Journal of the Indian Institute of Science
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEWS

Assembling Neural Circuits for Generating Movement

Vatsala Thirumalai

Abstract | Locomotion is essential for survival and animals need to move early in life to protect themselves from predators and to forage. Neural circuits controlling locomotion thus need to be functional even at stages when the nervous system is immature and developing. Studies from a wide variety of experimental model systems show that motor circuits are capable of generating functional output even though the mature adult circuit may not be present. The output from this immature circuit is however qualitatively different and in many ways, inflexible as compared to the adult. Also, network activity may be driven by different sets of mechanisms in the developing animal compared to the adult. For example, at early embryonic stages, network activity can be driven by electrical coupling between neurons, which gradually decreases as the animals mature, giving place to chemical neurotransmission. The electrical activity generated by early networks acts as a regulator that provides instructional cues for developmental events such as the correct outgrowth of axons to their targets and the selection of transmitter molecules used by a neuron. Early networks have homeostatic mechanisms in place to maintain their activity levels within an operating range. Neuromodulators, present in descending and sensory projections and as circulating hormones have profound effects on neural circuit development by affecting multiple developmental processes and by fusing and uncoupling networks to produce appropriate motor patterns.

1 Introduction

The human brain is estimated to consist of about 10^{11} neurons and at least 1000 times as many synapses. Yet, the pattern of connectivity between the astronomical numbers of neurons is precisely regulated over short and long distances. For example, the human spinal cord spans about 450 mm in length but not more than 13 mm in width. Yet, neurons within the spinal cord form specific connections along both the length and width of the spinal cord. When the circuitry is miswired or lost, functional output is affected as may happen, for example, in autism or spinal cord injury. How does the brain wire itself? What are the rules that govern how neurons form synapses with each other?

Circuits that control movement, i.e. motor systems, offer us an appealing model system in which to study these questions. This is primarily because movement is essential for survival and therefore circuits that control the generation of movement mature relatively early. Many animals locomote as soon as they are born. In still others, like mice and humans, locomotion matures during post-natal life but this delay is due to the late development of postural mechanisms that help in supporting body weight; the spinal pattern generators themselves are functional even before birth.[1] Secondly, the output of motor circuits is well-defined, patterned and stereotyped from one individual to another.[2] Thirdly, the electrical output from motor circuits is translated to a pattern

National Centre for Biological Sciences, Bangalore 560065, India.
vatsala@ncbs.res.in

of muscle contraction, and hence movement. When circuitry is perturbed, motor function and consequently locomotor behavior will be affected. Therefore, locomotor behavior can be used as an assay for investigating the functional consequences of perturbing circuitry. Fourthly, the organization of motor systems has been partially established and we now know the connectivity between several brain regions and spinal centres involved in the generation of movement (Fig. 1). This provides us with a preliminary "circuit diagram" to begin to ask how connectivity between these distant regions develops.

Neuromodulators: Substances that act on slower timescales relative to classical neurotransmitters, and may modify many parameters of neural function. Examples are amines like serotonin and peptides like endorphins.

Neocortex: Newly evolved cortex, found only in mammals. Neurons in neocortex are involved in higher cognitive processing. Neocortex consists of the outer layers of the cerebral hemispheres.

2 Overview of Motor Systems in Vertebrates and Invertebrates

Motor circuits have been studied in a wide variety of animal models ranging from the nematode *Caenorhabditis elegans* (*C. elegans*) to primates. Each model system provides its own set of unique advantages and limitations. For example, invertebrate preparations such as the crustacean stomatogastric nervous system (STNS, see box and Fig. 2) and the leech heart beat and swimming circuits are excellent for understanding how individual neurons are connected to form small circuits and how these small circuits operate. However, these preparations are not the preferred choice for classical genetics experiments in which molecular players governing the assembly of neural circuits can be identified. For such experiments, genetic model organisms such as *C. elegans*, *Drosophila*, zebrafish and mice are ideal. Circuit analysis in *C. elegans* and *Drosophila* was difficult if not impossible as their small neurons posed a challenge for electrophysiologists. However, with the advent of calcium and voltage sensing dyes and proteins, such experiments have become feasible.[3–5] With the latest optogenetic tools and imaging methods, it is now possible to perform circuit dissection and analyses in invertebrate as well as vertebrate preparations and understand the implications for motor behavior.

Interneurons: 'Inter'—between; neurons that are not primary sensory or motor in function but receive inputs from and send outputs to other neurons.

Optogenetics: Collection of techniques by which light-activated ion channel proteins are expressed in neurons and light is used to either switch on or off electrical activity in these neurons with high temporal precision.

Ipsilateral projections: Where the axon descends on the same side as the neuronal cell body.

What have we learnt from studying motor circuits in these diverse model organisms? Several key concepts have emerged:

1. Rhythmic motor behaviors such as walking, flying, swimming, chewing etc. are driven by oscillatory circuits that generate rhythmic electrical output even in the absence of rhythmic sensory or descending inputs. In other words, such circuits are intrinsically rhythmic and these are called central pattern generators[2] (CPGs).
2. Sensory and feed-forward inputs can significantly alter the phasing and periodicity of CPG output and thus make the motor pattern adaptable to environmental and internal needs.[6]
3. Sensory and descending projections not only release classical fast neurotransmitters but also slower-acting neuromodulators that can modify the motor pattern over short and long timescales.[7]
4. Descending projections to central pattern generators are many and varied: in mammals, their origin ranges from the neocortex to the hindbrain with thousands of projection neurons in each region. Lower vertebrates have fewer descending projections than mammals that originate from the more caudal regions of the brain[8] (Fig.1).
5. Descending projections may relay signals for motor pattern selection, initiation, termination and intensity of locomotion.[9]
6. The pattern can be generated by motor neurons themselves such as in the STNS, or it can be generated by a layer of premotor interneurons such as in the spinal cord. However, even in this case, the motor neurons can act as rhythm generators during embryonic and neonatal development.[10,11] Whether motor neurons are part of the rhythm generator in the adult spinal cord remains to be investigated.
7. In vertebrates, the CPGs for motor pattern generation are present in ventral spinal cord. Inhibitory interneurons, whose axons cross the midline, mediate left-right alternation. Midline crossing excitatory interneurons are also present and may mediate bilateral synchrony as in hopping. The pattern generator for hindlimb movement is in the lumbar spinal cord and that for forelimbs is in the cervical cord. Ipsilateral, longitudinally projecting interneurons mediate flexor-extensor alternation.[12]

In the ensuing article I will discuss recent key findings that throw light on how vertebrate and invertebrate motor circuits are assembled and how they function throughout development.

3 Gap Junctions in Developing Motor Circuits

Gap junctions are cytoplasmic continuities between two cells mediated by assemblies of proteins: connexins and pannexins in vertebrates and innexins in invertebrates. Six connexin or innexin molecules associate to form one half of the gap junction channel or 'hemi-channel'. The coming together of two hemi-channels forms a functional gap junction. Thousands of such assemblies form gap

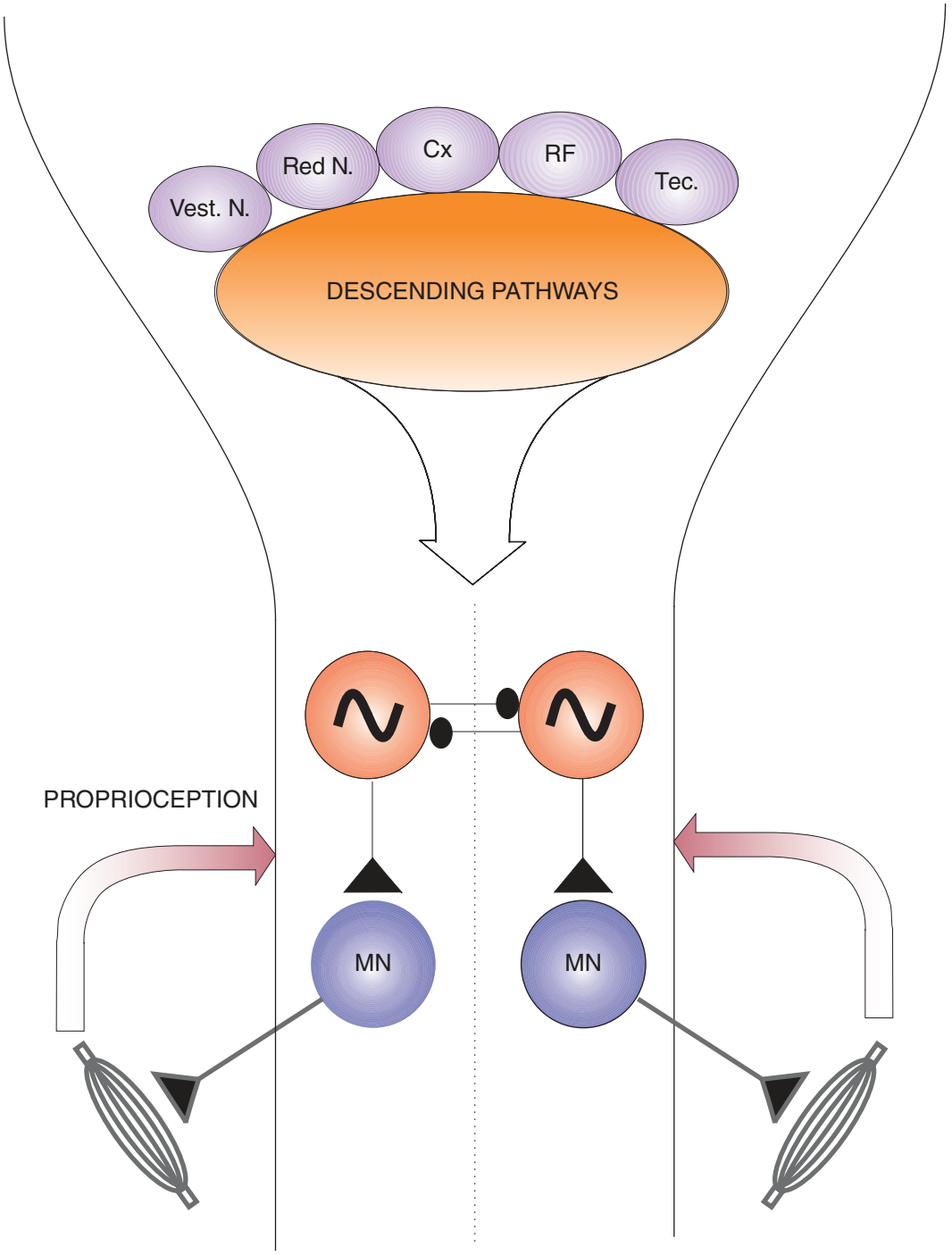

Figure 1: Abstract schematic of the organization of the adult vertebrate motor system. Movement is generated by the contraction of skeletal muscles. Motor neurons in the spinal cord send electrical input to muscles and drive co-ordinated muscle contraction. Proprioceptive feedback from the muscles is fed back into the spinal cord via the dorsal roots. The central pattern generators in each hemi-cord, denoted by '~', generate rhythmic activity and drive motor neurons rhythmically. Motor neurons are part of the CPG in developing vertebrates but their role in rhythm generation in adult networks needs to be explored (see text). Alternation between the left and right hemi-cords is driven by reciprocal inhibition mediated by commissural interneurons. Descending pathways control various aspects of CPG activity including initiation and termination of activity, speed of locomotion etc. In lower aquatic vertebrates, the reticular formation (RF) provides the bulk of the descending input. In mammals, five distinct pathways originating from the motor cortex (Cx, corticospinal), tectum (Tec, tectospinal), vestibular nucleus (Vest. N., vestibulospinal), red nucleus (Red N., rubrospinal) and the reticular formation (RF, reticulospinal) innervate the spinal cord from the brain.

Box 1 The Stomatogastric Nervous System (STNS)

The STNS of crustaceans is a network that controls the rhythmic movements of muscles in the foregut to enable swallowing, grinding and filtering of food. The crustacean foregut consists of an anterior oesophagus followed by a much broader cardiac sac, a gastric mill region and a pylorus (Fig. 2A). Food is swallowed by peristaltic movements of the oesophagus and then stored in the cardiac sac. The cardiac sac also serves as a mixing chamber where food is mixed with digestive enzymes. There are three teeth in the gastric mill region, two lateral and one medial. By moving the teeth in various patterns against each other, food is macerated and then sent on to the pylorus. Here, a set of comb-like filters sort the chewed food such that well-processed food is passed on to the midgut, but larger particles are retained in the stomach for further mastication. The processing of food in the stomach of crustaceans is thus a complicated but coordinated set of movements of various regions of the stomach, mediated by striated muscles on the stomach walls. The STNS generates the motor patterns that drive these rhythmic movements of stomach muscles. The STNS consists of four ganglia, the oesophageal (OG), the paired commissural (CoG) and the stomatogastric (STG). The OG houses the neurons that generate the oesophageal motor pattern. The OG and the paired CoGs send modulatory projections to the STG via the stomatogastric nerve (stn). The neurons that collectively generate the gastric mill and pyloric motor patterns are housed in the STG (Fig. 2B).

The pyloric network that generates the pyloric motor pattern is perhaps the best studied central pattern generating circuit today. Why is this network so desirable for the study of circuit dynamics and circuit development? Firstly, the wiring diagram of the pyloric network is known (Fig. 2C). This means that intuitive word models of how the circuit operates can be made, although our knowledge in this regard is not complete yet. Secondly, the same neurons can be recorded in different individuals. Thus, measurements can be made in identified neurons whose place in a circuit is known (Fig. 2D). Thirdly, the pyloric network is richly modulated by substances released by the projections of OG and CoG neurons. This offers an opportunity to study the effects of neuromodulation not only at the circuit output level, but also at the single neuron and synapse level. Fourthly, the pattern, though stereotypical, is still plastic. Several forms of long-term and short-term dynamics in intrinsic and synaptic conductances have been identified. The pattern itself shows homeostatic plasticity by recovering rhythmicity several hours after modulatory inputs are removed. Lastly, as a developmental model system, it offers the advantage of being rhythmically active at a very young embryonic stage. This makes it possible to follow the function of the network through much of development and study how the neurons and synapses change with the growing stomach and changing dietary needs of the animal. However, the single greatest disadvantage of the STNS for developmental studies is the extraordinarily long developmental time. It takes roughly 6–7 years for a lobster to grow from a zygote to an adult weighing about a pound. Another disadvantage is the extremely small size of the embryonic STNS and muscles, but a few heroic attempts recording activity from these tiny structures do exist (see text). The advent of calcium imaging in the STNS could see a surge in studies of development using this powerful model system.

Further Reading:

1. Harris-Warrick E by RM, Marder E, Selverston AI and Moulins M(1992) Dynamic Biological Networks: The Stomatogastric Nervous System. MIT Press, Cambridge, MA.
2. Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu. Rev. Physiol. 69:291–316.

junctional plaques at electrical synapses.[13] Electrical synapses have virtually no delay in transmission, unlike chemical synapses wherein the release, and binding of neurotransmitters, and the final gating of receptor channels involve significant delay. Because of the continuity of pre-synaptic and post-synaptic cytoplasm, current flow across the electrical synapse is instantaneous and usually bi-directional, although many electrical synapses show rectification, in which current flow is preferentially in one direction.[14]

3.1 *Gap junctions in the developing spinal cord*

One of the hallmarks of developing nervous systems is the extensive gap junctional connectivity between neurons. In the spinal cord, motor neurons innervating the same muscle are electrically

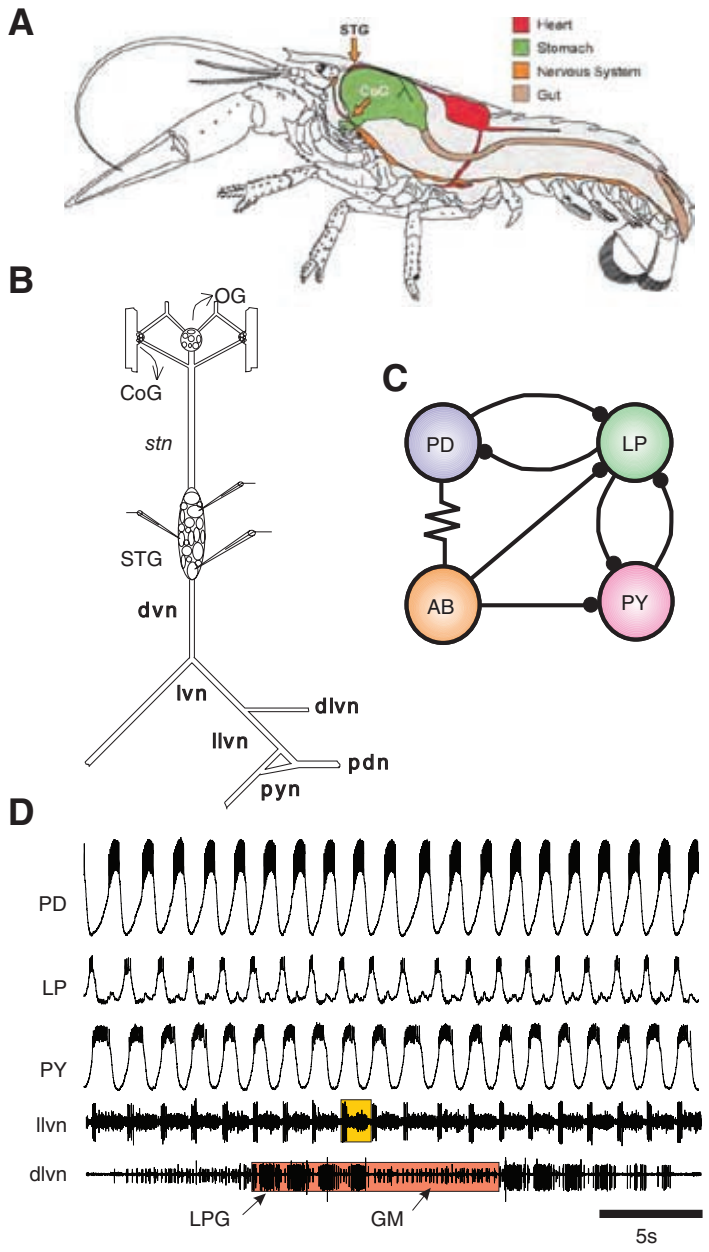

Figure 2: The stomatogastric nervous system of decapod crustaceans. A. Lateral view of a mid-sagittal section of the North Atlantic lobster *Homarus americanus*. The STNS sits on top of the stomach and controls movements of stomach muscles. Reproduced from[91] with permission. B. The STNS can be dissected free from the stomach and placed in vitro for several days. When the commissural ganglia (CoGs) and the oesophageal ganglion are present and connected to the stomatogastric ganglion (STG) via the stomatogastric nerve (*stn*), rhythmic activity can be recorded from the motor nerves posterior to the STG. Activity can also be recorded intracellularly from the motor neuron somata in the STG. Adapted from[74] with permission. C. Circuit diagram of the pyloric network. The Anterior Burster (AB) interneuron and the Pyloric Dilator (PD) motor neurons make up the pacemaker kernel of the pyloric CPG. The Lateral Pyloric (LP) and Pyloric (PY) motor neurons are followers. All chemical synaptic connections are inhibitory and shown with lines ending in filled circles. Resistor symbol indicates electrical synaptic connection. Adapted from[74] with permission. D. Pyloric and Gastric mill motor patterns. Intracellular recordings from the PD, LP and PY neurons show the triphasic pyloric motor pattern. The triphasic pattern is also seen in the extracellular nerve recording from the *llvn* nerve that carries the axons of the PD, LP and PY neurons. The gastric mill motor pattern can be seen from the rhythmic firing of the LPG and GM motor neurons whose axons are present within the *dlvn* nerve. Note the vastly different periodicities of the pyloric and gastric mill motor patterns. Reproduced from[92].

coupled to each other.[15] Such electrical coupling is thought to synchronize the firing of these motor neurons.[16,17] Mice that lack Connexin 40 (Cx40-/-) show reduced gap junctional coupling between motor neurons. The motor neurons also exhibit decreased temporal correlation in firing.[18] In contrast to adult animals in which each muscle fiber is innervated by only one motor neuron, neonatal muscle fibers are multiply innervated. In mice, by the second week of post-natal life, motor neuron terminals that elicit weak end plate potentials are lost while those that cause strong end plate potentials are maintained. This process gradually removes all poly-innervation in a classic process of synapse elimination and each muscle fiber retains terminals from only one motor neuron.[19] Interestingly, synapse elimination occurs during a time window when gap junctional connectivity between motor neurons also decreases dramatically,[15] suggesting a role for electrical coupling in maintaining poly-innervation. Consistent with this notion, in Cx40-/- mice, the synapse elimination process is accelerated and muscle fibers become singly innervated significantly earlier in life.[18] This suggests that gap junctional coupling between motor neurons enhances correlated firing between them. Such correlated activity may delay synaptic competition at the neuromuscular junction.

End plate potential: Voltage response due to binding of neurotransmitter to its receptor on the membrane of muscle fibers.

Pre-motor interneurons can also be electrically coupled and contribute to the generation of a co-ordinated rhythm. Interneurons located in the ventromedial border of the spinal cord express the homeobox transcription factor Hb9 as do motor neurons. The Hb9 interneurons (Hb9 IN) are excitatory neurons that release glutamate and their axons do not cross the midline (ipsilateral). Among other targets, Hb9 INs project to motor neurons and are co-active during locomotor-like activity. During early stages of development, Hb9 INs are electrically coupled to each other and continue to be coupled even during the second week of post natal life. Although there is a high degree of variability in the strength of coupling between Hb9 INs, action potential firing between coupled neurons is highly correlated serving to excite target motor neurons synchronously.[20]

Contralateral projection: Where the axon crosses the midline of the nervous system and projects on the side opposite to that of the cell body.

Transcription factor: A protein that recognizes specific DNA sequences, binds to them and regulates synthesis of RNA from DNA.

3.2 *Gap junctions can lay down the ground plan for future circuits*

The functional implications of perturbing gap junctional connectivity during development were elegantly demonstrated in the medicinal leech *Hirudo verbana*. Leeches respond to touch by bending their body wall away from the stimulus—this behavior is called local bending (LB) and is specific to the segment being touched. Muscles near the segment that is touched contract while those on the opposite side relax leading to a bend in the body wall. The circuit that generates LB has been worked out in the adult and thus provides a platform to study circuit development. There are four pressure-sensitive or P neurons in each mid body segment innervating the four quadrants of the body wall. These then excite a set of LB interneurons (LBI), which in turn excite a set of about 16 inhibitory and excitatory motor neurons (Note: as opposed to vertebrates which receive only excitatory input, many invertebrate muscle fibers receive both excitatory and inhibitory inputs). The inhibitory motor neurons inhibit excitatory motor neurons as well as their muscle targets. When one side of the body wall is touched, one or two P cells innervating that region respond to the mechanical stimulus and excite their downstream LBIs. The LBIs in turn excite excitatory motor neurons that innervate longitudinal muscles ipsilateral to the stimulus. This causes contraction of the touched side. In addition, LBIs also excite inhibitory motor neurons innervating longitudinal muscles contralateral to the side that was touched leading to relaxation of those muscles.[21,22]

The LB behavior begins as a spontaneous contraction of all longitudinal muscles in a segment leading to circumferential indentation (CI) of the body wall. At about 53% embryonic development (ED), the CI behavior is evoked by mechanical stimulus and at 60% ED, the CI is replaced by LB. This sequence of spontaneous CI followed by evoked CI and then evoked LB behaviors maps onto a neural circuit in which motor neurons are electrically coupled at first, then sensory neurons and interneurons are added to the circuit and finally the emergence of inhibitory synapses within the circuit switches the behavior from CI to a lateralized LB behavior. Thus the early ground plan for an LB circuit seems to be laid down purely through electrical synapses. Many of these electrical synapses are maintained in the adult while a few are replaced by chemical synapses.[23] Does this latter class of electrical synapses act as a placeholder for future chemical synapses? In a recent study,[24] double stranded RNA complementary to the gap junction protein innexin1 (ds-inx1) was injected into a single P-cell at around 50% ED, a stage when chemical synapses have not yet formed. This led to a reduction in gap junctional coupling between P-cells and its partners. Nearly a month after the ds-inx1 injection, when the dsRNA's effects had waned and electrical connectivity had returned to normal levels, the chemical synapses formed by the injected P-cell

onto its post-synaptic partners were still considerably weaker compared to sham-injected P-cells. This suggests that interfering with gap junctions at a time when they are needed to specify chemical synapses can lead to long-lasting defects in synaptic function. Further, in the ds-inx1 injected leeches, local bending behavior was rarely observed suggesting that a normal LB circuit had failed to form when electrical coupling was transiently disrupted during a critical period when chemical synapses usually form. This study stresses the importance of electrical synapses in providing instructive cues for the formation of future chemical synapses. How electrical synapses might prefigure chemical synapses is the million-dollar question that needs to be answered in the future.

4 The Role of Activity in Motor Circuit Assembly

Spontaneous electrical activity is a prominent feature of many developing neural circuits including the spinal cord, hippocampus and retina.[25] During the last part of the previous century, it was widely held that molecular pathways laid down the fundamental ground plan for the network and that electrical activity acted at much later stages in refining those connections. But recent work has blown away this claim by demonstrating that early developmental events such as neuronal proliferation, axon pathfinding and neurotransmitter phenotype can also be affected by electrical activity.[26] The electrical activity seen at these early stages is different in origin and kinetics from that seen at later stages.

4.1 *Spontaneous neural activity prior to synapse formation*

Soon after neural tube closure, spontaneous activity is seen in spinal neurons in the form of brief bursts of calcium called 'calcium spikes'.[27] Calcium spikes last only a few seconds and regulate several key events during the assembly of motor circuits including neuronal proliferation, migration, axon pathfinding, neurotransmitter specification, and neurotransmitter receptor specification.[28] This early form of activity is seen even before synaptic connections are made and arises from calcium activity in single neurons. Calcium spikes may be generated via transient receptor potential channels, calcium release from intracellular stores or metabotropic neurotransmitter receptors.[26] Calcium spikes may also be induced by morphogens present in the developing spinal cord. It is well known that the morphogen Sonic Hedgehog (Shh) is secreted from the notochord and floor plate and induces ventral cell identities in the developing spinal cord. A gradient of Shh signaling from ventral to dorsal spinal cord creates domains of neural progenitors distinguished by the transcription factors they express. Each progenitor domain then gives rise to multiple neuronal types.[29] Recently a role for calcium spiking in mediating the actions of Shh has become evident. Belgacem and Borodinsky[30] show that Shh binding to its receptor, *smoothened* (Smo) activates a signaling cascade that leads to increased calcium spike activity in ventral neurons of the *Xenopus* spinal cord soon after neural tube closure. Blockade of Shh signaling or of the calcium spikes leads to fewer GABAergic neurons, presumably due to altered differentiation of neural progenitors, as the total number of neural progenitors or neurons is not affected. These results show that the calcium-spike activity induced by Shh is required for Shh-mediated patterning of the spinal cord.

Calcium spikes are thought to specify neuronal subtypes through their frequency. For example, Rohon-Beard sensory neurons, motor neurons, and ventral interneurons all have distinct calcium spike frequency signatures. Rohon-Beard neurons have a low calcium spike frequency and use glutamate as their neurotransmitter. Motor neurons have low spiking activity initially but the frequency increases with development and they use acetylcholine as their transmitter. Interfering with the frequency of calcium transients leads to abnormal specification of neurotransmitter phenotype.[31] A developmental perturbation of calcium transients not only affects the specification of neurotransmitter, but also changes the post-synaptic receptor.[32]

Calcium spikes are cell-autonomous and thus are not at all or only weakly correlated across cell populations. Warp et al.[33] recently showed that in zebrafish embryos, at 18 hours post fertilization (hpf), calcium transients occur in islands of neurons in the spinal cord. Such activity was not synchronized across neurons. However, between 18hpf and 21hpf, clusters of neurons started to fire together and these domains slowly coalesced together to form a correlated ipsilateral network by 22hpf. When the uncorrelated calcium transients at 18hpf were silenced for an hour using optogenetic tools, the activity failed to mature to the correlated pattern seen at 22hpf, indicating that the early uncorrelated calcium spike activity is essential for normal circuit development.

4.2 *Spontaneous activity arising from network interactions*

In the study described above, the authors also show that applying gap junction blockers at 18hpf had no effect on the calcium transients as would

Morphogen: A signaling molecule that uses its local concentration or activity to trigger diverse developmental pathways or fates.

be expected of activity that is generated in single neurons autonomously. However, the same procedure blocked all activity at 22hpf showing that the correlated activity generated at 22hpf emerges from an electrically coupled network. Also, at 22hpf, the ipsi- and contralateral sides became anticorrelated, giving rise to alternating bursts of activity. How does the alternation between the ipsi and contralateral halves arise? Presumably there is crossed inhibition that causes one side to be active and the other silent. However, the canonical inhibitory neurotransmitters GABA and glycine are not inhibitory but depolarizing at these early developmental stages due to high intracellular chloride.[34] Yet, modeling studies show that such depolarizing GABA/glycine inputs can still be inhibitory by shunting adjacent excitatory inputs if both arrive within a short time window and the chloride reversal potential is below the action potential threshold.[35]

Reversal Potential: The reversal potential of an ion is the membrane potential at which there is no net current flow across the membrane.

Spontaneous network activity is also present in the embryonic chick and mouse spinal cords. Chick embryos at E3–4 (embryonic day 3 to 4) and mouse embryos at E11.5 to 12.5 display episodes of spontaneous depolarization when suction electrodes are applied to lumbar muscle nerves.[10,36–39] These episodes consist of bursts of action potentials occurring at about 2-minute intervals and are initially synchronous across the right and left sides. At these early stages, the episodes are generated by a predominantly cholinergic drive that is supplemented by GABA/glycinergic excitation and electrical coupling. Whether, like in zebrafish, there is an earlier form of spontaneous network activity driven purely by electrical coupling is not yet known. Current data demonstrate that both in the chick and in the mouse, chemical transmission is required for the earliest recorded spontaneous activity patterns.[10,36] This activity then matures to a pattern that is driven primarily by glutamatergic and GABAergic inputs at E10 in the chick and at E18 in the mouse.[40,41] Alternation between antagonist motor nerves emerges at this stage, even though GABA and glycine are still excitatory. Another feature of spontaneous network activity is activity dependent network depression—soon after an episode is fired, the excitability of the network is reduced and it is refractory to firing another episode even when electrically stimulated. Two factors are thought to contribute to network depression: one is hyperpolarization of neurons immediately following a burst, which slowly recovers over many tens of seconds. The second factor is depletion of intracellular chloride during an episode, which leads to a substantial decrease in chloride equilibrium potential and hence results in a reduction in amplitude of GABA/glycine synaptic currents soon after an episode than before it. Slow reaccumulation of intracellular chloride via the action of chloride cotransporters restores the network to its original state.[42–44]

5 Spontaneous Activity and Homeostatic Plasticity in Developing Motor Circuits

Spontaneous network activity is a ubiquitous feature of developing neural circuits and has been shown to be vital for the proper assembly of these circuits. When the frequency of spontaneous activity is either reduced or increased using pharmacological intervention, motor neuron axons make more pathfinding errors. Such interventions alter the expression levels of several axon guidance molecules.[45,46] Further, it appears that the depolarization during an episode and not the neurotransmitter signaling *per se* is important for this process. In an elegant set of experiments, chick spinal cords were made to express the light-activated algal protein channelrhodopsin2, so that spontaneous episodes could be triggered by light. In this preparation, when $GABA_A$ receptors were blocked, the frequency of spontaneous activity reduced by 50%. When the frequency was restored to normalcy by 'filling in' light-activated episodes, the pathfinding errors of motor axons were eliminated and the expression levels of axon guidance molecules became normal.[47] This demonstrates that the activity itself, and not the mechanism by which it is generated, is important for axon pathfinding. That activity itself, and not the precise network architecture that generates it, is key to proper circuit assembly, is borne out by the fact that network activity is robust to perturbations that block one or more transmitter systems. In the E3–5 chick spinal cord, when nicotinic acetylcholine and $GABA_A$ receptors are blocked with antagonists, spontaneous activity ceases as one would expect. However, the activity returns within 25 minutes but is now sensitive to atropine, which blocks muscarinic acetylcholine receptors.[36] This shows that when the activity resumes in the presence of nicotinic acetylcholine and $GABA_A$ receptor blockers, it is generated by acetylcholine binding to muscarinic acetylcholine receptors. This phenomenon is also seen in the E10 spinal cord where the rhythm is generated by glutamatergic and GABAergic drive. When NMDA and AMPA type glutamate receptor blockers were applied, spontaneous network activity was blocked completely, but it returned after about an hour and this recovered activity was insensitive to glutamate receptor blockers

implying that the new activity was not generated by glutamatergic drive. Instead, the recovered activity was sensitive to $GABA_A$ receptor blockers indicating that GABAergic drive took over and compensated for the absence of glutamatergic drive.[41] These results suggest that spontaneous network activity is robust and is capable of being generated by multiple mechanisms. Such plasticity that preserves network function is broadly termed 'homeostatic plasticity'.

5.1 *What is 'homeostatic plasticity'?*

Homeostasis derives from the Greek roots 'homoios' meaning similar and 'stasis' meaning 'standing still' and put together, this refers to the maintenance of a state by dynamic regulation of system parameters. In the context of the nervous system homeostasis refers to the ability of a neuron or network to maintain its output by regulating its membrane currents, synaptic strength etc. This ability of the nervous system to change its intrinsic and synaptic conductances so as to generate functional output is termed 'homeostatic plasticity'. Homeostatic plasticity is pervasive – it is present in invertebrates as well as vertebrates, during development and in adulthood, and in the periphery as well as in the central nervous system.[48,49]

In the developing spinal cord, synaptic currents are regulated homeostatically and this may play a role in the maintenance of spontaneous activity when one or more transmitter systems are blocked, as described above. In one study,[50] chick embryos at the E8 stage were treated with the sodium channel blocker lidocaine *in ovo*, for 48 hours. This treatment reduced spontaneous motility as well as network activity in the spinal cord. At the end of the treatment, when lidocaine was rinsed out, the frequency of spontaneous episodes in the isolated cord had increased suggesting increased excitability of the cord. How is the increased episode frequency possible in the face of post-episode depletion of intracellular chloride? The authors show that the amplitudes of $GABA_A$ synaptic currents in motor neurons return to their pre-episode values faster in lidocaine-treated preparations than in control. This suggests that depriving activity for a period of 48 hours causes faster re-accumulation of intracellular chloride perhaps via increased expression or function of chloride cotransporters. The increased chloride accumulation shifts chloride reversal to more depolarized values, and hence increases the amplitude of $GABA_A$ evoked and miniature post-synaptic currents[51] (mPSCs). Further, AMPA receptor mediated mPSCs increase in both amplitude and frequency as a result of reduced spontaneous activity.[50] Although a causal relationship is yet to be proven, it is likely that the increase in AMPA and $GABA_A$ mPSCs is responsible for the increase in observed frequency of spontaneous episodes.

5.2 *Homeostatic plasticity at the neuromuscular junction*

The *Drosophila* larval neuromuscular junction has proved to be immensely useful in understanding processes involved in the homeostatic regulation of synaptic efficacy.[52,53] Using genetic perturbations, when motoneuronal innervation of muscle fibers is decreased, the post-synaptic response per quantum of neurotransmitter (quantal size) is increased thus bringing the total response to normal levels. Note that quantal size changes can be brought about by post-synaptic alterations in receptor numbers or presynaptically by changes in neurotransmitter packaging density in vesicles or size of vesicles. By contrast, when muscle fiber innervation is increased, the presynaptic axon terminal decreases the probability of release of neurotransmitter at each of its boutons.[54] This compensatory decrease in neurotransmitter release at each synaptic contact results in the maintenance of the post-synaptic end plate potential. Perturbations in the opposite direction such as pharmacological blockade of post-synaptic glutamate receptors,[55] mutating the glutamate receptor subunit[56] or reducing muscle membrane excitability,[57] all lead to compensatory increases in the probability of transmitter release. It has been hypothesized that the level to which muscle fiber is maximally depolarized during an end plate potential is the sensor for homeostatic plasticity of this sort.[57] If the muscle fiber is not adequately depolarized to this set point, then a retrograde signal may be released which binds to Eph receptors on the presynaptic terminal. Activation of Eph receptors leads to a signaling cascade, which ultimately causes more calcium to flow into the terminal via voltage-sensitive calcium channels.[58,59] This then increases the probability of release of neurotransmitter to the extent that muscle depolarization is restored to the set point. Although the identity of the retrograde signal is not yet known, these experiments provide insight into how the invertebrate NMJ remains robust to perturbations that disturb its normal function.

Do such mechanisms exist at the vertebrate NMJ? Denervation supersensitivity, wherein a muscle fiber that has lost its motor innervation, inserts more nicotinic acetylcholine receptors (nAChRs) into its membrane, is an example of a homeostatic process at the vertebrate NMJ. The

increased number of nAChRs makes the fiber more sensitive to applied ACh[60] and hence the name denervation supersensitivity.[61] Homeostatic plasticity of the NMJ also occurs in *myasthenia gravis*, an auto-immune disease in which nAChR density is reduced—here also the probability of release increases as a compensation for the reduced receptor number.

6 Neuromodulation of Developing Motor Networks

Developing motor networks may express electrical activity, and even exhibit correct phase relationships between constituent neurons at later stages. But they often lack the flexibility of more mature networks. For instance, developing zebrafish larvae and *Xenopus* tadpoles show spontaneous bursting patterns in their motor neurons.[62,63] However, at early stages, these bursts consist of only single spikes whereas at later stages the bursts have variable number of spikes. The number of spikes in a burst determines the amplitude of muscle contraction in one cycle. Thus, body turns can be achieved by generating bursts that are bilaterally asymmetric. Also, with more cycle to cycle variability in burst durations and cycle periods, the stage 42 tadpoles have a more flexible motor pattern that allows them to adjust muscle contraction amplitudes during swimming. How is such flexibility brought about? One factor is the maturation of the intrinsic and synaptic properties of the motor neurons themselves.[64] Another class of critical regulators is the neuromodulatory environment of motor pattern generating circuits. The arrival of neuromodulatory projections into pattern generating circuits signals the maturation of these circuits in many invertebrate and vertebrate motor systems. In *Xenopus*, serotonergic projections into the spinal cord trigger the conversion of the early immature pattern to the mature pattern seen at stage 42. Ablation of these serotonergic fibers with the monoamine neurotoxin 5,7 dihydroxy tryptamine caused stage 42 tadpoles to generate the immature swimming pattern. The same effect was also seen when stage 42 tadpoles were acutely treated with a serotonin receptor antagonist suggesting that continuous release of serotonin is needed to express the mature swim motor pattern.[65,66] Serotonin is also known to influence various aspects of locomotor development in mammals: in neonatal rats, depletion of serotonin leads to defects in the posture and in the firing behavior of motor neurons while application of serotonin activates rhythmic motor patterns in neonatal rats and mice.[37,67,68]

6.1 *Neuromodulators can influence CPGs even before their projections are present*

In both *Xenopus* and in neonatal mice, exogenous serotonin exerts its actions at stages when the descending serotonergic fibers have yet to invade the spinal networks. This suggests that receptors for serotonin are present even before serotonergic inputs arrive. This phenomenon is also seen in the developing stomatogastric nervous system of decapod crustacea (see box). The stomatogastric ganglion (STG), which houses the motor neurons, is already rhythmically active by 50% embryonic development.[69] However serotonergic projections are not seen in the STG until the second larval stage, by which time the larva is already an independent forager. Does this mean that serotonin does not play a role in the generation of early motor patterns in the STG? On the contrary, exogenous serotonin reduced rhythm frequency and increased duration of bursts in embryonic preparations. Also, when the embryos were incubated with serotonin, serotonergic neurons took up and concentrated it in their nuclei, projection fibers and cell bodies. This loading of serotonin from the bathing medium was blocked when a serotonin transporter blocker was included in the medium. If indeed the developing STG can use serotonin as a "borrowed" transmitter, where is the endogenous source? The STG is situated in an artery and is constantly bathed by hemolymph. Serotonin is present in neurohemal structures such as the pericardial organs even in embryos and when released into the hemolymph, it can be taken up by the STG.[70] Thus, a neuromodulator can be functional even at stages in development when the adult complement of neuromodulatory projections is not yet present.

6.2 *Neuromodulators can repress network output*

While neuromodulators can be enablers for circuit maturation, in some cases they have also been shown to repress network output until later stages. In the stomatogastric nervous system, by 50% embryonic development (E50), neurogenesis in the STG is complete and rhythmic activity can be recorded from STG motor neurons. However, whereas the adult STNS generates four motor patterns with distinct periodicities, the embryonic STNS generates a single rhythm when modulatory inputs from anterior ganglia are left intact.[69] However, when the nerve connecting the STG to anterior ganglia is severed, isolating the embryonic STG from modulatory inputs, multiple motor patterns can be elicited after pharmacological stimulation.[71]

This result raises many questions: firstly, are all descending modulatory inputs present at the embryonic stages? Subsequent studies have shown that this is not the case. Although the projection neurons that innervate the adult STG are present in the embryo, they acquire their neuromodulatory phenotype gradually throughout embryonic and larval development.[72,73] Neurons that contain and release more than one neuromodulator in the adult may acquire these co-transmitters at distinct stages in life. Secondly, do neuromodulators have distinct functions in the embryo and in the adult? Or, are neuromodulators repressive in the adult also? Clearly this is not the case as many of the neuromodulators known to be present in projections to the STG can activate rhythmic activity in the isolated STG of adult crustacean.[7,74] Indeed several neuromodulators have distinct effects on the embryonic and adult STNS motor patterns.[75] The distinct actions could be due to differential distribution of neuromodulator receptors in the embryo, the extensive presence of electrical coupling in the embryonic STNS[76] or because circuit parameters are vastly different between embryo and adult such that the modulated states are different even though the 'unmodulated' states look similar.[77] Having said the above, a few neuromodulators do have similar effects on the embryonic and adult STNS rhythms.[75,78–80]

In light of these studies, it is difficult to propose the simple hypothesis that the embryonic STNS is functionally mature but neuromodulatory inputs suppress the expression of the adult motor pattern. The above studies taken together indicate that the embryonic network resembles the adult network in some aspects but is quite distinct and immature in many others. The combined influence of the neuromodulatory environment, intrinsic and synaptic properties of neurons and the sensory inputs arriving at these stages are likely to determine the immature output pattern of the embryonic STNS.

Neuromodulatory repression of early, active circuits is seen elsewhere also. Zebrafish larvae are quiescent after hatching but swim robustly by 5 days post fertilization (dpf). Corresponding with this behavior, fictive swim motor patterns recorded from 3dpf larvae are infrequent but by 5dpf, episodes of motor bursting are highly frequent. However, the 3dpf larvae were capable of generating spontaneous swim episodes at a high frequency when dopamine receptors were blocked or when dopaminergic inputs were ablated.[62] This suggests that the circuitry for sustaining rhythmic activity at high frequency is present at 3dpf but is inhibited by dopamine. How then is the high frequency of activity unmasked at 5dpf since dopaminergic neurons, projections and receptors are still present at this stage? At 5dpf, endogenously released dopamine is unable to suppress fictive swimming as evidenced by the lack of effect of dopamine reuptake blockers. This demonstrates that although dopamine is released and it binds to its receptors at 5dpf, dopaminergic signaling is insufficient to inhibit the swim circuit.[62] This may be because of other excitatory pathways that are absent at 3dpf and slowly mature by 5dpf.

In the bullfrog *Rana catesbaiana*, the circuit that controls lung breathing in the brain stem is already fully developed in the tadpole, when the animals are still gill-breathing. Brain-stem respiratory circuits are inhibited by GABA acting via $GABA_B$ receptors and are functional only after metamorphosis.[81] The expression of the air-breathing pattern may be due to changes in the efficacy of the $GABA_B$ inhibitory pathways, or due to maturation of excitatory pathways, which lead to a 'developmental disinhibition', similar to what is seen in the zebrafish swim pattern generator.

Metamorphosis: Developmental events that lead to drastic changes in body structure, behavior and habitat of many insects and amphibians.

6.3 *Neuromodulation can reconfigure neural circuits for new functions*

Metamorphosis is a major life event in holometabolous insects and anurans. Neuromodulation plays key roles in reconfiguring neural circuits during metamorphosis in anurans and insects. Holometabolous insects go through four life stages, i.e. the egg, larva, pupa and adult. Motor behaviors characteristic of each life stage are commanded by a nervous system that undergoes profound changes to generate stage-specific behavior. For example, crawling is the mode of locomotion for larvae while adults can walk and fly. How does an insect reconfigure its body to generate these behaviors? Larval muscles are histolyzed and adult muscles are generated during metamorphosis within the pupa. During this period, larval motor neurons retract their axons and dendrites and reinnervate adult muscles as they form. For example, the MN5 motor neuron in the mesothoracic ganglion of *Manduca* participates in slow larval crawling, but after metamorphosis, is transformed into a fast flight motor neuron. To make this possible, MN5 undergoes changes in membrane properties, dendritic architecture and the sub-dendritic targeting of synapses.[82,83] In insects, metamorphosis is co-ordinated by the steroid hormone 20-hydroxyecdysone. This hormone can induce programmed cell death of obsolete motor neurons in *Drosophila*[84] and in *Manduca*.[85]

Fictive motor pattern: Rhythmic motor neuronal electrical activity recorded from isolated nervous systems or from paralyzed preparations. Since in these cases the recorded electrical activity does not result in movement, these patterns are termed fictive.

Anurans undergo metamorphosis from aquatic tadpoles to amphibious adults. This transition

in habitat requires drastic changes in physiology. As we saw earlier, the mode of respiration changes from gill-breathing to lung-breathing. Among other changes, the eyes shift to a more frontal position and binocular vision is acquired. Locomotion by axial swimming is replaced by appendicular swimming and hopping. Tadpoles locomote by contracting the right and left sides of each muscle segment in alternation (axial swimming). But post-metamorphosis, the tail is lost and hopping movements generated by the limbs now take over. It is interesting that while the right and left sides alternate during axial swimming, they are synchronously activated during hopping with alternation between flexors and extensors of the same side.[86] This implies that the neural circuits governing locomotion have to be reorganized to serve the post-metamorphic frog. Unlike in insects where the larvae enter into a quiescent pupal stage in which all reorganization happens, in frogs, all reorganization needs to happen while the animal is still moving about. In late tadpole stages when both the hind limbs and the tail are present, the muscles of the hind limbs are innervated by flexor and extensor motor neurons but their activity is entrained by the axial swim pattern generator. The limb motor neurons are never active independently—flexors and extensors are synchronous on the same side and alternate with the contralateral side. Thus the limb pattern generator at this stage is subservient to the axial swim pattern generator of the tail. By stage 61, about 4 days later, the hind limbs are well-developed and as before, undulatory swimming is seen. However, the limb pattern generator can now be recruited independently of the axial swim generator. Moreover, the rhythm seen in the tail motor neurons is faster than that seen in the limb motor neurons. Additionally, the right and left flexors are now synchronous, alternating with the ipsilateral extensor motor neurons. Thus at this stage, the hind limb pattern generator is segregated from the axial swim pattern generator and resembles closely the mature pattern generator seen in froglets. Yet, at this stage, the limb pattern generator, when it is co-active with the swim pattern generator, can influence the frequency and duration of the swim bursts. Therefore at this stage, the two pattern generators seem to be segregated but still coupled.[87]

How does the spinal motor system drive developmental changes such that the axial swim network is gradually replaced by the limb pattern generator but at intermediate stages, the two circuits operate in a co-ordinated fashion? Pre-metamorphic tadpoles start showing gradual increases in their plasma levels of the thyroid hormones (TH), reaching a peak at about the stage when metamorphosis begins. After metamorphosis, plasma TH levels return to baseline again.[88] This raises the possibility that metamorphosis could be co-ordinated by TH. Several lines of evidence support this hypothesis: blocking TH synthesis blocks metamorphosis and supplying exogenous TH to pre-metamorphic tadpoles induces precocious metamorphosis.[88] Recently, TH was also shown to be critical for the proper innervation of the hind limb muscles. When TH levels were reduced by over-expressing an enzyme that degrades it, the tadpoles were paralyzed, had smaller nervous systems and died at metamorphic climax. Closer analysis revealed that the number of limb motor neurons was reduced and those motor neurons that were present failed to form functional connections with muscle.[89] These results demonstrate that functional TH signaling is required for the proper formation of post-metamorphic circuits in the CNS and in the periphery. The gaseous neurotransmitter nitric oxide (NO) could be one of the agents that mediates the actions of TH on the metamorphosing tadpole locomotor circuitry by exerting divergent effects on neurogenesis, synaptogenesis and cellular and synaptic conductances.[88] It is quite likely that several other neuromodulatory players are also involved,[90] perhaps all co-ordinated by TH. How these multiple neuromodulators result in the developmental reconfiguration of the locomotor network is a fascinating question to answer in the future.

7 Conclusions

In this article I have reviewed recent literature on motor system development. From studies on both invertebrate and vertebrate model systems, several key principles have emerged which are applicable to any developing nervous system.

1. Spontaneous activity is a key feature of developing neural circuits. Such spontaneous activity can be generated in a cell autonomous fashion in the early stages when synapses have not yet formed. This may be followed by a stage in which spontaneous activity is generated by multiple co-ordinated neurons which are connected to each other via gap junctions. At still later stages, spontaneous activity is driven by chemical synapses and finally by means of experience.
2. Spontaneous activity at all of these stages is essential for proper network function at later stages governing key events such as neurotransmitter phenotype acquisition, axon guidance and electrical properties of constituent neurons.

3. Developing networks may have an intrinsic set point that they try to maintain in the face of changes in activity. This homeostasis may help developing networks to stay within an operating range.
4. Gap junctions may not only affect circuit maturation by helping to generate network activity but they may also have a role in the specification of chemical synapses. Whether this process is independent of network activity remains to be seen.
5. Neuromodulators are essential regulators of circuit maturation. In some cases, neuromodulation may repress network function where the network is mature even before its function is needed. In other cases, neuromodulators may enable network maturation by triggering changes in network properties at the appropriate developmental stages.
6. The receptors for many neuromodulators seem to be expressed even before the neuromodulatory projections invade the motor circuit.
7. Neuromodulators can have different effects on the same network at different life stages. This may be due to a temporal regulation of receptor subtype expression.
8. Neuromodulators can fuse two networks together or can segregate a single network into two separate networks to suit the behavioral needs of the animal.

Acknowledgements

Supported by a Wellcome Trust—DBT India Alliance Intermediate fellowship. I wish to thank Prof. Eve Marder, Prof. Michael O'Donovan, Dr. Sanjay Sane, and the members of my lab, all of whose critical feedback was invaluable in making this manuscript better.

Received 20 November 2012.

References

1. Muir, G.D. Early ontogeny of locomotor behaviour: A comparison between altricial and precocial animals. *Brain Res. Bull.* **53**, 719–726 (2000).
2. Marder, E. & Calabrese, R.L. Principles of rhythmic motor pattern generation. *Physiol. Rev.* **76**, 687–717 (1996).
3. Olsen, S.R. & Wilson, R.I. Cracking neural circuits in a tiny brain: New approaches for understanding the neural circuitry of Drosophila. *Trends in Neurosciences* **31**, 512–520 (2008).
4. Looger, L.L. & Griesbeck, O. Genetically encoded neural activity indicators. *Current Opinion in Neurobiology* **22**, 18–23 (2012).
5. Faumont, S., Lindsay, T. & Lockery, S. Neuronal microcircuits for decision making in C. elegans. *Curr. Opin. Neurobiol.* **22**, 580–591 (2012).
6. Marder, E. Moving rhythms. *Nature* **410**, 755–755 (2001).
7. Marder, E. & Thirumalai, V. Cellular, synaptic and network effects of neuromodulation. *Neural Netw* **15**, 479–493 (2002).
8. Goulding, M. Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* **10**, 507–518 (2009).
9. Grillner, S., Wallén, P., Saitoh, K., Kozlov, A. & Robertson, B. Neural bases of goal-directed locomotion in vertebrates—An overview. *Brain Research Reviews* **57**, 2–12 (2008).
10. Hanson, M.G. & Landmesser, L. T. Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. *J. Neurosci.* **23**, 587–600 (2003).
11. O'Donovan, M.J. *et al.* Mechanisms of excitation of spinal networks by stimulation of the ventral roots. *Ann. N. Y. Acad. Sci.* **1198**, 63–71 (2010).
12. Kiehn, O. Development and functional organization of spinal locomotor circuits. *Current Opinion in Neurobiology* **21**, 100–109 (2011).
13. Söhl, G., Maxeiner, S. & Willecke, K. Expression and functions of neuronal gap junctions. *Nature Reviews Neuroscience* **6**, 191–200 (2005).
14. Furshpan, E.J. & Potter, D.D. Mechanism of Nerve-Impulse Transmission at a Crayfish Synapse. *Published online: 17 August 1957; doi:10.1038/180342a0* **180**, 342–343 (1957).
15. Walton, K.D. & Navarrete, R. Postnatal changes in motoneurone electrotonic coupling studied in the in vitro rat lumbar spinal cord. *J. Physiol. (Lond.)* **433**, 283–305 (1991).
16. Zhang, H.-Y., Li, W.-C., Heitler, W.J. & Sillar, K.T. Electrical coupling synchronises spinal motoneuron activity during swimming in hatchling Xenopus tadpoles. *J. Physiol. (Lond.)* **587**, 4455–4466 (2009).
17. Tresch, M.C. & Kiehn, O. Motor coordination without action potentials in the mammalian spinal cord. *Nat. Neurosci.* **3**, 593–599 (2000).
18. Personius, K.E., Chang, Q., Mentis, G.Z., O'Donovan, M.J. & Balice-Gordon, R.J. Reduced gap junctional coupling leads to uncorrelated motor neuron firing and precocious neuromuscular synapse elimination. *Proc. Natl. Acad. Sci. USA.* **104**, 11808–11813 (2007).
19. Colman, H., Nabekura, J. & Lichtman, J.W. Alterations in synaptic strength preceding axon withdrawal. *Science* **275**, 356–361 (1997).
20. Hinckley, C.A. & Ziskind-Conhaim, L. Electrical coupling between locomotor-related excitatory interneurons in the mammalian spinal cord. *J. Neurosci.* **26**, 8477–8483 (2006).
21. Kristan, W.B., Jr, Calabrese, R.L. & Friesen, W.O. Neuronal control of leech behavior. *Prog. Neurobiol.* **76**, 279–327 (2005).

22. Marin-Burgin, A., Kristan, W.B., Jr & French, K.A. From synapses to behavior: Development of a sensory-motor circuit in the leech. *Dev Neurobiol.* **68**, 779–787 (2008).
23. Marin-Burgin, A., Eisenhart, F.J., Kristan, W.B., Jr & French, K.A. Embryonic electrical connections appear to pre-figure a behavioral circuit in the leech CNS. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **192**, 123–133 (2006).
24. Todd, K.L., Kristan, W.B., Jr & French, K.A. Gap junction expression is required for normal chemical synapse formation. *J. Neurosci.* **30**, 15277–15285 (2010).
25. Blankenship, A.G. & Feller, M.B. Mechanisms underlying spontaneous patterned activity in developing neural circuits. *Nat. Rev. Neurosci.* **11**, 18–29 (2010).
26. Spitzer, N.C. Electrical activity in early neuronal development. *Nature* **444**, 707–712 (2006).
27. Gu, X., Olson, E.C. & Spitzer, N.C. Spontaneous neuronal calcium spikes and waves during early differentiation. *J. Neurosci.* **14**, 6325–6335 (1994).
28. Rosenberg, S.S. & Spitzer, N.C. Calcium Signaling in Neuronal Development. *Cold Spring Harb Perspect Biol* **3**, (2011).
29. Alaynick, W.A., Jessell, T.M. & Pfaff, S.L. SnapShot: Spinal cord development. *Cell* **146**, 178–178.e1 (2011).
30. Belgacem, Y.H. & Borodinsky, L.N. Sonic hedgehog signaling is decoded by calcium spike activity in the developing spinal cord. *Proc. Natl. Acad. Sci. USA.* **108**, 4482–4487 (2011).
31. Borodinsky, L.N. *et al.* Activity-dependent homeostatic specification of transmitter expression in embryonic neurons. *Nature* **429**, 523–530 (2004).
32. Borodinsky, L.N. & Spitzer, N.C. Activity-dependent neurotransmitter-receptor matching at the neuromuscular junction. *Proc. Natl. Acad. Sci. USA.* **104**, 335–340 (2007).
33. Warp, E. *et al.* Emergence of patterned activity in the developing zebrafish spinal cord. *Curr. Biol.* **22**, 93–102 (2012).
34. Ben-Ari, Y. Developing networks play a similar melody. *Trends in Neurosciences* **24**, 353–360 (2001).
35. Jean-Xavier, C., Mentis, G.Z., O'Donovan, M.J., Cattaert, D. & Vinay, L. Dual personality of GABA/glycine-mediated depolarizations in immature spinal cord. *PNAS* **104**, 11477–11482 (2007).
36. Milner, L.D. & Landmesser, L.T. Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity before target contact. *J. Neurosci.* **19**, 3007–3022 (1999).
37. Branchereau, P. *et al.* Development of lumbar rhythmic networks: from embryonic to neonate locomotor-like patterns in the mouse. *Brain Research Bulletin* **53**, 711–718 (2000).
38. Yvert, B., Branchereau, P. & Meyrand, P. Multiple spontaneous rhythmic activity patterns generated by the embryonic mouse spinal cord occur within a specific developmental time window. *J. Neurophysiol.* **91**, 2101–2109 (2004).
39. O'Donovan, M.J., Wenner, P., Chub, N., Tabak, J. & Rinzel, J. Mechanisms of spontaneous activity in the developing spinal cord and their relevance to locomotion. *Ann. N. Y. Acad. Sci.* **860**, 130–141 (1998).
40. Branchereau, P., Chapron, J. & Meyrand, P. Descending 5-hydroxytryptamine raphe inputs repress the expression of serotonergic neurons and slow the maturation of inhibitory systems in mouse embryonic spinal cord. *J. Neurosci.* **22**, 2598–2606 (2002).
41. Chub, N. & O'Donovan, M.J. Blockade and recovery of spontaneous rhythmic activity after application of neurotransmitter antagonists to spinal networks of the chick embryo. *J. Neurosci.* **18**, 294–306 (1998).
42. Chub, N. & O'Donovan, M.J. Post-episode depression of GABAergic transmission in spinal neurons of the chick embryo. *J. Neurophysiol.* **85**, 2166–2176 (2001).
43. Chub, N., Mentis, G.Z. & O'Donovan, M.J. Chloride-sensitive MEQ fluorescence in chick embryo motoneurons following manipulations of chloride and during spontaneous network activity. *J. Neurophysiol.* **95**, 323–330 (2006).
44. O'Donovan, M.J., Chub, N. & Wenner, P. Mechanisms of spontaneous activity in developing spinal networks. *J. Neurobiol.* **37**, 131–145 (1998).
45. Hanson, M.G. & Landmesser, L.T. Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron* **43**, 687–701 (2004).
46. Hanson, M.G., Milner, L.D. & Landmesser, L.T. Spontaneous rhythmic activity in early chick spinal cord influences distinct motor axon pathfinding decisions. *Brain Res. Rev.* **57**, 77–85 (2008).
47. Kastanenka, K.V. & Landmesser, L.T. In vivo activation of channel rhodopsin-2 reveals that normal patterns of spontaneous activity are required for motoneuron guidance and maintenance of guidance molecules. *J. Neurosci.* **30**, 10575–10585 (2010).
48. Turrigiano, G.G. & Nelson, S.B. Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* **5**, 97–107 (2004).
49. Davis, G.W. Homeostatic control of neural activity: from phenomenology to molecular design. *Annu. Rev. Neurosci.* **29**, 307–323 (2006).
50. Gonzalez-Islas, C. & Wenner, P. Spontaneous network activity in the embryonic spinal cord regulates AMPAergic and GABAergic synaptic strength. *Neuron* **49**, 563–575 (2006).
51. Gonzalez-Islas, C., Chub, N., Garcia-Bereguiain, M.A. & Wenner, P. GABAergic synaptic scaling in embryonic motoneurons is mediated by a shift in the chloride reversal potential. *J. Neurosci.* **30**, 13016–13020 (2010).
52. Davis, G.W. & Bezprozvanny, I. Maintaining the stability of neural function: a homeostatic hypothesis. *Annu. Rev. Physiol.* **63**, 847–869 (2001).
53. Davis, G.W. & Goodman, C.S. Genetic analysis of synaptic development and plasticity: homeostatic regulation of synaptic efficacy. *Curr. Opin. Neurobiol.* **8**, 149–156 (1998).

54. Davis, G.W. & Goodman, C.S. Synapse-specific control of synaptic efficacy at the terminals of a single neuron. *Nature* **392**, 82–86 (1998).
55. Frank, C.A., Kennedy, M.J., Goold, C.P., Marek, K.W. & Davis, G.W. Mechanisms underlying the rapid induction and sustained expression of synaptic homeostasis. *Neuron* **52**, 663–677 (2006).
56. Petersen, S.A., Fetter, R.D., Noordermeer, J.N., Goodman, C.S. & DiAntonio, A. Genetic analysis of glutamate receptors in Drosophila reveals a retrograde signal regulating presynaptic transmitter release. *Neuron* **19**, 1237–1248 (1997).
57. Paradis, S., Sweeney, S.T. & Davis, G.W. Homeostatic control of presynaptic release is triggered by postsynaptic membrane depolarization. *Neuron* **30**, 737–749 (2001).
58. Frank, C.A., Pielage, J. & Davis, G.W. A presynaptic homeostatic signaling system composed of the Eph receptor, ephexin, Cdc42, and CaV2.1 calcium channels. *Neuron* **61**, 556–569 (2009).
59. Müller, M. & Davis, G.W. Transsynaptic control of presynaptic Ca^{2+} influx achieves homeostatic potentiation of neurotransmitter release. *Curr. Biol.* **22**, 1102–1108 (2012).
60. Axelsson, J. & Thesleff, S. A study of supersensitivity in denervated mammalian skeletal muscle. *J Physiol* **147**, 178–193 (1959).
61. Cannon, W.B. & Rosenblueth, A. *The supersensitivity of denervated structures: a law of denervation*. (Macmillan Co.: 1949).
62. Thirumalai, V. & Cline, H.T. Endogenous dopamine suppresses initiation of swimming in prefeeding zebrafish larvae. *J. Neurophysiol.* **100**, 1635–1648 (2008).
63. Sillar, K.T., Wedderburn, J.F. & Simmers, A.J. The development of swimming rhythmicity in post-embryonic *Xenopus laevis*. *Proc. Biol. Sci.* **246**, 147–153 (1991).
64. Zhang, H.-Y., Issberner, J. & Sillar, K.T. Development of a spinal locomotor rheostat. *Proc. Natl. Acad. Sci. USA.* **108**, 11674–11679 (2011).
65. Sillar, K.T., Woolston, A.M. & Wedderburn, J.F. Involvement of brainstem serotonergic interneurons in the development of a vertebrate spinal locomotor circuit. *Proc. Biol. Sci.* **259**, 65–70 (1995).
66. Wedderburn, J.F. & Sillar, K.T. Modulation of rhythmic swimming activity in post-embryonic *Xenopus laevis* tadpoles by 5-hydroxytryptamine acting at 5HT1a receptors. *Proc. Biol. Sci.* **257**, 59–66 (1994).
67. Cazalets, J.R., Sqalli-Houssaini, Y. & Clarac, F. Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. *J. Physiol. (Lond.)* **455**, 187–204 (1992).
68. Madriaga, M.A., McPhee, L.C., Chersa, T., Christie, K.J. & Whelan, P.J. Modulation of locomotor activity by multiple 5-HT and dopaminergic receptor subtypes in the neonatal mouse spinal cord. *J. Neurophysiol.* **92**, 1566–1576 (2004).
69. Casasnovas, B. & Meyrand, P. Functional differentiation of adult neural circuits from a single embryonic network. *J. Neurosci.* **15**, 5703–5718 (1995).
70. Richards, K.S., Simon, D.J., Pulver, S.R., Beltz, B.S. & Marder, E. Serotonin in the developing stomatogastric system of the lobster, *Homarus americanus*. *J. Neurobiol.* **54**, 380–392 (2003).
71. Le Feuvre, Y., Fénelon, V.S. & Meyrand, P. Central inputs mask multiple adult neural networks within a single embryonic network. *Nature* **402**, 660–664 (1999).
72. Fénelon, V.S., Kilman, V., Meyrand, P. & Marder, E. Sequential developmental acquisition of neuromodulatory inputs to a central pattern-generating network. *J. Comp. Neurol.* **408**, 335–351 (1999).
73. Fénelon, V.S., Le Feuvre, Y. & Meyrand, P. Phylogenetic, ontogenetic and adult adaptive plasticity of rhythmic neural networks: a common neuromodulatory mechanism? *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **190**, 691–705 (2004).
74. Thirumalai, V. & Marder, E. Colocalized neuropeptides activate a central pattern generator by acting on different circuit targets. *J. Neurosci.* **22**, 1874–1882 (2002).
75. Rehm, K.J., Deeg, K.E. & Marder, E. Developmental regulation of neuromodulator function in the stomatogastric ganglion of the lobster, *Homarus americanus*. *J. Neurosci.* **28**, 9828–9839 (2008).
76. Ducret, E., Le Feuvre, Y., Meyrand, P. & Fénelon, V.S. Removal of GABA within adult modulatory systems alters electrical coupling and allows expression of an embryonic-like network. *J. Neurosci.* **27**, 3626–3638 (2007).
77. Marder, E. Variability, compensation, and modulation in neurons and circuits. *Proc. Natl. Acad. Sci. USA.* **108 Suppl 3**, 15542–15548 (2011).
78. Pulver, S.R., Thirumalai, V., Richards, K.S. & Marder, E. Dopamine and histamine in the developing stomatogastric system of the lobster *Homarus americanus*. *J. Comp. Neurol.* **462**, 400–414 (2003).
79. Rehm, K.J., Taylor, A.L., Pulver, S.R. & Marder, E. Spectral Analyses Reveal the Presence of Adult-Like Activity in the Embryonic Stomatogastric Motor Patterns of the Lobster, *Homarus americanus*. *J Neurophysiol.* **99**, 3104–3122 (2008).
80. Richards, K.S. & Marder, E. The actions of crustacean cardioactive peptide on adult and developing stomatogastric ganglion motor patterns. *J. Neurobiol.* **44**, 31–44 (2000).
81. Straus, C., Wilson, R.J. & Remmers, J.E. Developmental disinhibition: Turning off inhibition turns on breathing in vertebrates. *J. Neurobiol.* **45**, 75–83 (2000).
82. Duch, C. & Levine, R.B. Remodeling of membrane properties and dendritic architecture accompanies the postembryonic conversion of a slow into a fast motoneuron. *J. Neurosci.* **20**, 6950–6961 (2000).
83. Meseke, M., Evers, J.F. & Duch, C. Developmental changes in dendritic shape and synapse location tune single-neuron computations to changing behavioral functions. *J. Neurophysiol.* **102**, 41–58 (2009).

84. Winbush, A. & Weeks, J.C. Steroid-triggered, cell-autonomous death of a Drosophila motoneuron during metamorphosis. *Neural Dev* **6**, 15 (2011).
85. Weeks, J.C. & Truman, J.W. Independent steroid control of the fates of motoneurons and their muscles during insect metamorphosis. *J. Neurosci.* **5**, 2290–2300 (1985).
86. Sillar, K.T., Combes, D., Ramanathan, S., Molinari, M. & Simmers, J. Neuromodulation and developmental plasticity in the locomotor system of anuran amphibians during metamorphosis. *Brain Research Reviews* **57**, 94–102 (2008).
87. Combes, D., Merrywest, S.D., Simmers, J. & Sillar, K.T. Developmental segregation of spinal networks driving axial- and hindlimb-based locomotion in metamorphosing *Xenopus laevis*. *J. Physiol. (Lond.)* **559**, 17–24 (2004).
88. Rauscent, A. *et al.* Development and neuromodulation of spinal locomotor networks in the metamorphosing frog. *Journal of Physiology-Paris* **100**, 317–327 (2006).
89. Marsh-Armstrong, N., Cai, L. & Brown, D.D. Thyroid hormone controls the development of connections between the spinal cord and limbs during *Xenopus laevis* metamorphosis. *Proc. Natl. Acad. Sci. USA.* **101**, 165–170 (2004).
90. Rauscent, A., Einum, J., Le Ray, D., Simmers, J. & Combes, D. Opposing aminergic modulation of distinct spinal locomotor circuits and their functional coupling during amphibian metamorphosis. *J. Neurosci.* **29**, 1163–1174 (2009).
91. Marder, E. & Bucher, D. Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. *Annu. Rev. Physiol.* **69**, 291–316 (2007).
92. Thirumalai, V. Implications of cotransmission and neuromodulation for neural network function. Ph.D Thesis, Brandeis University, Waltham MA (2002).

Vatsala Thirumalai is currently an Assistant Professor at the National Centre for Biological Sciences, Bangalore. She graduated from Anna University, Chennai with a B.Tech in Biotechnology. She obtained a Ph.D in Neuroscience from Brandeis University, Waltham, MA in 2002 studying motor pattern generation and the role of neuromodulators in shaping neural circuits. She then completed postdoctoral fellowships from Cold Spring Harbor Laboratory, NY and the National Institutes of Health, Bethesda, MD. During this time, she studied the development of neural circuits controlling movement in zebrafish and mice. She continues to be deeply interested in the area of development of motor systems and their neuromodulation and focuses on these in her current research. http://www.ncbs.res.in/vatsala