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Development of neuronal circuits and behaviors in the medicinal leech

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ABSTRACT: We are studying the neuronal mechanisms responsible for establishing circuitry underlying the local bending response in the medicinal leech. Local bending replaces an embryonic behavior, circumferential indentation, during the time of initial chemical synaptogenesis in leech embryos. We found that the electrical connections among the motor neurons are established first, about 5% of embryonic time (almost 2 full days) before chemical connections form. The inhibitory connections from muscle inhibitors to muscle excitors are, we hypothesize, responsible for the emergence of local bending. We have also found that the central processes of the excitors—but not the inhibitors—have much longer central processes when their peripheral processes are kept from contacting their target muscles. This system should allow us to test ideas about how individual neurons find their appropriate targets to form functional neuronal circuits. © 2001 Elsevier Science Inc.

KEY WORDS: Bending response, Synaptogenesis, Muscle inhibitors.

INTRODUCTION

One of the central questions in developmental neurobiology is how neurons establish their highly ordered and precise patterns of connectivity to produce a nervous system capable of sensing the environment, processing information, and generating behavior [2,3,58,59,63,79]. In recent years, major advances have been made in discovering molecular mechanisms by which neurons make and maintain specific contacts [27,37,65], how the axons find their way to their targets [9,33,74,76], and ultimately make synaptic contacts [11,12,27,38]. How these mechanisms form a functioning system capable of producing appropriate behaviors is just starting to emerge. For the past several decades, studies of the development of the sensory side of nervous systems have emphasized the relative contributions of "hard-wiring" as opposed to "activity-dependence" in producing sensory systems [25,36]. More recently, studies are increasingly being directed at motor development, and some generalities are emerging. For instance, motor patterns emerge early in development, at a time when their behavioral significance is unclear [2]. For example, movement patterns by a chick can help it escape from its eggshell, but the movements arise well before hatching is feasible or desirable [3,26]. In fact, it appears that the first coordinated motor patterns produced by birds *in ovo* and by mammals *in utero,* which tend to be rhythmic and spontaneous, depend, at least in part, on connections between motor neurons that are unlikely to be important for producing behavior in postembryonic life [57]. One reasonable guess is that the neuronal networks producing these transient motor connections provide scaffolding for the ultimate formation of adult-like neuronal connections. Surprisingly, many of these motor patterns appear to depend upon electrical connections among motor networks, including among the motor neurons themselves [8,77]. As the spinal cord matures, both modulatory and standard chemical synaptic pathways take over the predominant role in producing motor patterns [5,7,13,58,70,71].

We study the neural development of behavior in the medicinal leech. We, too, find that behaviors emerge during embryogenesis in leech embryos before they are needed for any discernable purpose, and that some behaviors are completely embryonic: they appear relatively early in development and disappear before the animal "hatches." (In leech development, 10–25 fertilized eggs are laid into an egg case, improperly called a "cocoon," and develop into juveniles in about 30 days; they usually leave the deteriorating egg case—i.e., "hatch"—in the 5th or 6th week after they were laid.) We also find that the electrotonic connections among the motor neurons are formed first, followed by chemical connections. Because this circuitry is relatively simple, and because we can identify the neurons before they gain their adult connections, we will be able to tell whether they make their adult-like, "appropriate" connections right from the start or whether there is overgrow and retraction of connections. We have found that there are morphological consequences when excitatory—but not inhibitory—motor neurons are deprived of their peripheral connections to body wall muscles. In this paper, we will discuss our general approach, then focus on one particular neuronal circuit, the one that produces local bending in response to a light touch on the external body wall.

THE DEVELOPMENT OF BEHAVIORS

The Onset of Behaviors in Embryonic Leeches

Before starting to characterize the neuronal basis of behavioral development, it was necessary to know when the behaviors first appear. Embryos develop from fertilized egg to adult-like morphology and behavior in about 30 days at 20°C (faster at 25°C and slower at 18°C, which are the limits for normal development). For

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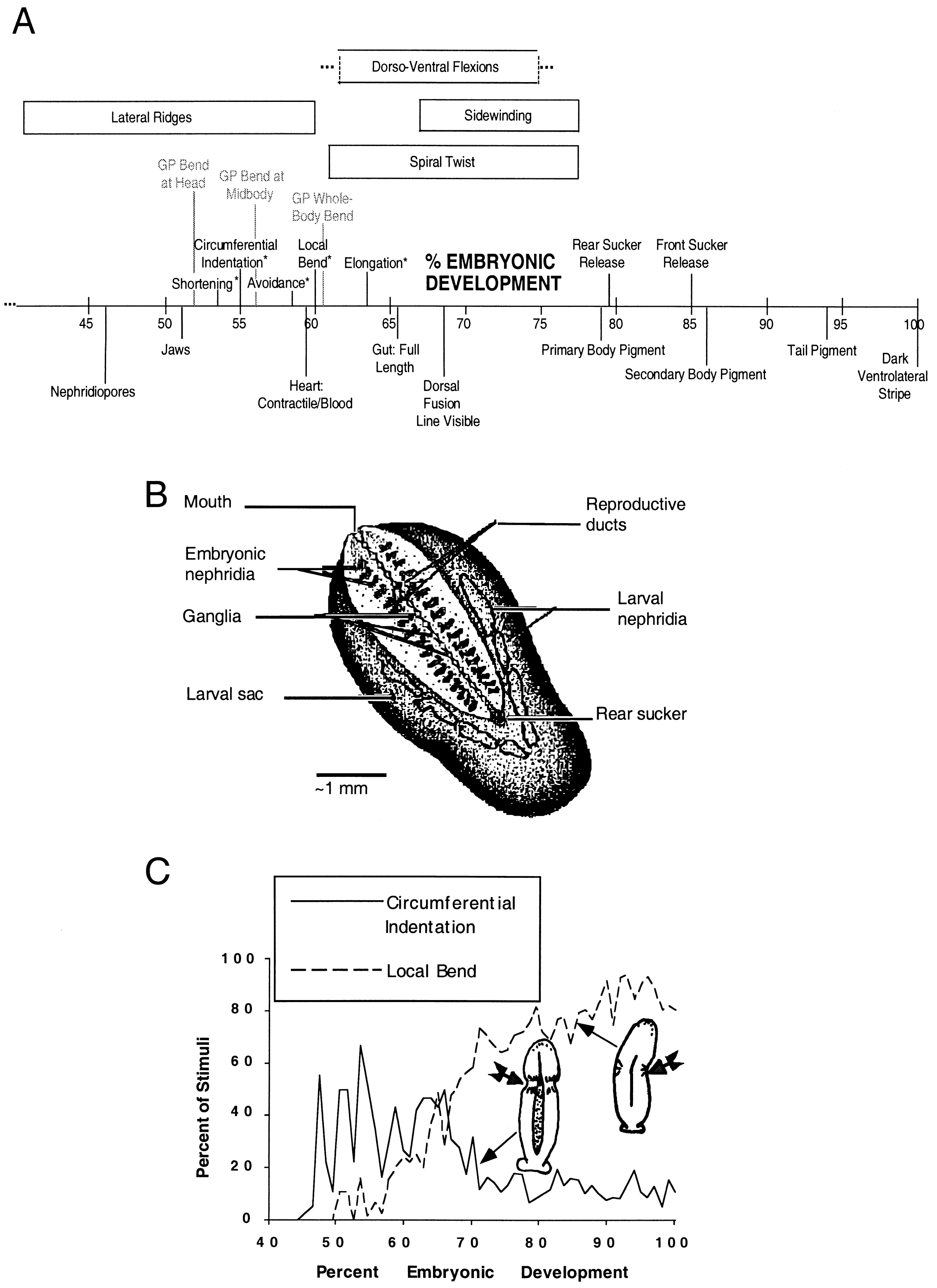

FIG. 1. Onset of behaviors in the medicinal leech. (A) Staging table of embryonic development (ED) [61]. Morphological features are placed below the time line and behaviors above it. Abbreviation: GP, germinal plate. (B) Drawing of an embryonic medicinal leech at about 50% ED, showing the germinal plate (the lighter structure located on the surface of the darker larval sac that contains the yolk). During development the germinal plate grows in length and width, and by 68% ED it encompasses the larval sac. The germinal plate produces most structures in the leech, including all of the neurons and muscles that generate overt behavior. (C) Circumferential indentation and local bending in leech embryos. Both behaviors are elicited by weak mechanical stimulation applied

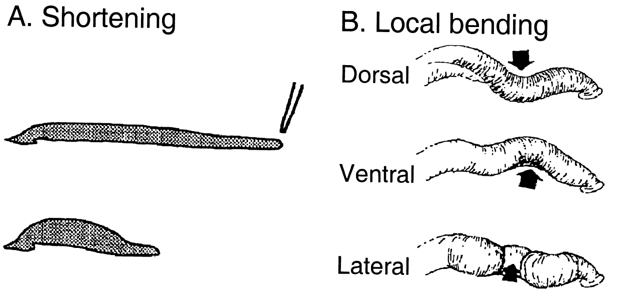

FIG. 2. Two behaviors of the medicinal leech, *Hirudo medicinalis*, that can be elicited by mechanosensory stimuli. These two adult behaviors that can also be elicited in young embryos, as shown in the Fig. 1. (A) Shortening is caused by a contraction of the longitudinal muscles in all body segments and is best elicited by stimulation at the front end of the animal. (B) Local bending is confined to one or a few segments and is generated by contraction of longitudinal muscles on the side that has been lightly touched, coupled with a relaxation of the longitudinal muscles on the side opposite to the touch. Diagram shows responses to a touch on the dorsal, ventral, and lateral surfaces of a single segment near the posterior end of the leech.

convenience, we express developmental stages as “% of embryonic development” or “% ED.” Well-formed ganglia first appear in the anterior of the embryo at about 30–32% ED. Cell division in the ganglia continues for another day until ganglia contain approximately 400 neurons, the adult number, whereupon all the neurons start to grow processes at nearly the same time [18,73]. During the next 12% of development (about 4 days), most neurons acquire their recognizable adult morphology [41–43], begin to produce their neurotransmitter [24], and make their initial synaptic contacts [41]. At about 51% ED, the first neuronally mediated behavior (shortening, or withdrawal) is seen [62]. At about 68% ED, the lateral edges of the germinal plate meet and fuse at the dorsal midline, transforming the animal into an elongated tube.

The first two adult behaviors that embryos produce are shortening and local bending [62], two behaviors whose neuronal basis we have characterized [39,45–49,67–69]. We have found that these behaviors first appear at particular stages (Fig. 1A). In fact, the earliest behaviors appear when the leech is a flat sheet of cells, the germinal plate, on the surface of the yolk sac (Fig. 1B). One of the first behaviors seen is circumferential indentation, a localized contraction of longitudinal muscles on both sides of the midline of the germinal plate that is independent of which side was stimulated (Fig. 1C). Because, within 2 days after circumferential indentation first appears, the same stimulus produces local bending, we hypothesize that circumferential indentation turns into local bending when inhibitory connections among motor neurons become fully established [62]. One of the motivations for studying the development of local bending is to test this hypothesis.

Characterization of Local Bending

When a leech is touched lightly on its skin, the longitudinal muscles contract at the site of the touch, and those on the other side of the animal relax, producing a bend in the segment stimulated (Fig. 2) [39]. We have characterized the interneurons and motor neurons required for this behavior [48,49], as well as the function of the system as a whole [45–47]; a schematic of the neuronal circuitry for this response is shown in Fig. 3. The four pressure-sensitive mechanoreceptor cells (P cells) have overlapping receptive fields equally spaced around the circumference of a segment. Each P cell makes excitatory synaptic contact with all 17 local bend interneurons (LBIs), and the synaptic strengths differ in a regular manner. One P cell or two P cells with adjacent receptive fields make strong synaptic connections onto a given LBI, whereas P cells with more distant receptive fields make weaker connections [49]. The LBI connections to motor neurons were not tested directly but the simplest explanation for the effects of P cells on

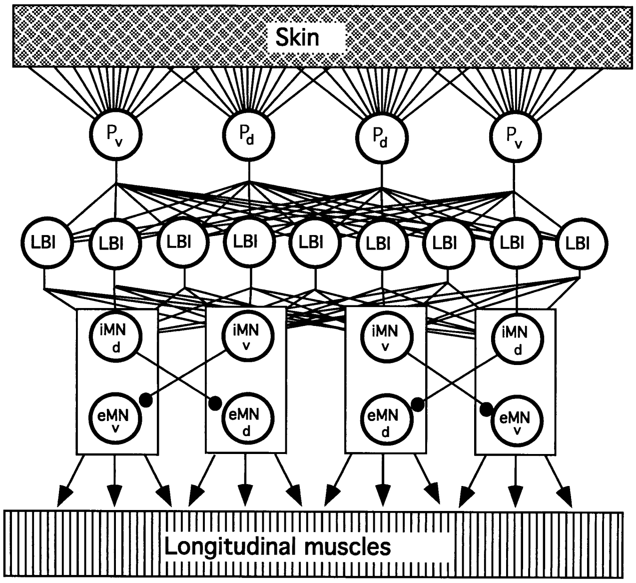

FIG. 3. Segmental circuitry underlying local bending. A total of four pressure sensitive mechanoreceptors (P cells) innervate the circumference of the body wall in overlapping receptive fields. The receptive field of each P cell is indicated by a subscript; P_d innervates dorsal body wall, P_v the ventral. All four P cells excite all seventeen identified local bend interneurons (LBIs) in a graded manner. (Nine of the seventeen identified LBIs are shown in this diagram.) The LBIs excite the excitatory motor neurons (eMNs) and inhibitory motor neurons (iMNs) is such a way that P cell activation most strongly excites eMNs innervating the same region of the body wall. For example, P_d cells activate eMNd and P_v cells activate eMNv. As a result, activating a P cell generates a local bend on the side of the animal that was touched. In addition to producing relaxation of longitudinal muscle fibers, the iMNs inhibit the eMNs that control longitudinal muscle on the same side of the animal: iMNds inhibit eMNds, and iMNvs inhibit eMNvs. (The *inhibitory* motor neurons to one set of muscles receive the same kind of interneuronal inputs as do the *excitatory* motor neurons to the antagonistic muscles; for example, both the iMNd and eMNv receive strongest input from interneurons with ventral receptive fields.) Motor neurons with the same input (e.g., the iMNd and eMNv) are enclosed in the same box in this diagram. Connections indicated by lines are excitatory; connections indicated by lines ending in closed circles are inhibitory.

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to the middle of the body, as indicated in the sketches of an embryonic leech at about 65% ED. The sketches show a dorsal view of the embryos. Arrows with expanded tails indicate the site of stimulation; anterior is up. The sketch on the left represents circumferential indentation, and the one on the right shows local bending. The graph plots the percentage of stimuli administered to the middle of the leech that produce circumferential indentation and local bending.

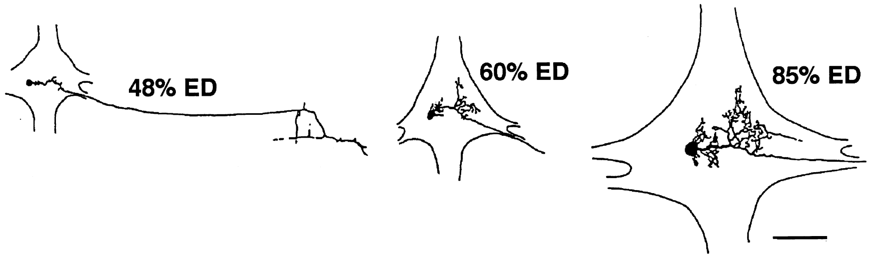

FIG. 4. Outgrowth of cell 3, a typical eMNd. Figures show *camera lucida* drawings of horseradish peroxidase (HRP)-filled neurons at three different stages of development. To save space, the peripheral arborization has been omitted from the drawings of 60% and 85% embryonic development (ED) embryos. The first process to sprout from the soma grows across the ganglion, into the posterior root, and out to the edge of the germinal plate (which is the future dorsal region of the leech). Although the axon produces a few small sprouts in the ganglion in this early phase, identifiable central branches arise only after the axon begins to branch in the periphery. Chemical synapses are detected by intracellular recording shortly before 60% ED; connections are adult-like by 85% ED. Scale bar: 50 μm. Adapted from an original drawing by J. Jellies.

the motor neurons [48] is to assume that each LBI directly excites all the longitudinal muscle motor neurons, with the synaptic strengths differing in the same orderly way as for the P → LBI connections: the strength of the synaptic connection falls off with the distance between the receptive field centers for the LBIs and the motor neurons. In the circuit, inhibitory motor neurons (iMNs) not only inhibit muscles peripherally, they also make inhibitory connections onto excitatory motor neurons (eMNs) that would excite that muscles (e.g., motor neurons that inhibit dorsal longitudinal muscles–iMNd—inhibit motor neurons that excite dorsal longitudinal muscle—eMNd; similarly iMNv inhibit eMNv). The connections in the circuit are completely feed-forward: there are no known backward-going connections between hierarchical layers nor are there significant lateral connections within a layer [49].

The relative simplicity of the local bend circuit, coupled with the early onset of the behavior, makes it a particularly favorable focus for a cell-by-cell study of the ontogeny of circuitry. Our exploration of this circuit has commenced with a study of neurons in the motor layer, primarily because all of the neurons are readily identifiable in embryos and because synaptic connections in this layer are very well characterized in the adult nervous system [60].

FORMATION OF SYNAPTIC CONNECTIONS

Development of Electrical Synapses Among Motor Neurons

At around 33% ED, all neurons begin to grow axons. Leech neurons are monopolar and each one grows its longest axonal process first. Almost every neuron sends at least one process out of the ganglion: the sensory and motor axons exit at least via nerve roots, and interneurons send processes into the interganglionic connectives. (Most interneurons make connections both within their own ganglion and in adjacent ganglia.) Sensory neurons, probably because they send their axons ipsilaterally, reach the periphery first; in fact, they appear to function as "pioneer fibers" for some of the peripheral nerves [22,30,42]. Figure 4 shows the anatomy of cell 3, an excitatory motor neuron to the dorsal longitudinal muscles, at three different stages of development. Neurons are able to produce action potentials by about 48% ED, a time that corresponds to the initial synthesis of neurotransmitters in neurons of other leech species [24].

About 5% ED (nearly 2 days) before chemical synaptic transmission is detectable, however, electrical connections first appear (Fig. 5). By 50% ED, the electrical connections among motor neurons can be detected either by electrophysiological recording or by the passage of neurobiotin from one motor neuron to several others [16]. Some of these early connections are known from adult ganglia, but others are novel. In addition some of the (perforce excitatory) electrical connections at this time are made between neurons that ultimately will be connected by inhibitory chemical synapses (FJ Eisenhart, unpublished data).

These observations provided strong motivation to explore the electrical connectivity among all the neurons to determine whether a pattern could be discerned in how neurons were transiently connected. Using paired electrophysiological recordings is both too tedious and too destructive in embryonic neurons. (To determine all the connections onto a single neuron, we would need to maintain the recording from one neuron while sampling dozens of others with a second electrode; embryonic neurons are too small and too fragile to endure longer than the time required to record from a few other neurons.) Instead, we took advantage of the discovery that Neurobiotin molecules cross leech gap junctions. We filled individual embryonic neurons with Neurobiotin through a sharp electrode then fixed the preparation when the dye had diffused along the processes of the impaled cell and across gap junctions into other neurons. In adult ganglia, the Neurobiotin passed into other neurons whose identity could be predicted based on published electrophysiological results; i.e., the dye-coupling was consistent with the known pattern of electrical coupling. This consistency is both qualitative (cells in the same anatomical position are stained in each ganglion) and quantitative (relative staining levels in coupled cells is consistent between preparations) [17]. In embryos, the pattern of staining emerges gradually from 50% to 57% ED, but many cells are labeled that will not be part of the mature pattern. By 74% ED, the transient coupling has disappeared, and the mature pattern of electrical connections is firmly established. Interestingly, transiently coupled neurons have no consistent functional relation to the injected neuron, suggesting that the transient coupling may be serving a nonsynaptic function. This result suggests that the dendritic processes in the leech ganglion act like outgrowing sensory axons in insect limbs, which make strong, but transient, electrical connections with "guidepost cells" at choice points along their path into the central nervous system (CNS) [4]. Alternatively, the electrical contacts could have a negative effect, instructing processes *not* to grow into particular locations, as has been seen for transient interganglionic axons of leech motor neurons [80].

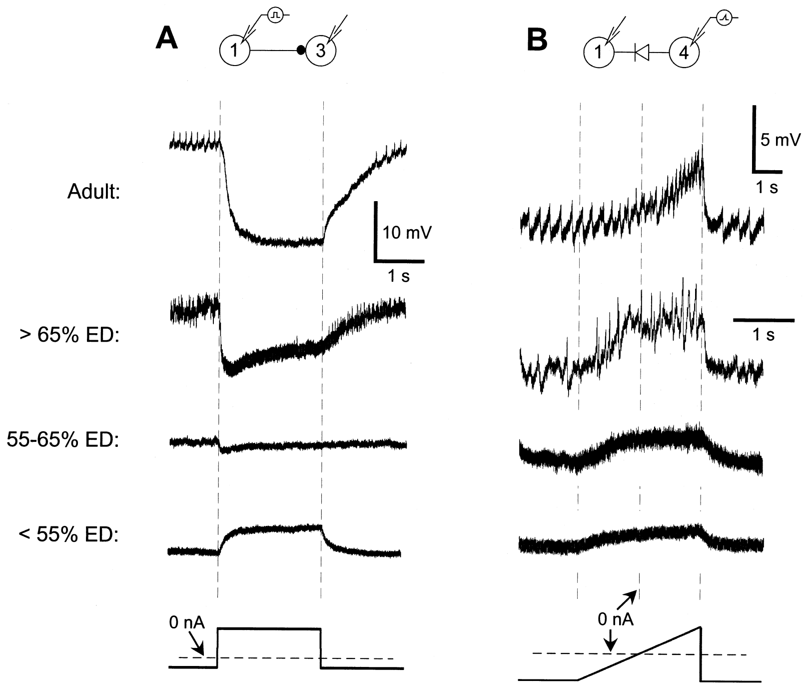

FIG. 5. Development of chemical (A) and electrical (B) synapses between longitudinal muscle motor neurons of the leech. (A) Development of the chemical synaptic connection from an inhibitory motor neuron (cell 1) onto an excitatory motor neuron (cell 3). The four recordings are from cell 3 in animals at different stages of development; the square wave at the bottom indicates when cell 1 was depolarized. (Cell 1 was held hyperpolarized between the depolarizing pulses.) In adult segmental ganglia, cell 1 makes an inhibitory chemical synapse that is graded and long-lasting. Before 55% embryonic development (ED), we saw no inhibitory postsynaptic potentials (IPSPs), although in some cases (as shown here), we observe excitatory postsynaptic potentials (EPSPs) due to "inappropriate" electrical coupling (i.e., the connection is never observed in adults). Chemical synapses are first observed between 55 and 65% ED, although the IPSPs are initially quite small and transient. At >65% ED, the chemical IPSPs are larger and can be sustained for several seconds, although they may still be transient. The parameters of the stimulus were: in the adult, −2 nA between depolarizing pulses of +6 nA; in embryos, −0.5 nA between depolarizing pulses of +1.5 nA. (B) Development of a previously undescribed rectifying electrical connection between an excitatory motor neuron (cell 4) controlling ventral muscles and an inhibitory motor neuron controlling dorsal muscles (cell 1). The top 4 recordings were made from cell 1 while cell 4 was being stimulated by the ramp of current shown in the bottom trace. In adults, stimulating cell 4 with a current ramp produces a ramp response in cell 1. The slope of the voltage ramp in cell 1 is greater on the positive phase of the current ramp (0 nA is marked by the indicated dashed lines) into cell 4 than on the negative phase, indicating that the electrical synapse passes positive current more effectively than negative current. (Reciprocal data for cell 1 stimulation are not shown.) Appropriate electrical synapses are present before chemical synapses, as shown by the small cell 4 to cell 1 response seen at <55% ED; however, the rectification of the electrical synapse is reversed: negative current passed more effectively than positive current. The electrical coupling grows stronger over development, as demonstrated by the larger size of the voltage responses in cell 1, but normal rectification is not seen consistently until adulthood. Stimulus intensities: adult, −2 to +2 nA; embryos, −1 to +1 nA.

Development of Chemical Synapses Among Motor Neurons

Recent studies have produced physiological and morphological evidence for electrical connections among neurons in a number of developing nervous systems [21,29,34,35]. In most cases, it is not known whether these connections are transient or permanent, and how they function in development. Some possibilities include: (a) they provide a means for neurons to exchange small molecules, thus allowing them to recognize one another and to determine whether to establish permanent contact; (b) they are the first step in the production of a chemical synapse; (c) they are used for pathfinding—as for guidepost cells in the grasshopper [4]—and have nothing to do with synapse formation per se.

By recording from pairs of neurons in leech embryos of various ages, we have found that chemical synaptic contacts are first recorded at 55% ED, nearly 2 days after the first electrical connections are made, but during the period when we see transient electrical connections (Fig. 5). We have followed the development of synaptic connections between identified pairs of neurons during this period, and although we have seen changes, we have yet to

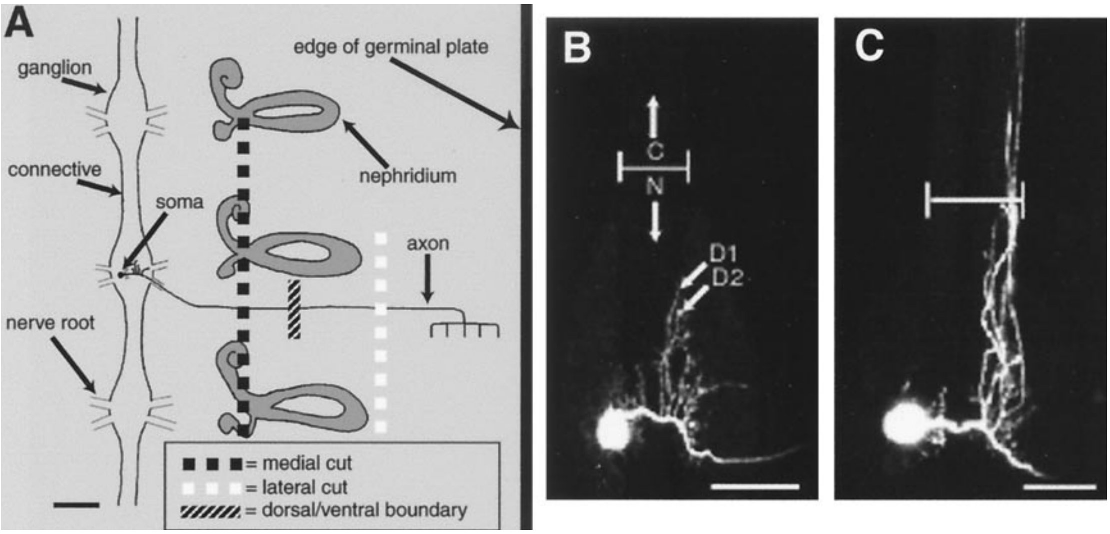

FIG. 6. Effects on the development of cell 3, an eMNd, of peripheral cuts that isolate the peripheral axon from its normal target field (adapted from [32]). (A) Diagram of cut locations in these studies. Longitudinal cuts were made through the germinal plate in either of the two locations indicated by dashed lines. As long as they were made in embryos, cuts caused central processes of cell 3 to extend into connectives. Equivalent cuts in adults left the central arborization of cell 3 unaffected. Extensions following lateral cuts were frequently retracted, whereas extensions following the more medial cuts typically were maintained into adulthood. (B) Structure of a normal cell 3 at 60% of development. (C) Structure of an extended cell 3. A longitudinal cut was made in the lateral position [see (A)] at 54% embryonic development. Embryo was filled, fixed, and viewed at 60% (2 days later). The anterior margin of the ganglion is indicated in (B) and (C) by an "H"-shaped line; Scale bars: 50 μm.

find chemical connections in embryos that would be considered inappropriate in light of known adult circuitry.

The experiments in the last two sections lead to three fundamental conclusions: (a) electrical synapses are rearranged during the development of this simple circuit; (b) the pattern of transient electrical synapses cannot be entirely explained based on the pattern of future chemical synapses, and (c) "inappropriate" connections are unusual, particularly in the formation of chemical connections. These observations suggest that synaptic rearrangements can serve functions other than those that have so far been hypothesized in vertebrate development. We suggest that they may subserve a developmental function, such as navigation around "guidepost" cells as mentioned in the previous section, or equally possibly the production of transient embryonic behaviors. They might in addition be analagous to the connections formed by thalamic neurons that make transient synapses onto cortical subplate neurons before continuing on to connect with neurons in cortical layers [66].

PERIPHERAL EFFECTS ON NEURONAL DIFFERENTIATION

Previously, we showed that Retzius neurons, motor-like neurons with the largest somata in leech segmental ganglia, as well as true motor neurons [1] depend critically upon the nature of their peripheral contact to determine their central morphology and the nature of their contacts with other neurons [19,20]. In most segments, Retzius neurons branch extensively among muscle fibers in the body wall, onto which they release serotonin, which modulates the amplitude and rate of muscle contraction [53]. In midbody segments 5 and 6, however, the peripheral branches of Retzius neurons encounter the primordia of the reproductive ducts—male in segment 5 and female in segment 6. This contact causes them to pull in some of their central processes [50], to make a new kind of acetylcholine receptor [40], and to accept contacts with different types of interneurons than those accepted by Retzius neurons in the other 19 midbody segments [51]. If the peripheral axons of Retzius neurons in segments 5 and 6 never contact embryonic reproductive ducts, they develop like "standard" Retzius neurons. Remarkably, even if their contact with the reproductive tissue is brief—certainly less than 12 h—all of the changes are permanent: these Retzius neurons have all the morphological, physiological, and connectivity properties of "reproductive" Retzius neurons for the entire life of the leech.

To see whether a similar plasticity was evident in the local bend circuitry, we examined the effect of removing a motor neuron's contact with its peripheral target. We focused on two motor neurons: cell 3, an excitor of the dorsal longitudinal muscles, and cell 1, an inhibitor of the same muscles (Fig. 3). At different locations in the periphery and in embryos of several different stages, we cut the nerve containing the growing axon of cell 3. (So far, this surgery has been performed at stages after the motor neurons have reached the muscles, but before functional neuromuscular junctions have formed.) This surgery produced dramatic overgrowth of cell 3's central dendritic branches (Fig. 6). Dendrites that normally are contained within the central neuropil grow into the interganglionic connectives and enter peripheral nerves that they normally never do [32]. Interestingly, cell 1, the inhibitor of longitudinal muscles, shows no central overgrowth in response to the same loss of peripheral targets. It may be significant that the excitatory motor neurons make chemical synaptic contacts only onto muscle fibers in the periphery, whereas the inhibitory motor neurons make chemical synapses both onto peripheral muscle fibers and onto motor neurons and interneurons within the CNS [6,60]. Hence, the overgrowth seen in the central process of only the excitors may take place because their entire chemical innervation has been disrupted. In contrast, loss of the peripheral axon leaves an inhibitory motor neuron with extensive chemical connections within the CNS. (As discussed in section 2B, the excitors have extensive

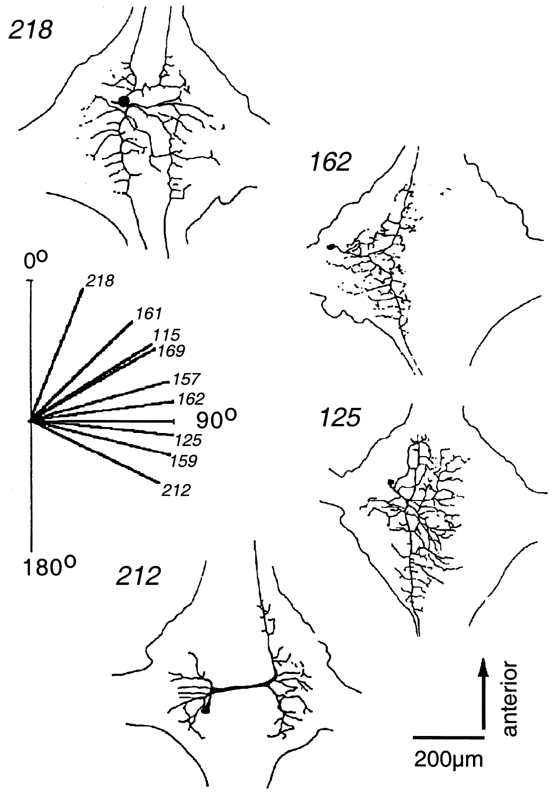

FIG. 7. Morphology of four local bend interneurons (LBIs) [49] compared with receptive field tuning for the 9 identified LBIs in one half of a ganglion [45]. The polar plot shows the center of the receptive field for each of the 9 different LBIs on one side of each segment. Each of the illustrated LBIs is shown near the position on the polar plot that represents the peak of its tuning curve in space. 0° on the plot is the dorsal midline, 180° is the ventral midline, and 90° is the lateral edge of the leech. Numbers identifying each LBI are in italics. Approximate outlines of the ganglion are indicated for each neuron.

electrical contacts with other motor neurons centrally; these contacts apparently do not keep their central processes from overgrowing their normal bounds.) These morphological changes were transient if the surgery was performed relatively early and in a location away from the ganglion. However, if the surgery was performed in later embryos or close to the ganglion, the extended central branches failed to retract and were instead retained into adulthood.

We wondered whether the extensive morphological changes were associated with changes in the establishment of electrical or chemical connections among the neurons. For instance, the processes of cell 3 that are most affected (i.e., the first two major branches on the side contralateral to the soma) are the ones know to be the only branches that receive input from the inhibitory motor neurons ([52; F. J. Eisenhart, unpublished data]). In preliminary experiments, the results are surprising: there appears to be no difference in either the pattern of electrical contacts made by the excitor (as judged by the motor neurons into which neurobiotin diffuses after its injection into cell 3) nor in the connection from the inhibitor onto the excitor (as measured by paired intracellular recordings). There might be some differences in the strengths of the connections or in the time course of their establishment, but the excitors make qualitatively correct connections despite very large changes in the extent of their central processes. Hence, it appears that the initial formation of synaptic contact of the motor neurons—unlike the case for the Retzius neurons—does *not* depend upon the establishment of appropriate peripheral contacts. This has been found, also, for the neuronal network controlling the leech heart: its motor pattern develops even after the heart tube is removed [31], so leech motor systems appear to develop normal function in the absence of target contact.

THE FUTURE: DEVELOPMENT OF THE SENSORY CONNECTIONS

The amount of plasticity in the formation of neuronal circuits appears to be extremely variable. For instance, the mammalian visual and somatosensory systems appear to depend heavily upon neuronal activity to establish their orderly arrangement of connections [36], whereas the targeting of motor neurons to their appropriate muscles seems more inflexible [14,15]. Locomotor patterns also seem to develop in many systems through several stages without apparent input from sensory or motor feedback [54,57,70]. However, this sensory vs motor dichotomy may be one of degree rather than absolute. For instance, basic mammalian cortical circuits appear to form even when part or all of the brain has been synaptically silenced [10,78], and although motor neurons find their proper target with great reliability, electrical activity at the neuromuscular junction is required for establishing the strength and number of terminals [11,75]. The amount of plasticity is independent of phylogeny, because both activity-dependent and activity-independent mechanisms are found in both vertebrates and invertebrates [56]. The nature of the mechanisms used to make synaptic connections appear to depend most heavily on the nature of the circuitry that is being established. It may be, for instance, that connecting motor neurons to muscles as part of fast reflexive pathways requires very specific point-to-point connectivity, suggesting that highly specific, highly controlled molecular mechanisms might be used to make these *cell-to-cell* connections. In other parts of the nervous system, a regular ordering of the projection from one area of the brain to another may be more important than ensuring that the details of the projections are precise. The ability to localize the source of a visual, auditory, or tactile object, for instance, depends upon "population coding" across neurons with overlapping receptive fields [23,28,44,72]. For such areas of the brain, competition that depends upon activity of the network is an efficient way to establish order in making *group-to-group* connections, in the same way that mammalian visual systems depend upon activity to form functional circuits [36].

The circuitry underlying the local bend reflex of the leech is based on population coding [45–47]. Each local bend neuron has a receptive field that covers about half of the circumference of one segment; its responsiveness is maximal at the center of its receptive field and falls off symmetrically on both sides (Fig. 3). Every P cell excites every LBI, and every LBI excites every MN, but the synaptic strengths vary in an orderly way that can be described as a cosine function:

$$W_i = W_{max}\cos\,(R_{pre} - R_{post})$$

in which W_i is the strength of a given synaptic connection, W_{max} is the maximum synaptic strength, R_{pre} is the center of the receptive field of the presynaptic neuron and R_{post} is the center of the receptive field of the postsynaptic neuron. This relationship is precisely the connection rule predicted by Salinas and Abbott [64] for neuronal circuits that use a population code to perform vector calculations to determine the direction of a movement. Examples are the circuits in mammals that responsible for eye movements

[72] and arm movements [23], as well as circuits underlying directional responses to threatening stimuli in insects [28,44]. This kind of population coding may, in fact, be quite general: the way to produce fine control of sensory discriminations and motor movements is to rely on overlapping connectivity of many broadly tuned neurons producing an output—sensory or motor—that depends upon the integrated activity in the whole population. Because the neuronal system for leech local bending is relatively simple, and because most of its elements are identified, we should be able to determine the cellular mechanisms by which this circuit is established embryonically.

If the leech nervous system were laid out in as orderly a fashion as indicated in Fig. 3, the developmental rule for constructing the patterned connectivity between hierarchical layers could be simple: grow toward the next layer and make strong connections with the first cell you encounter there, then compete with your neighbors for contact onto all the other neurons. In a synaptic layer built using this rule, the strengths of the connections would fall off with the distance from the receptive receptive field and might be well described by a cosine function. The physical arrangement of synapse-forming branches of leech neurons (Fig. 6) gives some credence to this possibility. For instance, the LBIs which receive their strongest synaptic input from both P cells on one side (e.g., cells 125 and 162) have a largely ipsilateral dendritic arbor, whereas those that receive their strongest inputs from P cells on both sides of the ganglion (e.g., cells 212 and 218) have a bilateral dendritic arbor. Appropriate connections could arise if the P cells and LBIs simply follow the same guidance cues. The P cell processes could then, at an appropriate time, connect to the nearest LBI process. Such a mechanism cannot, however, explain other features of the connectivity pattern. For instance, the two P cells on one side of the ganglion have very different receptive field centers (mid-dorsal and mid-ventral), and they make connections of very different strengths to each LBI even though their dendritic arbors and synaptic varicosities cover essentially identical areas of the neuropil [55]. It seems more likely that guidance cues direct the major processes to the proper area for establishing synaptic contacts and that activity-dependent mechanisms determine the strengths of contacts actually formed. If so, removing one of the sensory neurons or interneurons in this circuit should lead to a rearrangement, a filling-in, of synaptic contacts onto the next level. It will be interesting to see whether this system uses very different mechanisms at different levels: a fixed cell-to-cell strategy in making connections among motor neurons, and a more plastic group-to-group strategy at the sensory end of the circuit. This kind of information should give us insights into the logic of forming neuronal circuits, and, at the same time, help us to understand more about their functional organization.

SUMMARY

The brain is certainly the most complex organ in the body of most animals, so it should be no surprise that the mechanisms used to construct a functional brain during embryogenesis prove to be complex. Studying highly complex systems usually requires simplifications, either by focusing on only part of the whole system or by identifying a more tractable system that is qualitatively similar to the complex one. Taking this second approach, we hope to understand the developmental processes required for generating complex neuronal systems by looking at the development of several well-characterized circuits in the central nervous system of the leech. We have started by studying the development of the circuitry underlying the leech's simplest evoked behavior—the local bend response. The local bend has been shown in adults to depend on the pattern of activity in a population of neurons, making it a good model for many directed behaviors in higher animals. Such activity patterns that encode behavioral output in a "population code" seem to be similar in the adults of many phyla. We therefore expect the developmental mechanisms that establish connections in the local bend circuit to be similar to the development of connections among neurons that produce population codes in other species in which the process is harder to study. Minimally we expect our studies to generate techniques and approaches that will facilitate the study of development more complex nervous systems, ones that function more like our own.

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