From Synapses to Behavior: Development of a Sensory-Motor Circuit in the Leech

Antonia Marin-Burgin, William B. Kristan Jr., Kathleen A. French

Section of Neurobiology, Division of Biological Sciences, University of California, San Diego, La Jolla, California 92093

Received 14 March 2007; revised 30 May 2007; accepted 7 June 2007

ABSTRACT: **The development of neuronal circuits has been advanced greatly by the use of imaging techniques that reveal the activity of neurons during the period when they are constructing synapses and forming circuits. This review focuses on experiments performed in leech embryos to characterize the development of a neuronal circuit that produces a simple segmental behavior called "local bending." The experiments combined electrophysiology, anatomy, and FRET-based voltage-sensitive dyes (VSDs). The VSDs offered two major advantages in these experiments: they allowed us to record simultaneously the activity of many neurons, and unlike other imaging techniques, they revealed inhibition as well as excitation. The results indicated that connections within the circuit are formed in a predictable sequence: initially neurons in the circuit are connected by electrical synapses, forming a network that itself generates an embryonic behavior and prefigures the adult circuit; later chemical synapses, including inhibitory connections, appear, "sculpting" the circuit to generate a different, mature behavior. In this developmental process, some of the electrical connections are completely replaced by chemical synapses, others are maintained into adulthood, and still others persist and share their targets with chemical synaptic connections.** Develop Neurobiol 68: 779–787, 2008

Keywords: **FRET-voltage-sensitive dyes; imaging; invertebrate; dye-coupling; networks**

INTRODUCTION

During embryonic development, neurons form connections that ultimately create circuits that generate behaviors. In some instances these connections form in a temporal sequence that reflects the hierarchical organization of the finished circuit, with early connections shaping, or even prefiguring, the formation of connections that appear later. As neuronal connections form, many embryos generate behaviors, even before circuits are mature (Branchereau et al., 2000; Bekoff, 2001; Suster and Bate, 2002), and the type and strength of connections among neurons change over time (Cohen-Cory, 2002), suggesting that the nervous system becomes a functional network before it reaches its developmental endpoint.

Different approaches have been taken to investigate how neuronal circuits are established during development. In any case, however, understanding this process is greatly aided by detailed knowledge about how the circuit functions in mature animals, and as a result, most of the studies in vertebrates have focused on the development of sensory systems, particularly the visual system where the topographic maps are well understood (Katz and Shatz, 1996), or on motor systems such as the ventral spinal cord (Nishimaru and Kudo, 2000; Drapeau et al., 2002; O'Donovan et al., 2005) or the respiratory system (Viemari et al., 2003), from which a motor output can be recorded. In these studies of developing vertebrate neuronal

Correspondence to: A. Marin-Burgin (aburgin@ucsd.edu).

Contract grant sponsor: NIH; contract grant numbers: NS35336, MH43396.

Contract grant sponsor: Fundacion Antorchas.

Published online 28 March 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/dneu.20551

circuits, imaging techniques have been extremely useful (Momose-Sato et al., 2001; Niell and Smith, 2005; O'Donovan et al., 2005). They have revealed the acquisition of different patterns of activity among populations of neurons, as well as suggesting roles that different types of neurons play in generating those activity patterns.

Despite this progress, it has proven difficult to study the development of vertebrate circuits at the level of connections between individual cells, in part because a detailed understanding of many mature circuits in vertebrates is still in the future. There are, however, a few animals in which specific neuronal circuits are well understood at the level of each participating identified neuron and its connections (Marder and Calabrese, 1996; Kristan et al., 2005; Marder et al., 2005; Katz, 2007), and some laboratories are beginning to look closely at how those circuits are established during development (Casasnovas and Meyrand, 1995; Fenelon et al., 1999; Le Feuvre et al., 1999; Kristan et al., 2000; Marin-Burgin et al., 2005, 2006).

We have focused this article on experiments performed in our laboratory to elucidate the development of a simple neuronal circuit that produces a segmental behavior—local bending (LB)—in the leech. A detailed description of the adult circuit is available at the level of single neurons and their connections (Lockery and Kristan, 1990; Lewis and Kristan, 1998), providing a solid foundation for a study of how it develops. LB behavior first appears at about 59–60% of embryonic development (ED) [Fig. 1(a)] and is preceded by an embryonic version of the behavior called circumferential indentation (CI). CI consists of a ring of contraction around a single segment of the animal body in response to moderate touch delivered anywhere around the segment. Initially, CI appears and disappears spontaneously, but $\sim$1 day later it can be evoked by moderate touch to the skin (Reynolds et al., 1998; Marin-Burgin et al., 2006). Over the course of two more days of development, CI is gradually replaced by LB (Reynolds et al., 1998; Marin-Burgin et al., 2005), which persists into adulthood. LB is evoked by the same mechanical stimulation as CI, but unlike CI it is directed [Fig. 1(a)]: longitudinal muscles near the site touched contract, while those opposite the site of the touch relax, causing the segment to bend away from the site of the touch (Kristan, 1982).

The neuronal circuit that produces LB in each midbody segment is completely contained within the resident midbody ganglion (Kristan, 1982; Lockery and Kristan, 1990; Lewis and Kristan, 1998). LB is produced by a three-layered neuronal circuit: four mechanosensory neurons [Fig. 1(b), P cells, in green] excite 17 LB interneurons [Fig. 1(b), LBI, in red], which excite $\sim$16 pairs of inhibitory and excitatory longitudinal motor neurons [Fig. 1(b), in blue]. The only known lateral connections in the circuit are inhibitory and are made among the motor neurons (Ort et al., 1974), and the motor neurons make both electrical and chemical synapses with one another. The somata of neurons in this circuit occupy characteristic locations [Fig. 1(c)], facilitating their identification, even in embryos.

We have combined electrophysiology, anatomy, and imaging to characterize the assembly of this neuronal circuit. Our experiments revealed that the circuit is formed first by electrical synapses at the stage when touching an embryo generates CI (Eisenhart, 2000; Marin-Burgin et al., 2005, 2006). Chemical synapses, including inhibitory connections, appear later in development, modifying this circuit that initially includes only electrical connections. Adultlike LB behavior arises at about the stage when inhibitory chemical synapses can first be detected physiologically or with imaging. In this developmental process, some of the electrical connections are completely replaced by chemical synapses, whereas others are maintained, and still others share their targets with chemical synaptic connections.

DEVELOPMENT OF LB CIRCUIT

Development of Electrical Synapses

Because leech neurons can be identified by the location of their cell bodies and the morphology of their major processes, we have been able to follow the development of electrical coupling among neurons in the leech LB circuit. In embryos at different stages of development, we injected Neurobiotin, a small molecule that crosses gap junctions and reveals functional electrical connections in leech (Fan et al., 2005). The experiments showed: (i) electrical connectivity was established between identified neurons of the LB circuit in very specific patterns at different developmental stages (Marin-Burgin et al., 2006); (ii) embryonic electrical connections predicted connectivity between partner neurons in adults, although in some cases a pair of neurons lost their electrical connection and replaced it with a chemical synapse; (iii) the connections were established between layers of the circuit, i.e. sensory cells connected to identified interneurons, interneurons to motor neurons, and motor neurons mostly with other motor neurons (Reynolds et al., 1998; Marin-Burgin et al., 2006); and (iv) motor neurons were first dye-coupled among themselves, and sensory neurons and interneurons began to be dye-coupled with other neurons at least a day later. For

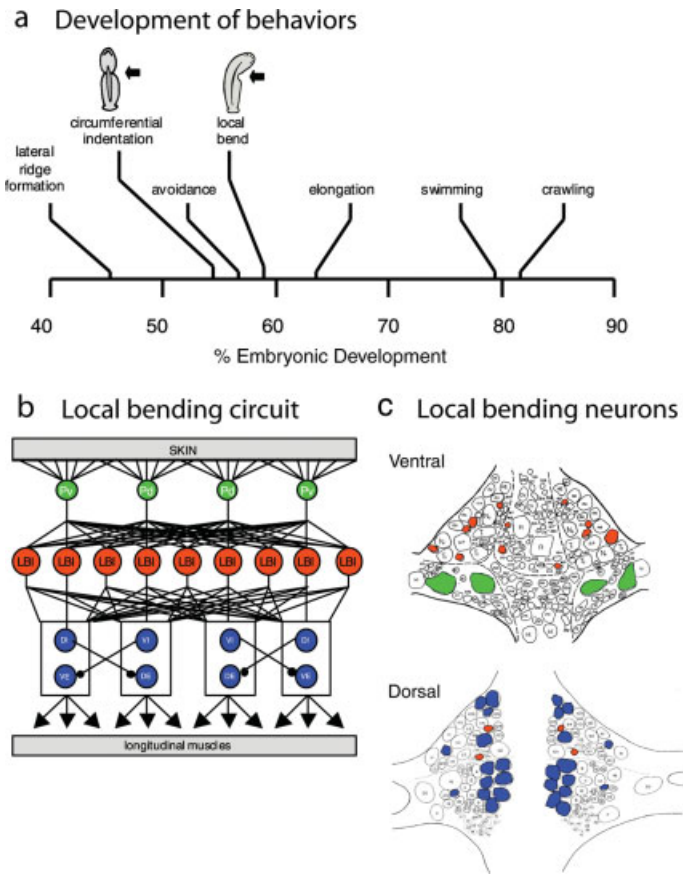

Figure 1 Development of behaviors and the local bending circuit of the leech. (a) Time line for development of behavior in leech (*Hirudo medicinalis*) embryos. Developmental progress is expressed as a percentage of total embryonic development (%ED); at room temperature development from egg deposition to hatching takes 30 days, so each day corresponds to roughly 3% ED. Drawings illustrate the transition from circumferential indentation (CI) to local bending (LB); arrow represents moderate touch in the midbody of the animal, which generates CI early and LB later. (b) Diagram of the neuronal circuit in a single midbody segment that produces the longitudinal component of local bending in that segment. The cross-section of the body wall is represented at the top and bottom of the diagram as though the segment has been cut longitudinally along the ventral midline and spread out. Four P mechanosensory neurons (P cells, in green) innervate the ventral (Pv) and dorsal (Pd) regions on each side of the body wall and make excitatory chemical synapses onto 17 identified local bending interneurons (LBI; only 9 LBIs are shown, and they are in red). LBIs make excitatory chemical synapses onto excitatory (dorsal excitatory, DE; and ventral excitatory, VE) and inhibitory (DI and VI) motor neurons (in blue), all of which innervate longitudinal muscle fibers in the segment. The only known inhibitory synaptic connections in the circuit (represented by black circles at the end of the lines connecting the neurons) are made between motor neurons: inhibitory motor neurons synapse onto muscles in the periphery, but they also make inhibitory chemical synaptic connections onto excitatory motor neurons that project to the same muscle. Excitation of a particular site of the skin excites one or two particular P cells. That P cell excites some of the LBI that will cause activation of excitatory motor neurons innervating that side. In addition, those LBI also activate inhibitory motor neurons on the contralateral side. As a result, the longitudinal muscles ipsilateral to the touched skin contract and the ones contralateral relax, producing the LB. (c) Standard maps of the ventral and dorsal surfaces of a midbody ganglion of the leech (Adapted from Muller et al., 1981) showing where the somata of the neurons that compose the local bending circuit are located (Lockery and Kristan, 1990). Colors indicate the same classes of neurons in Panels b and c. (Modified from Marin-Burgin et al., J. Comp. Physiol. 2006, 192, 123–133, Springer. Reproduced by permission).

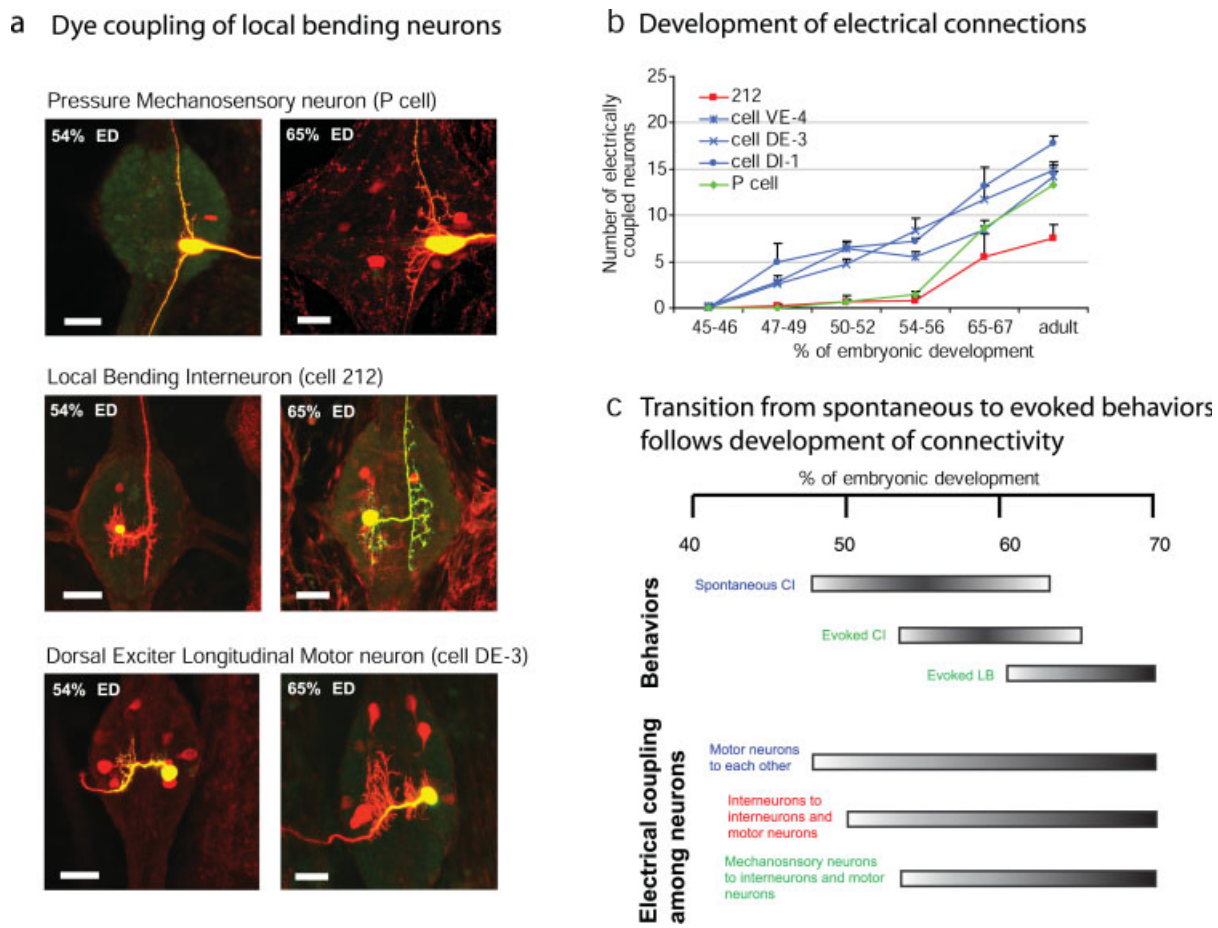

Figure 2 Development of connections in the local bending circuit. (a) Confocal images of mid-body ganglia from embryos in which a pressure-sensitive mechanosensory neuron (P cell), an LBI (cell 212), or a motor neuron (cell DE-3) was injected with both Alexa Fluor 488 (green) and Neurobiotin. Injections were made in embryos at either 54% ED or 65% ED. Neurobiotin was visualized with Steptavidin-Cy3 (red) after fixation. The injected neurons are yellow due to the overlap of green and red images. Neurons electrically coupled to the cell that was injected are red because Neurobiotin crosses gap junctions, but Alexa Fluor 488 does not. Scale bars: 100 μm. The processes from the injected neuron appeared red in most cases because Neurobiotin has a stronger signal (due to amplification in the post-injection histological processing) compared with Alexa Fluor 488. (b) Number of neurons labeled with Neurobiotin when a single neuron was filled with Neurobiotin/Alexa Fluor 488 (i.e., neurons that were dye-coupled to the injected neuron) at a variety of developmental stages. Data for P cells are shown in green, for interneurons 212 in red, and for motor neurons DI-1, DE-3, and VE-4 in blue. Values are mean $\pm$ SEM (modified from (Marin-Burgin et al., 2006)). (c) Correlation between the onset of connections in the local bending circuit and the first appearance of two behaviors, circumferential indentation and local bending. Electrical connections arise first among motor neurons. A day or two later mechanosensory neurons and interneurons also become electrically connected with other neurons. When only motor neurons are electrically coupled, embryos produce spontaneous CI, but no behavior is elicited in response to touch. Later, when sensory neurons and interneurons form electrical connections, touch elicits CI. Even later, touch elicits LB. (Figure modified from Marin-Burgin et al., J. Comp. Physiol. 2006, 192, 123–133, © Springer, reproduced by permission.).

example, at 54%ED sensory cells or interneurons showed little or no coupling with other cells, but motor neurons were coupled to a whole network of neurons [Fig. 2(a,b)].

The specificity in the spatial and temporal dynamics of constructing the connections correlates very well with the observed development of behaviors in embryos. We have found electrical connections

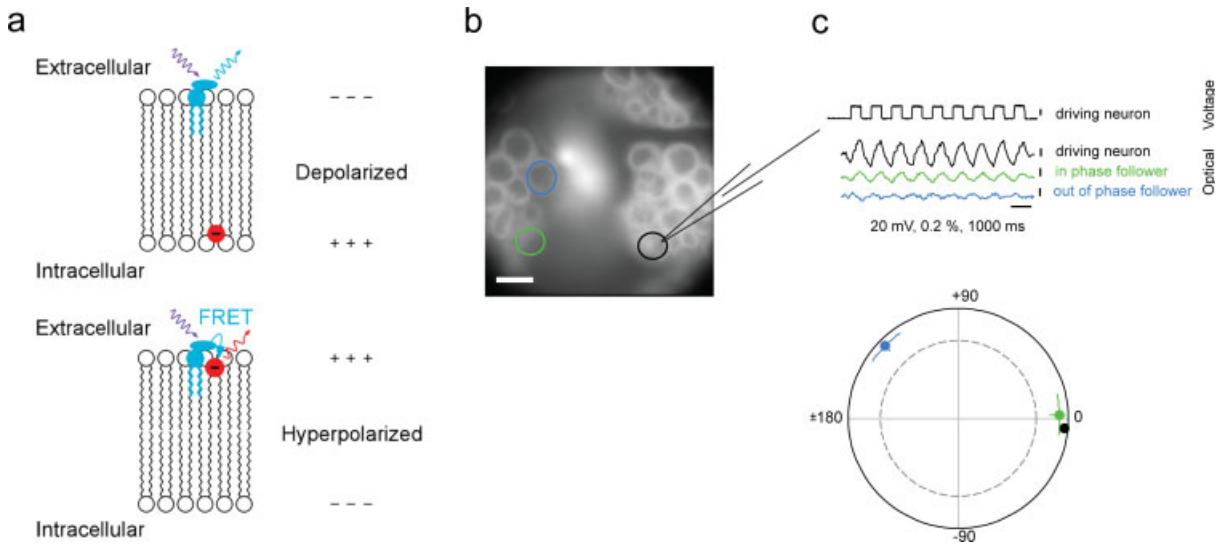

Figure 3 FRET voltage sensitive dyes to study connectivity. (a) Cartoon of the mechanism for recording the transmembrane voltage using fluorescence resonance energy transfer (FRET) from coumarin to oxonol. An impermeant amphiphilic coumarin conjugate (blue) is anchored in the outer leaflet of the cell membrane and is directly excited by the incident illumination. A negatively charged oxonol molecule (red) shuttles back and forth across the hydrophobic core of the membrane and is distributed between the outer and inner leaflets of the membrane according to the transmembrane potential. (b) This image shows the dorsal surface of a leech midbody ganglion stained with coumarin and oxonol. Scale bar, 100 μm. The average change in fluorescence recorded for the three outlined cells (black, green, and blue) is shown in part c. (c) Intracellular recording from the cell outlined in black in part b is shown at the top. The corresponding change in fluorescence is shown in black for that cell, and in green and blue for the other two cells outlined in part b. The polar plot at the bottom of the panel shows the phases of the optical signals with respect to the intracellular signal recorded from the cell outlined in black.

beginning at around 50%ED, and chemical inhibitory synapses (see Fig. 3) and excitatory synapses (K.L. Todd, personal communication) appeared 1–3 days later, suggesting that the earliest spontaneous behaviors that depend on muscle contraction coordinated by longitudinal motor neurons—such as spontaneous CI—are generated by an electrically coupled motor network that acts in the absence of sensory input [Fig. 2(b)]. Two days later, Neurobiotin injections show that sensory neurons and interneurons have become coupled with other neurons and are thus added into the circuit. At this stage, the same behaviors that initially were produced spontaneously can also be evoked by mechanical stimulation [Fig. 2(c)].

Finally, the pattern of early electrical connections among embryonic neurons of the LB circuit reflect the functional connections that have been characterized physiologically among neurons of the LB circuit in adults (Ort et al., 1974; Lockery and Kristan, 1990), suggesting that these early electrical connections prefigure a circuit within which appropriate chemical connections ultimately form.

The Use of FRET Voltage Sensitive Dyes

To record the activity of neuronal populations simultaneously, we have used FRET-voltage sensitive dyes. This technique combines two fluorophores [Fig. 3(a)]. An impermeant amphiphilic coumarin conjugate [blue molecule in Fig. 3(a)] binds to the outer leaflet of the cell membrane and is directly excited by the incident illumination. A negatively charged oxonol molecule [red molecule in Fig. 3(a)] shuttles back and forth across the hydrophobic core of the membrane and is distributed between the outer and inner leaflets of the membrane according to the transmembrane potential. The absorption spectrum of coumarin has minimal overlap with that of oxonol, so there is negligible direct excitation of the oxonol. On the other hand, the emission spectrum of the coumarin has substantial overlap with the absorption spectrum of the oxonol, so the coumarin efficiently transfers energy to nearby oxonol molecules on the outer leaflet via fluorescence resonance energy transfer (FRET). Changes in membrane potential are inferred from changes in the intensity of fluorescence emitted

by the directly excited coumarin and/or by the resonantly excited oxonol. Applying either molecule to desheathed leech ganglia produces strong staining of essentially all somata [Fig. 3(b)].

We use phase-sensitive detection to identify the postsynaptic followers of a driven neuron. We inject repeated square-wave currents into an identified neuron and then calculate the coherence between the transmembrane potential of the driven neuron and the optical records of all of the neurons in the field of view. A large coherence value indicates that a neuron is following the driven neuron. The phase of the coherence determines the sign of the connection, i.e., $\Delta\varphi \approx 0^\circ$ for excitatory connections and $\Delta\varphi \approx 180^\circ$ for inhibitory connections [polar plot in Fig. 3(c)]. A major advantage of using voltage-sensitive dyes, rather than the Ca^{2+}-sensitive dyes that have been used very effectively in many studies, is that the FRET voltage-sensitive dyes allow us to detect inhibitory responses, as well as excitation, in follower cells when we drive a particular neuron. Figure 3(c) shows an example of such a recording. In this experiment an intracellular electrode was used to drive the cell outlined in black [Fig. 3(b)]. The corresponding voltage and optical responses from the driven cell are shown in black. In addition, fluorescence changes in two other cells are also shown. One of the cells followed in phase with the driven neuron [Fig. 3(c), green trace] and the other cell also followed, but out of phase with the driven neuron [Fig. 3(c), blue trace]. A polar plot shows the phases of the follower neurons with respect to the driven neuron; in this example one cell is excited by the driven neuron (green) and the other is inhibited by it (blue).

This technique has been successfully used to track neuronal circuits in adult leeches (Cacciatore et al., 1999; Taylor et al., 2003; Briggman et al., 2005; Briggman and Kristan, 2006), as well as to study the development of connections in embryonic leeches (Marin-Burgin et al., 2005). Some of the experiments performed during development are described in the next section.

Development of Chemical Synapses

In adult LB behavior mechanical stimulation of one side of the animal evokes contraction of the side that was touched and relaxation of the contralateral side; the relaxation is produced by inhibitory motor neurons that inhibit both the contralateral muscles and the excitatory motor neurons controlling those muscles. It thus seemed that the switch from CI to LB must depend on the development of inhibitory chemical synapses. Simultaneous intracellular recordings from inhibitory motor neuron DI-1 and excitatory motor neuron DE-3 in embryos revealed that inhibitory postsynaptic potentials first appeared at about the same time as the switch from CI to LB (Marin-Burgin et al., 2005). In addition, the recordings showed that this pair of motor neurons were first connected by an electrical synapse that was gradually replaced by a chemical inhibitory one [Fig. 4(a)]. To observe this process of chemical synaptogenesis in an entire ganglion, instead of recording from just two neurons at a time, we used FRET voltage sensitive dyes (Cacciatore et al., 1999) to visualize, at single-cell resolution, the generation of chemical inhibitory synapses during development. Using the dyes, we imaged the activity of all neurons with somata on one surface of a midbody ganglion from either an embryonic or an adult leech while stimulating an individual neuron intracellularly. Figure 4(b) shows an experiment in which FRET voltage sensitive dyes were bath-applied to a midbody ganglion, and the activity of all visible cells was imaged simultaneously. An inhibitory motor neuron (dorsal inhibitor 1, cell DI-1) was driven by injecting depolarizing pulses at 1 Hz. At 58% ED, all of the neurons that significantly responded to the stimulated cell (colored in the image of the ganglion) were in phase with the stimulated cell [Fig 4(b), polar plot], indicating that all affected neurons were excited by the stimulated neuron, rather than inhibited. Approximately 3 days later, when cell DI-1was stimulated using the same protocol, the activity of some follower cells was in phase with the driven cell DI-1 (colored in green), whereas other cells responded out of phase (colored in blue). This pattern is similar to what we see in adults, and it indicates that some cells were excited (possibly via electrical synapses), and some were inhibited by the driven cell DI-1 (via chemical inhibitory synapses).

The development of physiologically active inhibitory synapses among motor neurons correlates temporally with the switch from CI to LB in behavior, and applying bicuculline methiodide (BMI, a GABA antagonist) to semi-intact preparations of juvenile leeches switches the response to touch from LB back to CI (Marin-Burgin et al., 2005).

We interpret the results summarized in Figures 2 and 4 to indicate that the LB circuit begins as a set of electrically coupled cells that transiently produce the CI behavior in embryos; these same neurons then produce the mature form of LB after inhibitory chemical synapses form.

COMPARISON WITH OTHER SYSTEMS

Although some aspects of the developing LB circuit may be unique properties of the leech nervous sys-

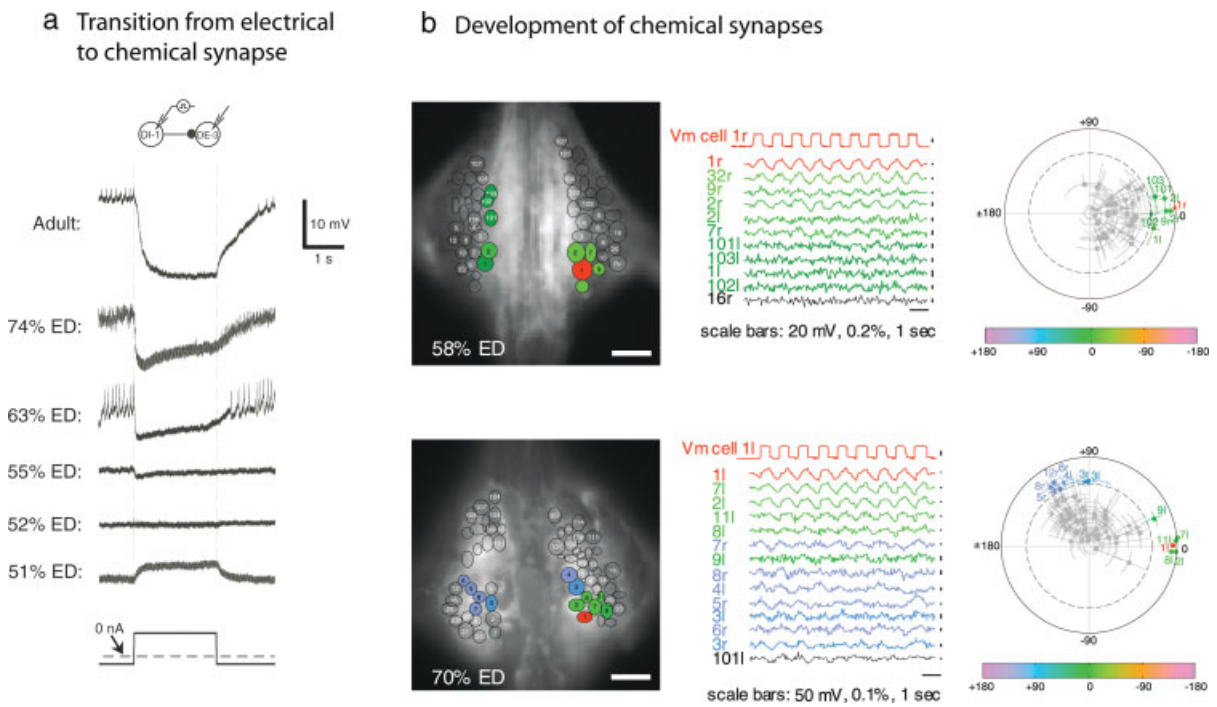

Figure 4 Development of chemical synapses. (a) Intracellular recordings from an excitatory motor neuron (cell DE-3) in response to stimulating an inhibitory motor neuron (cell DI-1) with current pulses of 2–4 nA. Recordings were made in a variety of developmental stages and in adults. (Recordings are from different individuals.) Electrical coupling (at 51% ED) gives way to chemical inhibitory transmission by 55% ED. At 52% ED the lack of a response in DE-3 could reflect the loss of the previous electrical connection, or alternatively it could indicate balanced electrical and inhibitory chemical connections. (b) FRET voltage-sensitive dye recordings of motor neuron activity. Left: Dorsal surface of midbody ganglia in which neuronal activity was recorded using voltage-sensitive dyes (see Cacciatore et al., 1999 for details). Hand-drawn ellipses indicate the boundaries of neuronal somata. An inhibitory motor neuron (cell DI-1, in red) was impaled and depolarized; neurons whose activity was significantly coherent with the driven cell are colored green (indicating that they were in phase with the driven cell) or blue (indicating that they were out of phase with the driven cell). Results from two developmental stages are shown; adult responses did not differ from those at 70% ED. Scale bars: 50 μm. Middle: Records of the electrical and fluorescence signals from the most coherent neurons shown in the images in the left panel. Colors correspond to phasing of the responses: green records were near 0° phase relative to the stimulated cell (that is, they depolarized when the driven cell was depolarized), and blue records were at 90–150° (i.e., they hyperpolarized when the driven cell was depolarized). Black records indicate non-coherent neurons. Scale bars are displayed below and to the right of the records. Signals are lined up in the order of their coherence values, with the maximum value at the top. In all cases, stimuli were depolarizations lasting 500 msec and delivered at 1 Hz at an intensity that varied with stage: 0.3 nA at 58% ED, 0.8 nA at 70% ED. Right: Polar plots show the coherence phase (the angle from 0°) and magnitude (distance from the center of the plot) of the responses of all neurons observed in the image. Values greater outside the dashed line are coherent at the 95% confidence level with the stimulus. (Figure modified from Marin-Burgin et al., J Neurosci 2005, 25, 2478–2489 ©, Society for neuroscience, reproduced by permission.)

tem, the sequence of events that we have observed during its construction may well be general mechanisms that can be found in many other systems. For example, the development of locomotion in zebrafish also is based on a transition from electrical to chemical transmission that produces a change in behavior (Drapeau et al., 2002). In fact, a growing body of morphological and physiological evidence indicates that electrical synapses may be broadly distributed in both juvenile and adult vertebrate brains (Bennett, 2000a,b) and spinal cords (Kiehn and Tresch, 2002).

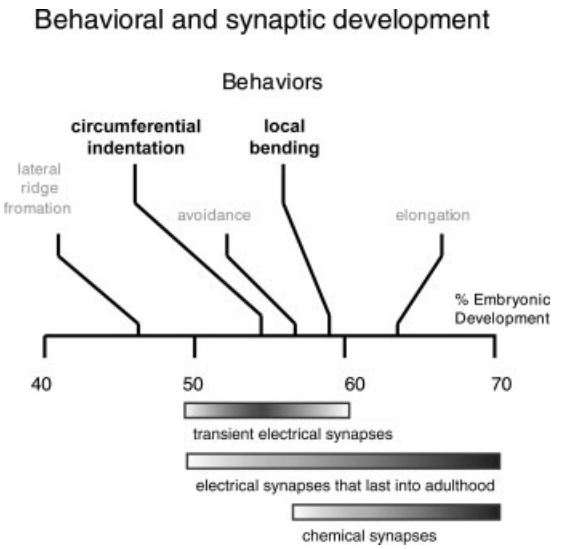

Figure 5 Connectivity among neurons is reflected in the behaviors expressed during development. Time line for development of behavior in embryos compared with the times of onset and expansion of electrical and chemical synapses. Developmental progression is given as a percentage of total embryonic development (%ED, Reynolds, 1998).

Although electrical synapses are known to persist into adulthood, the electrical synapses in embryos may perform different functions than they do in adults. Before chemical synapses are established, embryonic circuits may rely on electrical transmission to coordinate activity, as for example in generating waves of activity in the embryonic retina (Roerig and Feller, 2000), organizing motor output in the spinal cord (Tresch and Kiehn, 2000), or even determining the functional identity of neurons (Borodinsky et al., 2004). Because electrical activity may be crucial in fine-tuning the connectivity within neuronal circuits, this orchestration of activity mediated by electrical connections could exert a powerful influence on the final form of circuitry (Katz and Shatz, 1996). In addition, the transfer of small molecules that readily pass through gap junctions may provide chemical, as well as electrical, coordination of the developing neurons.

SUMMARY

The results presented here depended heavily on the unique features of FRET-based voltage-sensitive dyes, which allowed us to record simultaneously from all of the neurons on one surface of an embryonic ganglion and which reported both excitatory and inhibitory responses in those neurons when a single neuron was driven electrically. These experiments, combined with the results of pairwise electrophysiological recordings and Neurobiotin injections, revealed several rules for how the neuronal circuit that produces LB behavior in the leech is constructed during development (see Fig. 5): (1) all connections between the neurons in the circuit begin as electrical synapses; these electrical connections form first among motor neurons, followed by those connecting sensory neurons to interneurons and interneurons to motor neurons; the connections establish a very specific pattern that prefigures the functional connectivity of the mature LB circuit; (2) chemical synapses arise later in development, and their appearance causes the embryo to switch from one behavior to another (i.e., from CI to LB); (3) transient (or "inappropriate," when compared with adult connections) electrical connections disappear at around the same time that chemical connections became established.

The experimental tractability of the leech nervous system during its entire development has permitted this detailed description in the leech of a general pattern of synaptogenesis that has been hypothesized in many other animals with more complex nervous systems. On the basis of our understanding of the sequence of events as the circuitry is constructed, we can now use molecular techniques, such as RNAi technology (Dykes et al., 2004; Todd et al., 2005) to test directly the hypothesis that electrical connections are a necessary first step in chemical synaptogenesis.

Authors thank Dr. J. Glover for his comments on the manuscript.

REFERENCES

Bekoff A. 2001. Spontaneous embryonic motility: An enduring legacy. Int J Dev Neurosci 19:155–160.

Bennett MVL. 2000a. Electrical synapses, a personal perspective (or history). Brain Res Rev 32:16–28.

Bennett MVL. 2000b. Seeing is relieving: Electrical synapses between visualized neurons. Nat Neurosci 3:7–9.

Borodinsky LN, Root CM, Cronin JA, Sann SB, Gu XN, Spitzer NC. 2004. Activity-dependent homeostatic specification of transmitter expression in embryonic neurons. Nature 429:523–530.

Branchereau P, Morin D, Bonnot A, Ballion B, Chapron J, Viala D. 2000. Development of lumbar rhythmic networks: From embryonic to neonate locomotor-like patterns in the mouse. Brain Res Bull 53:711–718.

Briggman KL, Abarbanel HDI, Kristan WB Jr. 2005. Optical imaging of neuronal populations during decision-making. Science 307:896–901.

Briggman KL, Kristan WB Jr. 2006. Imaging dedicated and multifunctional neural circuits generating distinct behaviors. J Neurosci 26:10925–10933.

Cacciatore TW, Brodfuehrer PD, Gonzalez JE, Jiang T, Adams SR, Tsien RY, Kristan WB, et al. 1999. Identification of neural circuits by imaging coherent electrical activity with FRET-based dyes. Neuron 23:449–459.

Casasnovas B, Meyrand P. 1995. Functional differentiation of adult neural circuits from a single embryonic network. J Neurosci 15:5703–5718.

Cohen-Cory S. 2002. The developing synapse: Construction and modulation of synaptic structures and circuits. Science 298:770–776.

Drapeau P, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Brustein E. 2002. Development of the locomotor network in zebrafish. Prog Neurobiol 68:85–111.

Dykes IM, Freeman FM, Bacon JP, Davies JA. 2004. Molecular basis of gap junctional communication in the CNS of the leech Hirudo medicinalis. J Neurosci 24: 886–894.

Eisenhart FJ. 2000.Development of specific chemical and electrical synapses in the longitudinal motor neuron circuit of the leech. Doctoral Thesis in Neurobiology Section. La Jolla, CA: University of California, San Diego.

Fan RJ, Marin-Burgin A, French KA, Friesen WO. 2005. A dye mixture (Neurobiotin and Alexa 488) reveals extensive dye-coupling among neurons in leeches; physiology confirms the connections. J Comp Physiol A 191:1157–1171.

Fenelon VS, Kilman V, Meyrand P, Marder E. 1999. Sequential developmental acquisition of neuromodulatory inputs to a central pattern-generating network. J Comp Neurol 408:335–351.

Katz LC, Shatz CJ. 1996. Synaptic activity and the construction of cortical circuits. Science 274:1133–1138.

Katz PS. 2007. Evolution and development of neural circuits in invertebrates. Curr Opin Neurobiol 17:59–64.

Kiehn O, Tresch MC. 2002. Gap junctions and motor behavior. Trends Neurosci 25:108–115.

Kristan JWB, Calabrese RL, Friesen WO. 2005. Neuronal control of leech behavior. Prog Neurobiol 76:279–327.

Kristan WB Jr. 1982. Sensory and motor neurons responsible for the local bending response in leeches. J Exp Biol 96:161–180.

Kristan WB Jr, Eisenhart FJ, Johnson LA, French KA. 2000. Development of neuronal circuits and behaviors in the medicinal leech. Brain Res Bull 53:561–570.

Le Feuvre Y, Fenelon VS, Meyrand P. 1999. Central inputs mask multiple adult neural networks within a single embryonic network. Nature 402:660–664.

Lewis JE, Kristan WB Jr. 1998. A neuronal network for computing population vectors in the leech. Nature 391: 76–79.

Lockery SR, Kristan WB Jr. 1990. Distributed processing of sensory information in the leech II. Identification of interneurons contributing to the local bending reflex. J Neurosci 10:1816–1829.

Marder E, Bucher D, Schulz DJ, Taylor AL. 2005. Invertebrate central pattern generation moves along. Curr Biol 15:R685–R699.

Marder E, Calabrese RL. 1996. Principles of rhythmic motor pattern generation. Physiol Rev 76:687–717.

Marin-Burgin A, Eisenhart FJ, Baca MS, Kristan WB Jr, French KA. 2005. Sequential development of electrical and chemical synaptic connections generates a specific behavioral circuit in the leech. J Neurosci 25:2478–2489.

Marin-Burgin A, Eisenhart FJ, Kristan WB, French KA. 2006. Embryonic electrical connections appear to pre-figure a behavioral circuit in the leech CNS. J Comp Physiol A 192:123–133.

Momose-Sato Y, Sato K, Kamino K. 2001. Optical approaches to embryonic development of neural functions in the brainstem. Prog Neurobiol 63:151–197.

Niell CM, Smith SJ. 2005. Functional imaging reveals rapid development of visual response properties in the zebrafish tectum. Neuron 45:941–951.

Nishimaru H, Kudo N. 2000. Formation of the central pattern generator for locomotion in the rat and mouse. Brain Res Bull 53:661–669.

O'Donovan MJ, Bonnot A, Wenner P, Mentis GZ. 2005. Calcium imaging of network function in the developing spinal cord. Cell Calcium 37:443–450.

Ort CA, Kristan WB Jr., Stent GS. 1974. Neuronal control of swimming in the medicinal leech part 2 identification and connections of motor neurons. J Comp Physiol 94:121–154.

Reynolds SA, French KA, Baader A, Kristan WB Jr. 1998. Development of spontaneous and evoked behaviors in the medicinal leech. J Comp Neurol 402:168–180.

Roerig B, Feller MB. 2000. Neurotransmitters and gap junctions in developing neural circuits. Brain Res Rev 32:86–114.

Suster ML, Bate M. 2002. Embryonic assembly of a central pattern generator without sensory input. Nature 416:174–178.

Taylor AL, Cottrell GW, Kleinfeld D, Kristan WB Jr. 2003. Imaging reveals synaptic targets of a swim-terminating neuron in the leech CNS. J Neurosci 23:11402–11410.

Todd K, Nelson S, Kristan WB, French KA. 2005.Injecting siRNA decreases electrical coupling strength between leech neurons. In: Society for Neuroscience Abstract, Atlanta.

Tresch MC, Kiehn O. 2000. Motor coordination without action potentials in the mammalian spinal cord. Nat Neurosci 3:593–599.

Viemari J-C, Burnet H, Bevengut M, Hilaire G. 2003. Perinatal maturation of the mouse respiratory rhythm-generator: in vivo and in vitro studies. Eur J Neurosci 17:1233–1244.