman and Riesen, 1978; Rutledge et al., 1974; Spinelli et al., 1980; Tieman and Hirsch, 1982). One explanation of these results is that the inductive effect of contacting afferents on dendritic growth is due to the functional activity of these synaptic contacts (Borges and Berry, 1978; Smith, 1981). Alternatively, such effects may be the result of effects on the maintenance (Changeux and Danchin, 1976; Smith, 1981; Tieman and Hirsch, 1982) or modification of differentiated branches (Rutledge et al., 1974; Uylings et al., 1978).

The data from the present study argue against a role for the activity of afferent synapses in the initial induction of dendritic arborizations. Analyses of nine different measures of motoneuron dendritic size and complexity reveal no significant differences between control and experimental groups. Although the separation of the mean values of these measures is quite distinct at certain points, the inter-animal variability is such that these differences are not statistically significant. It is possible that treatment effects may have been masked by age-related heterogeneity in degree of development of sampled neurons (see Lamborghini, 1980; Nordlander, 1984). Even in this case, however, the similarity of these measures between control and experimental groups suggests only a minimal role of synaptic activity on initial dendritic outgrowth.

In 1941, Paul Weiss stated that the basic neural assemblies responsible for coordinated motor output "differentiate by virtue of the developmental dynamics of the growing organism in forward reference to their future function, but without the benefit of exercising that function during their formative period" (Weiss, 1941, p. 87). In the 45 years since that statement was made, however, an enormous amount of experimental data from both *in vivo* and *in vitro* systems have variously demonstrated both the integral role that functional activity may play in many developing systems (e.g., Bergey et al., 1981; Harris, 1981; Janka and Jones, 1982; Oppenheim, 1981; Oppenheim et al., 1978; Pettigrew, 1978; Stryker, 1981), and also provided examples of conditions under which impulse activity is not related to neural development (e.g., Crain, 1980; Crain et al., 1968; Harris, 1980).

It may be argued that the remarkable degree to which the *Xenopus* motor system functions normally soon after the animal's removal from the drug environment is the result of a specific adaptation for effective behavioral performance very early in development (cf. Bixby and Spitzer, 1984; Kullberg et al., 1984). These data do not necessarily lead to the prediction, therefore, that neural assemblies that develop within time frames that allow for the intercession of functional activity in refinement of their output will show a similar perfection of performance after prolonged neural blockade. However, these studies, when taken in conjunction with previous investigations, serve to emphasize the remarkable degree to which initial neural projections and connections form in "forward reference" to their ultimate function.

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