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From circuits to behaviour: motor networks in vertebrates

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Abstract

Neural networks in the hindbrain and spinal cord generate the simple patterns of motor activity that are necessary for breathing and locomotion. These networks function autonomously, producing simple yet flexible rhythmic motor behaviours that are highly responsive to sensory inputs and central control. This review highlights recent advances in our understanding of the genetic programs that control the assembly and functioning of the hindbrain and spinal circuits that are responsible for respiration and locomotion. In addition, we highlight the influence that target-derived retrograde signaling and experience-dependent mechanisms have on establishing connectivity, particularly with respect to sensory circuits in the spinal cord.

Introduction

In charting the course “from circuits to behaviour”, neurobiologists have often used motor systems such as those that control respiration and locomotion to study the neural basis of behaviour [1-8]. Recent advances in our understanding of the dedicated genetic programs that govern the development of the embryonic nervous system have greatly facilitated these efforts. Using new genetic approaches for circuit analysis, rapid progress is being made toward elucidating how respiratory and locomotor networks are assembled and configured to produce their signature motor behaviours.

Circuits in the hindbrain that control breathing

The rhythmic motor activity for breathing appears to be generated by two “autonomous” interconnected centres in the medulla, the preBötzinger complex (preBötC) [9] and the parafacial respiratory group /retrotrapezoid nucleus (pFRG/RTN) [8,10] (Figure 1). Both oscillators are proposed to play complementary roles by driving inspiratory and expiratory movements, respectively, and they work in concert to produce a reliable respiratory rhythm throughout life.

Rhythm generation: preBötC

Studies of the preBötC have largely focused on the mechanisms that underlie rhythm generation ([6,7,9] and references therein). Although many preBötC cells show pacemaker-like activity, there appears to be no obligate requirement for “pacemaker” neurons [6,7,11]. Instead there is a growing consensus that rhythm generation in the preBötC represents an emergent network

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property, in which synaptically coupled excitatory cells with varying cellular properties all contribute to burst generation. Recurrent excitatory synaptic connections via NMDA, mGluR and AMPA synapses that activate calcium-activated cation (I_{CAN}) currents are essential for burst production [6,7,11-14]. Other currents such as the persistent Na^+ (I_{NaP}) and $I_A K^+$ currents are also likely to contribute to excitability and rhythmogenesis [6,7,11,15].

The cellular composition of the preBötC is heterogeneous. Subsets of excitatory preBötC neurons that are derived in part from MafB⁺ progenitors express various combinations of somatostatin (Sst) and the substance P/neurokinin-1 receptor (NK1R) [16-20]. Confirmation of their role in respiratory rhythm generation has come from the recent finding that silencing Sst⁺ neurons in the preBötC produces a persistent absence of breathing (apnea) [18], together with previous studies showing the loss of NK1R⁺ neurons leads to deficits in breathing and sleep apneas [19,20].

The emergent pFRG/RTN

The pFRG/RTN [7,8,10] located adjacent to the facial motor nucleus also contains neurons important for respiration, which are phase-locked to motor neurons (MNs) involved in expiratory respiratory movements. A series of recent studies now provide strong evidence that the pFRG/RTN arises from an embryonic structure termed the embryonic parafacial oscillator (e-pF) [21••,22•]. The e-pF is largely comprised of VGlut2⁺ neurons that arise from Egr2⁺ (Krox20) progenitors and express Lbx1, Atoh1 and Phox2b [8,21••,22•,23]. Consequently, mutations in any of these genes leads to defective development or the loss of the e-pF and the corresponding pFRG/RTN in older animals [21••,22•,23].

The e-pF exhibits “respiratory-like” oscillations that precede rhythmic activity in the preBötC [21••]. This rhythmic activity is lost in mice that lack Phox2b⁺ neurons in the parafacial region [21••,22•]. The observation that the preBötC rhythm is activated after the e-pF, and is initially synchronous with it, led to the suggestion that the e-pF entrains the preBötC [21••]. Consequently, while a preBötC driven rhythm does develop in mice that lack an e-pF [21••, 22•-24•], it is slower and less reliable. Although initially synchronous, the pFRG/RTN and preBötC rhythm becomes offset so as to drive expiratory and inspiratory muscle movements, respectively, a process likely linked with the development of chloride-mediated inhibition [22•].

Interestingly, the cellular mechanisms that produce a rhythmic “respiratory” output from the e-pF differ from those in the preBötC. Phox2b⁺ e-pF neurons are electrically coupled and exhibit endogenous bursting properties that are sensitive to inhibitors of I_{NaP} and I_h currents (see [21••] for details), two currents that are associated with pacemaker neurons. This configuration has been referred to as the “belt-and-suspenders principle” [25], with the different organization and cellular properties of each rhythmic centre engendering the respiratory neural network with a degree of functional redundancy. The different mechanisms underlying rhythm generation also allows for differential modulation of the pFRG/RTN and preBötC, thereby providing an additional level of flexibility and robustness to the respiratory neural network.

The pFRG/RTN is a key centre for CO₂ chemosensitivity

The pFRG/RTN also has an important role in CO₂ chemosensitivity, translating high CO₂ levels into enhanced ventilation. Congenital central hypoventilation syndrome (CCHS) is characterized by an absent or blunted response to high CO₂ or low O₂ blood levels (hypercapnia or hypoxemia) and is caused by a mutation in the human *Phox2b* gene [26]. Phox2b is expressed in the e-pF and pFRG/RTN [21••,22•,23,24•,27] and mice carrying a common CCHS mutation have markedly fewer Phox2b⁺ cells in the pFRG/RTN [28]. These mice exhibit respiratory

abnormalities similar to those seen in severe cases of CCHS [28]. When a conserved Phox2b response element was used to ectopically express channelrhodopsin in the ventral medulla of rats [29], photostimulation of the pFRG/RTN increased both the amplitude and frequency of phrenic nerve discharge in a CO₂-dependent manner, demonstrating that pFRG/RTN neurons mediate the respiratory response to hypercapnia. The observation that mice lacking Phox2b⁺ e-pF neurons show no increase in facial MN bursting in response to low pH challenge that mimics hypercapnia provides further evidence that the e-pF represents the anlagen of the pFRG/RTN [22•].

The e-pF appears to be made up of a strikingly homogenous population of cells that are VGlut2⁺/Lbx1⁺/Atoh1⁺/Phox2b⁺. These e-pF cells exhibit a rather specific spectrum of ionic conductances. Later on the pFRG/RTN comprises of multiple functional subtypes subserving rhythmogenic expiratory/pre-inspiratory and chemosensitive roles [27]. Most of these chemosensitive neurons express Phox2b [8,27], although some neurons exhibit tonic firing while others are rhythmic within the mature pFRG/RTN. With the possible exception of galanin, no markers have been found for subsets of pFRG/RTN neurons that might define for example tonic versus rhythmic neurons.

Modulation of respiratory rhythmicity – additional elements of the breathing circuitry

In addition to chemosensory regulation, respiration is tightly regulated by a number of neuromodulators including opiates, acetylcholine, serotonin (5-HT) and substance P [7,8,30]. Although the serotonergic neurons of the Raphe were suggested to play a central role in CO₂ chemosensation, their role in the hypercapnic response instead appears to be modulatory. Indeed, the selective ablation of these cells blunts but does not abolish the respiratory response to hypercapnia [31] as do mutations affecting the e-pF/pFRG/RTN [21•,22•-24•]. Moreover, this responsiveness can be rescued by perfusing 5-HT or activating 5-HT_{2A} receptors [32]. The pFRG/RTN and preBötC, together with respiratory-related MNs, express multiple 5-HT receptors, including 5-HT_{2A}. These serotonergic neurons also release substance P, which increases respiratory drive. As with 5-HT, the targets of substance P action are likely to be pFRG/RTN and preBötC neurons that express NKR1 [21•,33]. 5-HT/substance P-mediated neuromodulatory drive prevents apneas and increases ventilation and is critical during the early neonatal period for proper viability.

Respiration and pattern generation

Respiration is under strong modulatory control. In addition to modifying the breathing frequency, the reconfiguration of the respiratory motor pattern during bouts of sighing, gasping and coughing are orchestrated in part by neuromodulators [30]. For example, cholinergic transmission modifies the pacemaker properties of neurons in the preBötC during sighing [34]. Breathing also needs to be coupled with vocalization and locomotion, each of which involves highly coordinated patterns of pharyngeal, diaphragm, intercostal/abdominal and limb muscle activity. Giraudin *et al.* [35] showed complex respiratory-related behaviours can be studied in a reduced hindbrain-spinal cord preparation. In doing so, they observed intercostal and abdominal muscle efferents exhibit temporally appropriate patterns of inspiratory and expiratory activity, which were subject to coordination and resetting from limb sensory afferents [35].

Motor circuits in the spinal cord: lessons from the “V” interneurons

Recent studies have used a combination of molecular genetics, electrophysiology, optical techniques and behavioural testing to delve more deeply into how motor circuits are assembled

and function [2-4]. The core premotor components of vertebrate locomotor circuitry are largely derived from six cardinal classes of embryonic interneurons (INs), the ventrally-settling V0, V1, V2a, V2b and V3 INs and the dI6 INs (Figure 2). These populations can be distinguished and manipulated on the basis of their unique transcription factor (TF) codes (Figure 2b; see [3,4,36] for details). Previous studies addressing the role V0 and V1 INs play in shaping locomotor activity revealed that V0 INs regulate left-right alternation [37], whereas V1 INs control the duration of the step cycle and speed of walking movements [38]. Three recent studies now describe the contribution V2a and V3-derived INs make to left-right stepping behaviours and the precision/robustness of the motor output.

V2a INs and left-right coordination in limbed vertebrates

Ablation of the V2a INs in Chx10-DTA mice disrupts left-right alternation [39••] in a manner similar to that previously reported for the loss of V0 commissural interneurons (CINs) [37] (Figure 2a). The V2a INs apparently provide excitatory drive to the spinal circuits controlling left-right alternation with some V2a INs forming direct synaptic contacts with Evx1⁺ V0_V CINs [39••] (Figure 2b). It is unclear, however, whether V2a connections onto Evx1⁺ V0_V CINs regulate left-right alternation, as mice lacking the Evx1⁺ V0_V CINs do not show deficits in left-right alternation [37]. In all likelihood, the V2a INs provide additional inputs to inhibitory V0_D CINs, as well as other inhibitory CIN subtypes. Crone *et al.* [40••], by analyzing the walking gait of awake behaving Chx10-DTA mice, found that V2a INs regulate left-right stepping at faster “trotting” speeds. This speed-dependent coordination suggests that the V2a-CIN inhibitory pathways may be preferentially active at higher walking speeds.

Behavioural “modules” in the spinal cord

Subsets of circumferential descending interneurons (CiDs), the embryonic homologues of V2a INs, are differentially active at faster swimming speeds [41,42••] with ventrally- and dorsally-located CiDs being differentially active during slow and fast swimming, respectively [43]. Multipolar descending commissural neurons (MCoDs) that are selectively active at lower swimming speeds are born late and located ventrally in the spinal cord [42••,44]. Intriguingly, this pattern of neuronal activity during swimming broadly recapitulates the temporal sequence of neuronal differentiation with INs active at faster speeds being born earlier [44].

Rather than being recruited in a graded manner according to the size principle [45], INs are differentially recruited during different motor-related tasks [40••,42••-44]. In the turtle one can find locomotor-related INs that are active either during scratching or swimming, while others are recruited during both behaviours [46]. Likewise, V2a INs maintain left-right stepping at faster speeds [40••] (Figure 2b). Consequently, task- or gait-dependent differential recruitment of INs may be a general feature of the spinal motor circuitry in vertebrates.

Rhythm generation in mammals

Mice lacking V2a INs generate a near normal pattern of pharmacologically-induced fictive locomotion [39••] suggesting other classes of ipsilateral excitatory INs, e.g. Hb9 INs might be responsible for generating the locomotor rhythm. Glutamatergic Hb9 INs exhibit cellular properties that can sustain rhythmic activity, and they are rhythmically active during fictive locomotion [47,48]. However, calcium imaging revealed a delay in the onset of Hb9 IN activity relative to ipsilateral motor bursting during fictive walking, which argues that these cells are unlikely to initiate rhythm generation [49].

The Sim1-expressing V3 CINs do appear to contribute to rhythm generation, in so far as the locomotor rhythm is less organized and robust when these cells are inactivated [50••]. Exactly how V3-excitatory pathways engender robustness and regularity to the motor rhythm is not clear, but as noted with the preBötC and pFRG/RTN, mutual excitation between oscillatory

centres may be an important mechanism. While spinal cords lacking V3 transmission exhibit a near normal alternation pattern, the relative duration of motor bursts on each side of the cord was highly variable (Figure 2a) arguing V3 INs, or a sub-population thereof, have an additional role in producing a balanced motor rhythm (Figure 2b). Indeed, acute attenuation of V3-neuronal activity in awake-behaving mice results in uncoordinated hindlimb movements that are marked by an uneven gait [50••].

Rhythm generation in swimming vertebrates

Studies in swimming vertebrates have identified INs that may contribute to rhythm-generation [51•,52•,53•]. In a functional screen in zebrafish, Wyart *et al.* [53•] used Gal4-drivers and a novel light activated glutamate channel to selectively activate Kolmer-Agduhr (KA) neurons. Upon doing so, tail beating movements were initiated that recapitulated swimming movements [53•]. KA neurons have cilia that contact the central canal [54] and may serve to translate mechanical stretch or chemical changes in the cerebrospinal fluid into rhythmic movements during early development when GABA transmission is excitatory [55,56]. Their contribution to spontaneous activity following reversal of the chloride potential and in other vertebrates remains unclear.

Efforts to identify the neurons that drive the locomotor rhythm in the frog tadpole have focused on excitatory dIN neurons in the hindbrain and spinal cord, which have a descending axon. The suggestion is that spinal dINs are, in fact, the premotor excitatory neurons that drive the locomotor rhythm and may be homologous to the V2a INs. Li *et al.* [51•] found that in addition to being synaptically interconnected, dINs are electrically coupled with each other. Such coupling between premotor reticulospinal neurons and spinal dINs would facilitate caudal-ward propagation of rhythmic activity along the axis during swimming. Further support for dIN involvement in rhythm generation comes from the finding that reticulospinal dIN activity precedes that of other locomotor neurons during the swimming cycle [52•]. In this context it is worth noting that the genetic program that defines neuronal subtypes such as V2a INs in the developing spinal cord also operates in the hindbrain [57] and that mice lacking V2a INs exhibit clear deficits in brainstem-initiated fictive locomotion [39••].

Assembly and function of sensorimotor circuits

Specification of motor neuron subtypes

Coordinated locomotion in limbed vertebrates is highly dependent on sensory feedback. An important sensorimotor pathway in mammals is the Ia monosynaptic reflex pathway between Ia proprioceptive afferent neurons that measure stretch in a particular muscle and the pool of α -MNs that innervate that same muscle [58]. The choice of α -MN versus γ -MN fate is a key step in configuring the Ia monosynaptic pathway, as γ -MNs that control intrafusal muscle fiber tension are not innervated by muscle spindle afferents [59]. γ -MNs require glial cell line-derived neurotrophic factor (GDNF) for their survival [60], although it is not clear whether GDNF or other peripheral signals act instructively to specify distinct MN identities. *Err3*, an orphan nuclear receptor, is restricted to γ -MNs postnatally [61]. However, since γ -MNs are already present embryonically, this gene is unlikely to have a role in subtype specification [60].

Genetic control of sensorimotor pool connectivity

The development of sensorimotor reflex circuits has been investigated in the context of Ia sensory connections. The picture that has emerged is one in which proprioceptive connectivity is largely determined by genetically hardwired programs. The analysis of various ETS TFs has been particularly informative. *Er81*, which is broadly expressed in proprioceptive sensory neurons in mice, regulates axonal invasion of the ventral horn [62]. *Pea3* is expressed in a more

restricted pattern, being confined to cutaneous maximus (Cm) and latissimus dorsi (Ld) MNs at forelimb levels [63,64] (Figure 3a), where it coordinates many aspects of motor pool development, including migration and aggregation of Cm MNs. The latter events may be through regulation of *cad8* and *cad7* in Cm MNs [63] (Figure 3b).

Cm and Ld MNs are somewhat atypical, in that they do not receive direct monosynaptic Ia inputs from their corresponding muscle Ia afferents, whereas triceps (Tri) MNs do (Figure 3a). This pattern of connectivity is disrupted in the *Pea3* mutant, with Cm (and Ld) MNs receiving inappropriate monosynaptic innervation from Tri sensory afferents [65] (Figure 3b). Cm MN dendrites also show marked morphological changes that accompany the formation of inappropriate Tri Ia-Cm MN connections. A recent study reveals that sensorimotor connectivity may be controlled by dedicated repellent signaling pathways. Semaphorin (Sema) 3E, a downstream target of *Pea3*, blocks the formation of monosynaptic connections between Cm afferents and Cm MNs [66••] (Figure 3b), by activating PlexinD1-signaling in sensory neurons, presumably preventing them from contacting Cm MN dendrites. Conversely, ectopic expression of Sema3E in the Tri motor pool converted a significant fraction of the monosynaptic inputs from Tri sensory afferents to polysynaptic inputs [66••] (Figure 3b). This suggests that the partnership between Sema3E/PlexinD1 prevents Cm sensory afferents from forming monosynaptic connections. Interestingly, the formation of inappropriate monosynaptic connections between Cm proprioceptive afferents and Cm MNs in *Sema3E/PlexinD1* mutants takes place without any overt change in the dendrite morphology or cell body positioning of Cm MNs, indicating that these two processes can be dissociated. While Ld MNs express *Pea3*, they do not express Sema3E. Consequently, Sema3E does not function as universal determinant of polysynaptic versus monosynaptic connectivity in forelimb motor pools that direct Ia inputs. Nonetheless, a similar repellent pair may control Ld sensory afferent-MN connectivity.

The molecular program triggered by *Pea3* also controls the innervation of Cm and Ld muscle targets by Cm and Ld MNs, respectively [62,63]. However, our knowledge of motor pool specification remains sketchy, and aside from FoxP1 and the Hox TFs, few TFs that might participate in motor pool specification have been identified. *Nkx6.1* is one of such a factor: adductor pool MNs fail to properly innervate their hindlimb target muscles in mice lacking *Nkx6.1* [67].

Specificity of presynaptic inhibition connectivity

Further evidence for genetic control of synaptic connectivity in the spinal cord comes from a recent study looking at the development of presynaptic GABA synapses [68]. Classic studies have described two forms of synaptic inhibition in the spinal cord: postsynaptic inhibition mediated by GABAergic and glycinergic axo-dendritic or axo-somatic synapses, and presynaptic inhibition where axo-axonic GABAergic synapses appose proprioceptive and cutaneous afferent fibres [69]. Inhibitory INs mediating presynaptic inhibition arise from two dorsal populations, dI4 and dIL_A neurons [68,70-72]. These populations are specified by the Ptf1a basic helix-loop-helix and Lbx1 homeodomain TFs. In contrast, ventral postsynaptic inhibitory cell types such as V1-derived Ia inhibitory interneurons (IaINs) and Renshaw cells (RCs) are generated in a Ptf1a-independent manner [73,74]. The anatomical (and functional) dichotomy in spinal inhibitory synapses therefore has its origins in the divergent genetic programs that specify two broad classes of inhibitory neurons in the embryonic cord. The observation that Ptf1a⁺ GABA axons transiently contact MNs, but fail to form mature functional synapses, indicates that target-derived signals participate in the formation and maturation of GABA⁺ presynaptic synapses. More tellingly, brain-derived neurotrophic factor (BDNF) emanating from sensory afferents is necessary for presynaptic localization of GAD65 in these axo-axonic synapses.

Learning from modular organization of nociceptive withdrawal reflex circuits

While there is a strong genetic component to the wiring up of proprioceptive pathways, activity plays a prominent role in configuring cutaneous and nociceptive reflex pathways. In rodents, the nociceptive withdrawal reflex (NWR) is functionally organized into modules [75,76] that transform sensory input from a receptive field into protective body movements. Far from showing an adult-like organization at birth, the modular structure of the NWR emerges from a plastic somatotopic sensory map in the dorsal horn that is sharpened by experience-dependent rewiring of afferent synaptic connections [77,78]. In this way, somatosensory imprinting entrains the NWR to elicit the appropriate motor behavioural response to noxious stimuli in the adult. The plasticity mechanisms that establish NWR connections, while not well understood, involve both long-term potentiation and NMDA-dependent mechanisms that shape the sensitivity distribution of NWR receptive fields [76,79]. This occurs in part via the reorganization of cutaneous afferent terminals in the lumbar spinal cord [80•].

The cellular organization of cutaneous sensory pathways in the cord is still poorly understood. Cutaneous afferent inputs exhibit a vertical columnar organization that creates a sensory map of the body surface within the dorsal horn. Overlaid on this is a lamina specific pattern of afferent innervation, with nociceptive and mechanoreceptive afferents terminating on INs in the substantia gelatinosa (lamina I and II) [80•] and in lamina III and IV, respectively. The outputs from these first order INs then converge on reflex encoder (RE) INs in the intermediate cord that are aligned with the motor pools they control [81]. The first order sensory neurons in lamina I-IV that make up these NWR modules are derived from Lbx1⁺ INs [70,71]. Moreover, second order sensory INs in lamina V and VI, which likely include so-called RE INs, also express Lbx1. For this reason, assessing the transcriptional programs that are downstream of Lbx1 will undoubtedly provide important insights into the molecular logic that underlies the diversification and function of specialized sensory IN cell types in the cord. Such studies are key to understanding how sensorimotor circuits in the spinal cord are wired up to generate the complex motor behaviours that underlie protective reflexes, postural control and locomotion.

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Figure 1.

Respiratory centres in the medulla that are responsible for the breathing rhythm and chemosensitivity. **(a)** Schematic of the neonate hindbrain showing the location of the major excitatory regions involved in respiratory rhythm generation, the e-pF/pFRG/RTN (embryonic parafacial nucleus/parafacial respiratory group/retrotrapezoid nucleus; orange), the PBC (preBötzinger complex; red, green/yellow), as well as the VRG (ventral respiratory group). **(b)** Structure of the e-pF and preBötzC. Neurons in the e-pF (orange) express a combination of VGlut2, Lbx1, Atoh1 and Phox2b [22•-24•] and they exhibit uniform pacemaker properties [21••]. Neurons in the preBötzC express different combinations of NKR1 and Sst [17-19], and exhibit various constellations of cellular currents [6,7,11-13]. Serotonergic neurons in the Raphe are indicated in blue with projections (arrows) to multiple structures including the pFRG/RTN and preBötzC. The e-PF and later pFRG/RTN are connected by excitatory (red) and inhibitory (black) connections, although the neural nature of these connections is not clear. Dashed lines indicate putative reciprocal excitatory and inhibitory connections between both rhythmic centres. e-pF/pFRG/RTN and preBötzC neurons on either side of the medulla are also mutually connected and excited (red).

Figure 2.

Organization and function of the locomotor central pattern generator (CPG) (a) Schematic of the mouse neonatal spinal cord illustrating lumbar segments 2 (L2) and 5 (L5) involved in flexor and extensor motor control, respectively. In the ventral spinal cord, CPG networks that contribute to generate rhythmic motor activity and control left-right alternation are located in laminae VI-IX [36] (pink; left). Summary of flexor-related activity recorded *in vitro* from left and right L2 ventral roots. The pattern of left-right alternation in wild-type, Chox10-DTA [39••] and Sim1-Cre; R26-TeNT [50••] mice during induced fictive locomotion is shown schematically (right). (b) Table summarizing the expression of transcription factors that define ventral (V0-V3) interneuron classes and their subclasses, neurotransmitter markers, axonal

projections, known connections and function [37,38,39••,40••,50]. Adapted from [82]. IaIN, Ia inhibitory interneuron; Calb, Calbindin; GABA, GABAergic; Glu, glutamatergic; Gly, glycinergic; Parv, Parvalbumin; RC, Renshaw cell.

Figure 3.

Motor pool organization and sensorimotor connectivity in the spinal cord. **(a)** Schematic representation depicting a longitudinal view of forelimb motor pools in the mouse spinal cord (left). The cell bodies of motor neurons (MNs) that send axons to specific limb muscles are contained within the lateral motor column (LMC). Motor pools are generated at specific rostrocaudal positions within the brachial LMC. At C7-T1 levels, motor pools projecting to the cutaneous maximus (Cm, green) and triceps (Tri, red) muscles can be molecularly defined by the expression of ETS transcription factors. At the intrasegmental level, the Cm motor pool can be distinguished by the expression of *Pea3* [63]. GDNF from the intrafusal muscle spindle induces the expression of *Pea3* in Cm MNs [64], which in turn activates *Sema3E* gene expression [63]. Note that Cm MNs receive polysynaptic input from Ia proprioceptive afferents (open line) whereas Tri Ia proprioceptive afferents make direct monosynaptic contacts to Tri MNs [65] (right). **(b)** Summary of the connectivity patterns in Cm and Tri reflex pathways in wildtype, *Pea3* mutants and mice with altered *Sema3E* expression. In wildtype, Tri afferents contact homonymous MNs. In *Pea3* mutants, Cm MNs receive contacts from Tri afferents [65]. In *Sema3E* mutants, Cm MNs receive monosynaptic contacts from Cm afferents [66•].

Ectopic expression of *Sema3E* prevents monosynaptic connections between Tri afferents and Tri MNs [66•]. Role of *Pea3* in the genetic program controlling Cm sensorimotor connectivity, cell body position and dendrite arborization [63,65,66•] (bottom). For details see text.