Walking with Salamanders: From Molecules to Biorobotics

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How do four-legged animals adapt their locomotion to the environment? How do central and peripheral mechanisms interact within the spinal cord to produce adaptive locomotion and how is locomotion recovered when spinal circuits are perturbed? Salamanders are the only tetrapods that regenerate voluntary locomotion after full spinal transection. Given their evolutionary position, they provide a unique opportunity to bridge discoveries made in fish and mammalian models. Genetic dissection of salamander neural circuits is becoming feasible with new methods for precise manipulation, elimination, and visualisation of cells. These approaches can be combined with classical tools in neuroscience and with modelling and a robotic environment. We propose that salamanders provide a blueprint of the function, evolution, and regeneration of tetrapod locomotor circuits.

Salamanders as a Model Organism for Studying Locomotion

Two major challenges in neuroscience are to decipher how the interplay between central and peripheral mechanisms controls locomotion in four-legged animals (tetrapods) and to delineate the reorganisation of motor circuits after spinal lesion. In this review, we outline the reasons why salamanders are ideally suited to address these two problems. First, salamanders are the only tetrapods capable of regenerating their locomotor circuits after full transection at adult stage [1–3]. Second, they are the closest extant representatives of the first tetrapods that transitioned from aquatic to terrestrial life [4]. This key evolutionary position makes salamanders ideal to provide a bridge between discoveries in fish (such as zebrafish) and mammals (such as mouse). Salamanders swim underwater and walk on ground [4], allowing researchers to investigate to what extent body dynamics and sensory feedback shape these locomotor patterns. Third, high precision dissection of their neural circuits is becoming possible thanks to new tools that will allow visualisation and optogenetic and chemogenetic manipulations that can be combined with classical neuroscience tools and advanced modelling and robotic environments.

It is thus timely to highlight the recent advances in salamander research at the intersection of genomics, systems neuroscience, modelling, and robotics, which altogether provide a unique opportunity to decode locomotor control in the intact and regenerated tetrapod nervous system. In this review, we systematically place these approaches and recent results into a cross-species comparative setting, with a special focus on zebrafish and mouse as comparative models for which most data on the genetic identity of locomotor circuits are available. For closer examination of the locomotor circuitries of other related animal models such as lamprey, cat, and Xenopus embryo, we refer readers to prior reviews (lamprey: [5], cat: [6], Xenopus embryo: [7]).

The Salamander Nervous System: A Bridge between Fish and Mammalian Models

Salamander locomotor circuits include the main features found in all vertebrates (Figure 1A,C). Their spinal cord contains a central pattern generator that produces walking- and swimming-like motor patterns when isolated from sensory and brain inputs [8–11]. The spinal circuits receive
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descending projections from brainstem reticulospinal neurons [12]. Reticulospinal neurons relay the locomotor command generated by the mesencephalic locomotor region (MLR), a brainstem region that controls locomotor speed and gait transitions in vertebrates ([13,14]; for review, see [15]). Catecholaminergic neurons are present in salamanders. Dopaminergic neurons modulate locomotor activity [16,17]. Such modulatory influences likely involve the ascending projections to the basal ganglia and descending projections to the MLR, an anatomical organisation that is conserved from lamprey to mammals [18,19].

The salamander fills a void among vertebrate genetic animal models (Figure 1): they are the only limbed vertebrate that can regenerate their locomotor system. Zebrafish do regenerate, but do not have legs (e.g., [20]), although interestingly, fish fins are controlled by neuronal circuits that share homology with those controlling legs in tetrapods [21] (see also [22]). The MLR has not been found yet in zebrafish but the circuitry is expected to be present, as it is the case in the phylogenetically older lamprey (for review, see [15]). In limbed mammals, and particularly mice, the locomotor circuitry is currently being mapped in detail, but these animals do not naturally regenerate.

Historically, much of what we know about the vertebrate locomotor circuitry at the cellular level was uncovered by studies in lamprey (for review, see [5]). These studies also uncovered in exquisite detail that the sensorimotor circuits in the brainstem (for review, see [15]), basal ganglia (e.g., [23]), and cortex (e.g., [24]) were most likely already present in the lamprey ancestors some 560 million years ago. Thus, the lamprey studies are an irreplaceable inspiration for the studies in genetically tractable vertebrate models, which are increasingly used to dissect locomotor circuitries. Experiments in zebrafish using genetic tools have uncovered a modular organisation of spinal cell populations controlling speed (Figure 1B, for review, see [25,26]), the role of reticulospinal neurons in providing excitation to spinal swimming circuits [27] and in the control of steering movements [28], and a key role for mechanosensory feedback in the control of swimming [29–31] (Figure 1B). In mice, several cell types have recently been genetically defined, including MLR cells controlling locomotor speed and gait transitions [32–34], reticulospinal cell types relaying locomotor commands [35–37] or steering commands to the spinal cord [38], and spinal cell types involved in locomotor rhythmogenesis and coordination (for review, see [39]) (Figure 1D). However, how these cell populations control locomotor speed and gait transitions in limbed vertebrates is not fully resolved. Moreover, repairing those circuits after spinal cord injury remains a major challenge.

The salamander is well suited to address both the function and regeneration of neuronal circuits using classical tools in neuroscience research (Figure 2). Salamanders display walking and swimming movements and therefore provide an opportunity to understand the coordination of axial and limb movements (Figure 2A–D). Neurons in the reticulospinal formation are active during walking and swimming [40,41]. In semi-intact preparations, where the brain is exposed and the body is free to move, low intensity MLR stimulation evokes walking, whereas higher intensities evoke swimming [13] (Figure 2E,F). Activation of reticulospinal neurons in one side of the brain induces ipsilateral body bending, as expected during steering movements (Figure 2I,J [42]) based on observations in mice [38], zebrafish [28], and lamprey [43]. Hindbrain reticulospinal neurons respond to MLR stimulation [44,45]. As in other vertebrates, the salamander MLR sends a descending bilateral glutamatergic drive that controls the activation level of reticulospinal neurons, as shown using calcium imaging in isolated brainstem preparations [14,42] (Figure 2K). The spinal circuits involved in locomotor rhythmogenesis and coordination are tractable for studies in isolated spinal cord preparations. These circuits are organised as a double chain of ‘unit burst generators’ [46] distributed in the spinal segments controlling the trunk muscles (Figure 1A) [9], limb muscles

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**Glossary**

- **Egr3**: Early Growth Response 3, a molecular marker of mouse muscle spindles.
- **ISL1**: ISL LIM-Homeobox 1, a molecular marker of mouse spinal neurons projecting to trunk motoneurons.
- **ISL2B**: ISL LIM homeobox 2B, a molecular marker of Rohon-Beard neurons, which are large glutamatergic mechanosensory neurons involved in touch-evoked swim responses in zebrafish.
- **Lbx1**: Ladybird Homeobox 1, a molecular marker of spinal neurons projecting to trunk motoneurons.
- **MMC**: motoneurons of the medial motor column. The medial part (MMCm) innervates ventral axial muscles in mice.
- **PKD2L1**: Polycystin 2 Like 1 Transient Receptor Potential Cation Channel, a molecular marker of Kolmer-Agduhr cells, the cerebrospinal fluid-contacting GABAergic neurons whose cilia in the central canal detects cerebrospinal fluid movements and which send input to the swimming circuitry.
- **RORE**: Related Orphan Receptor Alpha, a molecular marker of spinal neurons relaying cutaneous inputs.
- **RORB**: Related Orphan Receptor Beta, a molecular marker of mouse spinal neurons relaying sensory inputs during locomotion.
- **SHOX2**: short stature homeobox protein 2, a molecular marker of mouse isilaterally projecting excitatory neurons involved in locomotor rhythmogenesis and interlimb coordination.
- **V0**: commissural neurons expressing Develop brain homeobox 1 (Dbx1) and involved in left-right alternation during locomotion.
- **V0C**: cholinergic neurons expressing Dbx1 and pituitary homeobox 2 (PITX2), modulating motoneuron firing during locomotor activity.
- **V0D**: dorsal commissural inhibitory neurons positive for Dbx1, for Paired box protein 7 (PAX7), and involved in the control of fast swimming in zebrafish and in left–right hindlimb alternation at low locomotor frequencies in mice.
- **V0V**: ventral commissural excitatory neurons positive for Dbx1, Homeobox even-skipped homolog protein 1 (Esx1), negative for Paired box protein 7 (PAX7), and involved in slow swimming in zebrafish and in left–right hindlimb alternation.
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in limbed vertebrates, but the lack of genetic tools has long hampered progress. A model is ideally suited for bridging knowledge gaps between cellular function and motor behavior. Nongenetic neural tracing methods and immunofluorescence techniques have long been established in salamanders (e.g., [14,15,55]). In principle, the salamander model is ideally suited for bridging knowledge gaps between cellular function and motor behavior in limbic vertebrae, but the lack of genetic tools has long hampered progress.

Genetic Tools for Salamander Research

A major advance in salamander research during recent years is the extension of the genetic toolbox. Genome and transcriptomes of several salamander species have been sequenced and annotated (e.g., [56–62]). Among salamanders, the Mexican axolotl (Ambystoma mexicanum) and the Iberian newt (Pleurodeles waltl) are currently the two most commonly used species with significant genomic information and several genetically modified lines available. The Iberian newt and the axolotl have similar generation time (9–12 months) [3]. While the axolotl, a neotenic animal, remains fully aquatic during its entire life cycle, the Iberian newt undergoes metamorphosis and walks on land [3,4,10]. Hence, newts are well-suited for studying and modeling of swimming, walking, and the transition between the two (Figure 2A–F). Nevertheless, the molecular resources available from other salamander species are essential to explore both the degree of evolutionary conservations as well as the species-specific innovations that characterize the molecular profile and function of vertebrate locomotor circuits.

Comparative analysis of key marker genes expressed by neuronal subtypes in locomotor circuits indicates a higher degree of conservation between mammals and the Iberian newt, than between Iberian newt and zebrafish (data based on [57], Table 1). In addition, using transgenesis and CRISPR/Cas9-mediated genome editing, it has become feasible to lineage trace cells both in bulk as well as clonally (i.e., identifying which cells originate from one single cell in a particular lineage) (Figure 3F). It has also become routine to carry out systematic gene perturbation studies during both salamander development and regeneration. In the CNS specifically, cell proliferation and migration could be assessed during brain development at clonal resolution (e.g., [63]). Inducible Cre recombinase has been knocked into gene loci to trace cells and to mutate specifically targeted genes during spinal cord regeneration using CRISPR/Cas9-mediated technology (e.g., [64,65]). Altogether, these recent advances provide a solid ground for creating genetically modified salamanders in the near future, suitable for optogenetic and chemogenetic approaches in order to systematically dissect the structure and function of the salamander locomotor circuitry in the intact and regenerated CNS.

Spinal Cord Regeneration in Salamanders

Spinal cord regeneration studies in amphibians have a long history [66]. Among four-legged vertebrates, only salamanders have been found to repair the injured spinal cord throughout their entire life cycle. Although anurans (frogs and toads) have significant regenerative potential as larvae, metamorphosis leads to substantial loss of their regenerative capacity. Two main injury models that are most frequently applied are complete spinal cord transection and tail amputation (e.g., [2]). Salamanders respond to both types of injury by remarkable restoration of central nervous tissue and functionality. In this review, we will focus on complete spinal cord transection.
Salamanders can recover control of locomotor muscles distal (below) the lesion after a few weeks (Figure 3A–C) [12] and this is associated with regeneration of the spinal cord (Figure 3D) [67,68].

A distinctive feature of CNS regeneration in salamanders, as in all regeneration-competent anamniotes, is the lack of glial scar formation after injury (e.g., [69–71]). Most studies in this context indicate an inhibitory role for astrogliosis in the regeneration of the mammalian CNS (e.g., [72]). Recent data in mammals provided alternative views, which suggest that cells in the scar produce factors that can stimulate axon growth and limit secondary loss of axons [73,74]. The absence of scar in regenerative species is generally consistent with the idea that scar formation has an inhibitory function.
A major component of the mammalian scar are cells expressing the intermediate filament, glial fibrillary acidic protein (GFAP) [75], the astrocytes. The salamander CNS is largely devoid of GFAP expressing cells intermingled with neurons both normally as well as after lesion, which may be one important reason behind the absence of injury responsive glial scar formation. In fact, the only cell types that express GFAP in the salamander CNS are the ependymoglial cells that line the cerebrospinal fluid-containing ventricular system (e.g., [3,17]) (Figure 3E).

In terms of morphology and gene expression profile, ependymoglial cells are closely related to the embryonic radial glial cells in mammals [3]. In mammals, except for a few regions in the adult CNS where neurogenesis is maintained postnatally, such as the dentate gyrus of the hippocampus and the lateral ventricles in the forebrain, radial glial cells gradually disappear during
ontogeny (e.g., [76,77]). In contrast, salamanders, as well as other anamniotes, retain ventricular radial glial-like cells in large numbers [2]. In fact, ependymoglial cells line the entire ventricular system in the salamander CNS. Ependymoglial cells have several functions during salamander CNS regeneration. First, they can give birth to new neurons to replace those lost after injury (e.g., [17,78]) and they have been shown to be multipotent progenitors after spinal cord injury [79–81]. In addition, longitudinal extensions of ependymoglial cells provide support for the growing and reconnecting axons in the spinal cord, eventually forming a tube-like structure at the lesion site [82,83]. Interestingly, the regenerating meningeal cells have been proposed to provide guidance to the growing axons [84] (Figure 3E).

Important remaining questions are to what extent the salamander neurons in the CNS are inherently different from their mammalian counterparts in their ability to regrow axons and what are the molecular underpinnings of these differences. While systematic comparisons have not been carried out (to our knowledge), an interesting candidate is the tumour suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN), deletion of which renders regenerative traits to adult mammalian corticospinal neurons [85]. While axonal regrowth and lack of scar formation are common features of anamniotes, there are considerable differences in the patterns of axonal growth. In zebrafish, another commonly used regeneration model, reforming axons are mostly found in the grey rather than the white matter [86]. In salamanders, when tracers were injected into the injured axons, the pattern of labelling suggested that all regrowing axons travelled exclusively in the white matter [84]. Axonal regeneration eventually leads to the recovery of motor function, which requires the \textit{de novo} formation of descending connections and does not seem to rely on changes in spinal cord reflexes [88]. Most outgrowth of axons appear to come from cells around the transection site; however, descending axons that cross the lesion site can have their cell bodies far more rostrally to the lesion site (above the lesion) and originate from the brainstem [12,67,68].

Is spinal cord regeneration a perfect restoration of the original status? Surprisingly, while salamanders display coordinated locomotor movements after regeneration [12] (Figure 3A–C), cellular analyses show incomplete regeneration of neurons (Figure 3D,G–J). The number of neurons giving rise to regenerated descending axons after complete transection at the thoracic/lumbar level was quantified following injections of a neural tracer distally to the lesion in the salamander \textit{Notophthalmus viridescens}. Two months after injury, at a time point the animals showed locomotor recovery, 37% of brainstem cells had regenerated through the lesion compared with the nonlesioned animals (Figure 3I) and 28% of cells from the spinal segments innervating the forelimbs had regenerated through the lesion [67] (Figure 3G). The number of regenerated neurons caudal to the lesion sending ascending projections was not quantified but was reported to be reduced as well [67] (Figure 3H). A question is whether the regenerating axons originate from new neurons appearing following the

| Cell type | Chx10 | Dbx1 | Egr3 | PkD2L1 | Vglut2 | ChAT |
|-----------|------|------|------|--------|--------|------|
| V2a neurons | 69    | 70   | 76   | 74     | 89     | 76   |
| V0 neurons |      | 70   | 76   |        | 88     | 75   |
| Muscle spindles |      |      | 64   |        |        |      |
| Cerebrospinal fluid-contacting neurons (Kolmer-Agduhr cells) | | | | | | |
| Glutamatergic neurons | | | | | | |
| Cholinergic neurons | | | | | | |

| Cell type | Human homology (%) | Mouse homology (%) | Zebralish homology (%) | Iberian newt | Human homology (%) | Mouse homology (%) | Zebralish homology (%) |
|-----------|--------------------|--------------------|------------------------|-------------|--------------------|--------------------|------------------------|
| V2a neurons | 69 | 70 | 59 | Chx10 | 69 | 70 | 59 |
| V0 neurons |      | 70 | 55 | Dbx1 |      | 76 | 55 |
| Muscle spindles |      |      | 64 | Egr3 | 77 | 78 | 64 |
| Cerebrospinal fluid-contacting neurons (Kolmer-Agduhr cells) | | | | PkD2L1 | 74 | 73 | 70 |
| Glutamatergic neurons | | | | Vglut2 | 89 | 88 | 85 |
| Cholinergic neurons | | | | ChAT | 76 | 75 | 69 |

Table 1. Key Molecular Markers of Vertebrate Sensorimotor Circuits Show a Conservation Level That Is Higher between Salamander and Mammal Than between Salamander and Zebrafish (Data Based on [57])
Recovery of voluntary locomotor behaviour after full spinal cord transection

(A) Intact

(B) 14 Days post injury

(C) 126 Days post injury

Cells involved in spinal cord regeneration

(D) Regenerated spinal cord

(E) Meningeal cells

Glial cells

Neuron

Fate mapping using transgenesis

(F) Daughters of cell 1

Daughters of cell 2

Daughters of cell 3

Daughters of cell 4

Regrowth of lesioned fibers after full spinal cord transection

(G) Descending intraspinal fibers

(H) Ascending intraspinal fibers

(I) Descending brain fibers

(J) Ascending sensory fibers

(See figure legend at the bottom of the next page.)
lesion? Whereas the regenerated brainstem projections originate from lesioned brainstem neurons (i.e., not new neurons or collaterals from undamaged neurons), whether all the regrowing axons in the spinal cord originate from lesioned spinal neurons remains to be determined [12,67]. The amount of both white and grey matter was significantly diminished in both the rostral as well as the caudal vicinity of the lesion site (Figure 3D). Hence, it is likely that not all neuronal connections need to be re-formed to achieve functional recovery [67]. Our preliminary computational modelling work suggests a redundancy between sensory feedback and central pattern generators that could explain this observation ([87], see later). Another striking feature is that the ascending sensory axons from the sciatic nerve that travel through dorsal columns in intact animals do not appear to regenerate after spinal cord transection [83,84] (Figure 3J). Whether the sensory information processed distally to the lesion site reaches the brain through newly formed relay circuits derived from other spinal neurons remains to be determined [67]. It will be exciting to decipher in the future the minimal axonal regeneration that is sufficient to provide ascending sensory signals and descending motor commands to generate co-ordinated locomotor movements in salamanders. The combination of the available tools ranging from molecular methods to biorobotics provide an excellent opportunity for addressing this question.

**Salamander Robots, Numerical Models, and Brain–Machine Interfaces**

The computational modelling environment in salamander research is rich at several levels of abstractions, from coupled oscillators to detailed neuron models [4,88,89]. Recently, models for the circuitry underlying locomotor and turning movements in salamanders have also been developed [90,91]. The first model of the salamander spinal cord was based on the idea that the spinal circuits generating the fast swimming movements are inherited from phylogenetically older vertebrates, such as the lamprey, whereas the slower walking movements would result from the interactions of the swimming circuit with slower circuits controlling limb movements [92]. A similar idea was presented in [93]. These hypotheses were then tested using a salamander robot controlled by a spinal cord model based on coupled oscillators, with two additional hypotheses: (i) that the limb oscillators saturate (become tonic or are deactivated) when they receive a descending drive above a threshold; and (ii) that the limb oscillators are intrinsically slower than the axial oscillators when receiving the same level of drive [4]. Steering and speed were controlled by simulated descending drives originating from a simulated brainstem circuitry. The switch from walking to swimming in the salamander semi-intact preparation [13] was recapitulated with the robot when increasing the simulated brainstem activity [4,94] (Figure 4A–B). Slow walking movements are generated with low brainstem drive, which activates the limb oscillators that impose their slow oscillations to the axial oscillators. With high brainstem drive, the limb networks stop oscillating and only the swimming circuit is rhythmically active, producing a travelling wave with fast oscillations. At the cellular level, the putative circuitry underlying gait transition was explored using a brainstem–spinal cord network built with three-compartment Hodgkin and Huxley model neurons with >10 conductances [95]. A single synapse from excitatory neurons of the limb circuit to the swimming circuit was sufficient to recapitulate the switch between walking and swimming.

![Figure 3](image-url)
patterns observed when brainstem activity is increased in semi-intact preparations [13,95]. The neurons involved need to be identified in future biological experiments.

An important issue in locomotor control is the dynamic integration of sensory feedback during locomotion. A simulation study based on spiking neurons controlling a mechanical model of the salamander showed that (proprioceptive) limb sensory inputs detecting the late stance phase are essential to generate the transition from trotting to walking [96]. In another study, dynamical integration of local hydrodynamic force sensing by the spinal cord circuitry was sufficient for swimming to emerge in simulation and in an anguilliform robot driven by a spinal cord model based on coupled oscillators [87]. This intriguing effect results from body asymmetry (in particular a flexible tail fin) coupled with the lag associated with sensorimotor integration (the force sensors are located caudally to the motors). It is preserved even when the spinal cord model is unable to generate oscillatory activity according to our preliminary findings [87]. In other words, sensory feedback and the central pattern generators appear to be redundant mechanisms that both contribute to generate swimming movements [87]. This indicates that the spinal locomotor circuits (or at least the circuits for axial muscles) are highly robust against lesions, which has an important implication for regeneration after a spinal transection. Indeed, it is likely that there are multiple possible regrowth patterns of descending axons that can restore locomotion in salamander. These regrowth patterns might not need to reach very specific target neurons, as long as they can provide sufficient stimulation to the spinal circuits below the lesion to initiate and modulate locomotion. Future modelling studies can investigate this interesting hypothesis.

This richness of the modelling and robotics environment allows for unique work at the interface between biology and robotics, such as brain/machine interfaces. In a recent study, the activity of reticulospinal neurons recorded using calcium imaging in an isolated salamander brain was used to control the locomotor speed and direction of a salamander robot driven by a model of the spinal cord based on coupled oscillators [42] (Figure 4C). The amplitudes of reticulospinal responses were used to control robot speed (Figure 4D). The direction of the robot locomotor movements was driven by the ratio of reticulospinal activity on the left and right sides (Figure 4E). A novel aspect of this approach is the reliance on calcium signals, which are relatively rarely used in the field of brain/machine interfaces. Although calcium imaging signals show slower dynamics than electrophysiological recordings, they present the advantages of providing: (i) the activity of identifiable cell types (based on genetic marker expression and/or projection patterns); (ii) the spatiotemporal organisation of the recorded activity; and (iii) the opportunity to spot neurons that are rarely active. More generally, future closed-loop experiments that interface the salamander nervous system and a robot (or a biomechanical simulation) could be useful for investigating motor control signals in a controlled setting and for designing neuroprosthetic interfaces.

Salamander robots are useful scientific tools beyond the understanding of the architecture of the neural networks. A salamander robot that can closely mimic salamander locomotion was designed based on high-resolution cineradiography recordings of the movements of the salamander skeleton [94] (Figure 4A–B). This robot, initially designed to emulate salamander locomotion, was used as starting point (and point of comparison) for constructing a robotic replica of the Orobates, a stem amniote, and to quantitatively reconstruct the likely walking movements of this primitive tetrapod using its fossilised footprints [97]. This underlines the serendipitous usefulness of robots in several fields, including paleobiology.

Implementing computational models of neural circuits in robots (or in musculoskeletal simulations) offers the interesting opportunity to investigate closed-loop interactions between
Emulating salamander movements with robots

(A) Stepping

(B) Swimming

(C) Brain/robot interface

(D) Control of robot speed with brainstem calcium signals

(E) Control of robot direction with brainstem calcium signals

(See figure legend at the bottom of the next page.)
the nervous system, the body, and the environment, and to suggest new biological hypotheses (for review, see [98,99]). One interesting hypothesis is that the respective role of central pattern generators, sensory feedback, and descending commands has changed during evolution (Figure 5). This hypothesis is built upon ours and others’ work to emulate, using robots and neuromechanical simulations, the locomotor movements of lamprey, salamander, mouse, cat, and human (for review, see [99,100]). From an engineering point of view, the constraints that one faces in the efforts to generate stable locomotion suggest that the complexity of walking on ground and the transition from sprawling to erect postures required an increased role for the sensory inputs and descending commands (Figure 5). Indeed, animals like lamprey, zebrafish, and salamander are mechanically more stable and require less balance control than mammals. In particular, a sprawling animal such as the salamander has a low centre of gravity and a large support polygon (the polygon linking all contacts points to the ground) when moving on ground. It therefore presents relatively little risk of falling and turning over. Mammals such as mouse, cat, and in particular human, have a high centre of gravity and small support polygons. Therefore, they require good proprioceptive and vestibular sensory information to monitor the status of their body and more complex descending commands for visually guided foot placement and for maintaining balance. Our experience in building numerical and robotic models shows that lamprey and salamander locomotion can to a large extent be controlled by central pattern generators in open-loop (i.e., without sensory feedback) and with minimal modulation from descending signals (e.g., with only two drive signals, one to each side of the spinal cord models to modulate speed, heading, and type of gait). In contrast, our work on modelling human locomotion shows that sensory feedback is essential and that central pattern generators are merely beneficial for biped walking [101]. In particular, these analyses indicated that central pattern generators simplify the control of walking speed (compared with purely sensory driven locomotion) and that their contribution is most beneficial when applied to proximal (hip) muscles compared with distal (ankle) muscles. Of note, even though this has not been definitively confirmed, there is ample indirect evidence that central pattern generators exist in humans (for review, see [102]). Our view is therefore that the central (the central pattern generators) and peripheral (sensory feedback loops) control mechanisms in the spinal cord are strongly redundant and therefore offer robustness against lesions. We also hypothesize that all vertebrates share the same building blocks in their spinal cords, but that the respective roles of central and peripheral mechanisms might be different in different animal species and that they have changed during evolution, with generally a more important role of central pattern generators in anamniotes and a more important role of sensory feedback (and descending commands/modulation) in higher vertebrates. Computational methods and robotics will allow one to test this hypothesis and will facilitate investigation of the interplay between the different components underlying locomotion, in particular by taking into account the mechanical properties of the body (e.g., fin properties for swimming [103]) and of the environment (e.g., interactions with granular media like sand [98]).

Concluding Remarks and Future Perspectives

The recent availability of genetic tools in salamanders make this species a unique model organism in which several fundamental aspects of locomotion can be addressed. This

Figure 4. Interfacing Robots with Spinal Cord Models and Brain Activity to Emulate Salamander Locomotion. (A,B) Comparison of snapshots of the salamander Pleurodeles waltl and Pleurobot during walking from the side (A, two panels on the left) and from above (A, two panels on the right) and during swimming (B) (adapted from [94]). (C) Interfacing a salamander brain with a spinal cord model generating the walking pattern controlling Pleurobot movements. (D) Control of robot speed with calcium (Ca\(^{2+}\)) signals from reticulospinal (RS) neurons of the middle reticular nucleus. In the bottom panels, each line represents the Ca\(^{2+}\) response (ΔF/F) for one RS neuron. Stimulation of the mesencephalic locomotor region (MLR) evoked bilateral Ca\(^{2+}\) responses in RS neurons. The average RS activity on each brain side was sent to left and right oscillators of the spinal cord model. Such symmetrical activation of RS cells evoked forward locomotion. The stimulation of the MLR activated RS cells symmetrically and therefore evoked forward locomotion. Increasing the activity of RS neurons on one side with microinjections of glutamate evoked steering movements ipsilaterally to the injection (adapted from [42]). Abbreviations: BL, Body length; SVL, snout-vent length.

Outstanding Questions

What is the organisation of the salamander central pattern generator at the cellular level? Which cells are targeted by descending commands? Which cells are targeted by sensory inputs?

To what extent is the richness of locomotor patterns due to modulation of descending pathways versus modulation of sensory inputs? Are some sensory modalities more important than others in adapting the locomotor patterns to the environment?

After spinal cord lesion, to what extent is the reconnection pattern a replication of the one before lesion? How many different reconnection patterns can lead to functional recovery?

What are the respective roles of the central pattern generator, the sensory feedback, and the descending commands in functional recovery following spinal cord injury?

Can the understanding of functional recovery in the salamander help design robot locomotion controllers that can handle body lesions and loss of communication between robot parts?
includes the evolution of locomotor circuits during the transition from water to land, the identification of the circuits allowing transitions between swimming and walking, and the regeneration of locomotor circuits after lesion in tetrapods. Salamander research also benefits from a rich computational-modelling environment. With the tools available today, it is becoming feasible to make the communication between biology and modelling/robotics bidirectional and dynamic. For example, an integrative approach could involve a neuromechanical simulation environment that replicates real-life animal experiments in silico, to test and validate hypotheses gained from optogenetic and chemogenetic manipulations of targeted circuits in the intact spinal cord and at various time points after injury. Such knowledge should allow building full-scale numerical models of the locomotor system in the intact and regenerated spinal cord. Such experiments could be used to delineate a minimal reconnection map between spinal neurons after spinal cord transection that is required to recover major functions. These approaches could also help inform experiments examining to what extent recovery is associated with the rewiring of a specific circuitry and to what extent the system allows deviation from the original status. Further genetic, systems neuroscience, and modelling studies of the salamander will help in decoding how these mechanisms are implemented and how they contribute to this species’ remarkable ability to recover after spinal cord lesions. In addition, bio-inspired robotics could also pave the way for the development of robot controllers that can adapt to lesions and morphological changes, as salamanders do (see Outstanding Questions).

Figure 5. Hypothesis about the Evolution of Locomotor Control in Vertebrates. As a first approximation, locomotion in vertebrates is due to the interaction of musculo-skeletal properties, sensory-feedback loops, central pattern generators, and descending commands from supraspinal centres. We propose that the increase in the complexity of locomotor performance in different animal species increased the role of sensory feedback and descending commands in the control of locomotor movements (adapted from [100]). From aquatic to terrestrial vertebrates, the complexity of locomotor programs increased following the appearance of the limbs and the need to coordinate axial and limb movements. In mammals, the higher centre of gravity constitutes an additional level of complexity to walk while maintaining balance. Such a task is even more difficult to achieve in bipeds. We hypothesize that, while all vertebrates share similar circuits, the role of central pattern generators might be more important in anamniotes than in amniotes, relative to sensory feedback and descending commands. Finally, we would like to stress the importance of musculo-skeletal properties in locomotion (which we have hence included in this diagram). Indeed, the musculoskeletal system has its own complex dynamics that is well-tuned for locomotion, see, for instance, the surprising swimming movements of a dead trout in a turbulent flow [138]. The body represents an essential bridge between the nervous system and the environment and, hence, is a key component to consider when studying interactions between central and peripheral mechanisms.
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