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Resilience of Neural Networks for Locomotion

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Abstract

Locomotion is an essential behavior for the survival of all animals. The neural circuitry underlying locomotion is therefore highly robust to a wide variety of perturbations, including injury and abrupt changes in the environment. On the short term, fault tolerance in neural networks allows locomotion to persist immediately after mild to moderate injury. On the longer term, in many invertebrates and vertebrates, neural reorganization including anatomical regeneration can restore locomotion after severe perturbations that initially caused paralysis. Despite decades of research, very little is known about the mechanisms underlying locomotor resilience at the level of the underlying neural circuits and coordination of central pattern generators (CPGs). Undulatory locomotion is an ideal behavior for exploring principles of circuit organization, neural control and resilience of locomotion, offering a number of unique advantages lending experimental accessibility and modeling tractability. In comparing three well-characterized undulatory swimmers, lampreys, larval zebrafish, and *Caenorhabditis elegans*, we find similarities in the manifestation of locomotor resilience. To advance our understanding, we propose a comparative approach, integrating experimental and modeling studies, which will allow the field to begin identifying shared and distinct solutions for overcoming perturbations to persist orchestrating this essential behavior.

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Introduction

Neural systems possess remarkable resilience, leading to persistence of effective behaviors despite alterations in connectivity, activity, or environment. Nowhere is this more apparent than in locomotion, a behavior that is critical for gathering food, evading predation, finding mates, and overall survival. Across phyla, following mild or moderate injury, motor circuits are fault tolerant -- often continuing to generate adequate locomotor behaviors immediately after the perturbation. Following severe injuries to the invertebrate nerve cord or vertebrate spinal cord, many species recover some degree of locomotion through longer-term reorganization of motor circuits via neural regeneration and other physiological mechanisms (Yanik *et al.*, 2004; Morgan and Shifman, 2014; Rasmussen and Sagasti, 2016; Morgan, 2017). Even in humans, where spinal cord damage notoriously results in permanent loss of movements, recent work shows that epidural stimulation coupled with exercise training can overcome paralysis in some chronic spinal cord injury patients, resulting in adaptive control of locomotion (Harkema *et al.*, 2011; Angeli *et al.*, 2018; Wagner *et al.*, 2018). Thus, resilience in locomotor neural networks appears to be a highly conserved phenomenon that enables animals to overcome a wide range of injuries and conditions, thereby enabling persistence of the essential behavior. However, the neural mechanisms underlying locomotor resilience are still poorly understood. Here, we review some fundamental observations on locomotor resilience and discuss open questions that are ripe for mechanistic exploration.

In all animals studied to date, descending input activates neuronal oscillators, termed central pattern generators (CPGs), to generate rhythmic locomotion (Brown, 1911; Wilson, 1961; Marder and Bucher, 2001; Ijspeert, 2008; Bucher *et al.*, 2015). While CPGs can generate a motor pattern without sensory input, they also receive proprioceptive sensory inputs that can strongly modulate or stabilize their output (Wilson, 1961; Rossignol *et al.*, 2006). The motor program activates muscles in a spatiotemporal sequence for propulsion, as well as a more subtle tuning of the body's effective mechanical responses to its environment (Blight, 1977; Long, 1998; Berri *et al.*, 2009; Tytell *et al.*, 2018), a coordination that produces fluent and robust motion (Dickinson *et al.*, 2000). Propagation of alternating activity of antagonistic muscles is a common feature across species of undulators and legged locomotors alike (Cohen, 1988; Grillner and El Manira, 2020). Rostro-caudal coupling of CPGs along the body axis enables the propagation of such contralaterally alternating neural and muscle activity **(Figure 1A)**, highlighting the common organizing principles of the neural circuits and their function.

For undulatory locomotion, the coordinated metachronal patterns are relatively simple and likely ancestral. Undulators, from microscopic nematodes to 13 meter-long extinct snakes, generate thrust by propagating mechanical waves along their body, most commonly against the direction of locomotion (Gray, 1953; Cohen and Boyle, 2010). Across this range of sizes, animals experience vastly different physics, yet produce similar movements, suggesting similar requirements for pattern generation and resilience, and, potentially, comparable underlying mechanisms. All locomotion, and undulatory movement in particular, arises from the interaction between the dynamics of the body and the physics of the environment, an interaction that places strong constraints on the movement. When analyzing undulatory locomotion, the body axis provides a convenient reference for

interpreting and comparing muscle activity and locomotor phase across individuals as well as across disparate species. Furthermore, the neural and muscular activity that propagate the traveling wave along the neuraxis is cyclic, making it highly amenable to imaging, physiology, and behavioral recording, as well as analysis and comparison across individuals and species.

We focus on undulatory locomotion in three well-established model systems: lampreys (family *Petromyzontidae*), larval zebrafish (*Danio rerio*), and nematodes (*Caenorhabditis elegans*) **(Figure 1B)**. These model species offer a range of sizes and speeds, and are complementary in our current knowledge and accessibility to techniques. Their nervous systems differ in size, number of neurons and connections, and in many details, but analogies can be drawn among the core components such as descending input, local circuit elements (e.g. cross-inhibition, proprioception), motor-units, and axial musculature **(Figure 1A)**. The lamprey CNS has experimental advantages that include large, identified neurons in the brain and spinal cord with known roles in locomotion, which facilitates imaging and physiology, and it is perhaps the best established model for neural regeneration of the three species (Rovainen, 1976; Selzer, 1978; Cohen *et al.*, 1986; Davis and McClellan, 1994; Buchanan, 2001; Oliphint *et al.*, 2010). The larval zebrafish is transparent and amenable to genetics and electrophysiology, as well as modern methods of optogenetics (for circuit activation or inactivation) and functional imaging while simultaneously measuring behaviors in semi-restrained and freely moving animals (McLean and Fetcho, 2011; Portugues *et al.*, 2013; Albadri *et al.*, 2017; Severi *et al.*, 2018; Marques *et al.*, 2020). *C. elegans* offers a relatively small, comprehensively identifiable and fully mapped nervous system (White *et al.*, 1986; Haspel and O'Donovan, 2011; Reilly *et al.*, 2020; Brittin *et al.*, 2021), as well as established genetic and transgenic methods (Biron and Haspel, 2015; Corsi *et al.*, 2015; Haspel *et al.*, 2020), and optical transparency permitting analysis of circuit function and behavior in individuals and populations of animals. This level of details also presents an opportunity for whole-animal modeling (Bargmann and Marder, 2013; Sarma *et al.*, 2018; Cohen and Denham, 2019). The nematode locomotion interneurons are analogous to reticulospinal neurons that provide descending input; its motoneurons are analogous to spinal interneurons, integrating sensory and descending inputs, while generating and coordinating motor programs; and its muscle arms are analogous to spinal motoneurons (Haspel *et al.*, 2020). While we focus on these three models, where possible we also extend these comparisons to other vertebrate and invertebrate undulatory swimmers, as well as legged animals.

Understanding resilience of locomotion in these three undulators, therefore, provides an opportunity to study the interplay between neural control, biomechanics and sensory feedback. While undulatory locomotion has long provided an important foundation for understanding the basic neural mechanisms underlying locomotor behaviors, comparatively little is known about the neural mechanisms that restore locomotor behaviors after perturbations such as injury or environmental changes. We thus propose new avenues of investigation that build upon that foundation to identify both shared and distinct mechanisms underlying resilience of undulatory locomotion.

Locomotor Networks are Fault Tolerant to Acute Perturbations

Locomotor circuits often continue to function immediately after perturbation or failure of individual components. Such fault tolerance appears to be conserved, because partial lesioning of either descending axons or local circuit neurons in lampreys, zebrafish (vertebrates), and *C. elegans* (an invertebrate) does not suddenly halt movement, but instead results in altered but functional locomotor behaviors. The disruption of locomotion can range in severity depending on which neurons are perturbed, allowing the behaviors to continue despite alterations in speed, body form, or gait.

Fault tolerance is well-described in lampreys and larval zebrafish, two leading models for the study of vertebrate locomotion from cells through circuits to behavior (Buchanan, 2001; Fetcho and McLean, 2010; Berg *et al.*, 2018; Grillner and El Manira, 2020). Lampreys can retain functional swimming immediately following substantial damage to their spinal cords, before any regeneration can occur. For example, partial transection of medial spinal tracts, comprising descending reticulospinal axons, acutely alters but does not halt swimming **(Figure 2A)** (McClellan, 1988). This result can be mimicked in a simulated model of a swimming lamprey, where active force generation stops at the lesion site, but the mechanical wave propagates passively to the tail **(Figure 2B)**. If the mechanical wave propagates across the lesion, local sensory input may be sufficient to activate and synchronize spinal circuits below the lesion even without descending control (Wallen, 1982). Similarly, hemi-lesions sparing half of the rostral spinal cord often result in seemingly normal swimming without any directional bias (McClellan, 1988), as well as normal alternating muscle activity (Shaw *et al.*, 2010). In comparison, partial lesions of the lateral spinal tracts in lampreys cause a loss of muscle activity and swimming (McClellan, 1988; Shaw *et al.*, 2010), suggesting that lateral spinal tracts are more important for maintaining locomotion. While the reason for this difference is unknown, one possibility is that the lateral tracts in lamprey spinal cord may comprise the axons of "start" or "maintain" RS neurons that fire at the beginning and throughout the duration of swimming activity (Juvin *et al.*, 2016), which could be tested by tracing axonal projection patterns from the midbrain somata to their respective positions within the spinal cord.

Similarly, larval zebrafish are quite tolerant to spinal cord damage, as are many species during early development. Single-cell somatic ablations of subsets of descending reticulospinal neurons, or caudal spinal transection **(Figure 2C)**, do not stop locomotion but instead change locomotor frequency, timing, or swim speed (Orger *et al.*, 2008; Huang *et al.*, 2013; Severi *et al.*, 2014; Liu *et al.*, 2019). Likewise, removal of specific classes of spinal interneurons has demonstrated the resilience of swimming, which can persist in the absence of local circuit elements. For example, ablation of V2a glutamatergic interneurons alters swim speed without a total loss of swimming ability (McLean *et al.*, 2007; McLean *et al.*, 2008; Sternberg *et al.*, 2016; Menelaou and McLean, 2019). Silencing or activation of GABAergic and glycinergic interneurons can impact swimming speed, cycle period, or rostral-caudal propagation while locomotion is maintained (Fidelin *et al.*, 2015; Callahan *et al.*, 2019; Kimura and Higashijima, 2019; Satou *et al.*, 2020). In fish and amphibians, a pair of large bilateral hindbrain neurons called Mauthner cells (M-cells) mediate the fastest of startle responses (Korn and

Faber, 2005; Sillar, 2009; Hale *et al.*, 2016), and are likely necessary for any escape response (Hecker *et al.*, 2020b). Yet even after M-cell ablation and loss of the fast startle response, fish retain the ability to perform other types of locomotor behaviors including swimming, demonstrating the complexity of the circuitry underlying distinct types of locomotor movements (Hecker *et al.*, 2020b); when one type is lost, others may persist.

In *C. elegans*, interneuron axons along the ventral nerve cord provide the main source of descending input to locomotion motoneurons (Chalfie *et al.*, 1985; White *et al.*, 1986; Altun *et al.*, 2009; Cohen and Denham, 2019; Haspel *et al.*, 2020). Ablation or inactivation of specific classes of interneurons induce direction-related effects, leaving one direction intact (Chalfie *et al.*, 1985; Wicks and Rankin, 1995; Kawano *et al.*, 2011), while ablating a single GABAergic head interneuron (namely, RIS) acutely reduces stopping events (Turek *et al.*, 2013). Only ablation of all premotor interneurons stops locomotion entirely (Zheng *et al.*, 1999; Gao *et al.*, 2018). Within the local circuits of the ventral nerve cord, ablation of about half of the 56 neurons that comprise two of six cholinergic and excitatory motoneuronal classes, eliminates one direction of locomotion, but spares the other (Chalfie *et al.*, 1985). Similarly, eliminating or inactivating all 19 neurons that comprise the two GABAergic inhibitory motoneuronal classes (or interrupting the synthesis of GABA) eliminates rapid crawling and swimming, but leaves slow locomotion intact **(Figure 2D)** (McIntire *et al.*, 1993; Deng *et al.*, 2021). Virtual ablations in computational models suggest a number of subtle and redundant inhibitory mechanisms (McIntire *et al.*, 1993; Deng *et al.*, 2021), which have yet to be tested experimentally.

Across the animal kingdom, such fault tolerance within locomotor networks is not limited to undulatory swimmers. For example, the ophiuroid brittle star, an echinoderm, produces highly modified yet effective gaits of locomotion following a series of amputations that sequentially reduce the number of arms from six to one (Kano *et al.*, 2019). Similarly, many crabs and spiders change locomotor patterns to move effectively with multiple legs amputated (Pfeiffenberger, 2017; Wilshin *et al.*, 2018). The new, compensating, motor programs are generated immediately and innately. Another example of locomotor fault tolerance occurs during extreme perturbations in external forces. For example, zebrafish, lungfishes, and *C. elegans* all maintain effective locomotion when researchers change the viscosity of their typical substrates (e.g. water or other Newtonian and non-Newtonian fluids), even by several orders of magnitude (Horner and Jayne, 2008; Berri *et al.*, 2009; Fang-Yen *et al.*, 2010; Danos and Lauder, 2012). Fish can also swim efficiently even in extremely turbulent water (Liao, 2007). Impressively, when a running cockroach experiences a lateral force more than 10 times larger than its normal thrust, caused by a miniature backpack cannon, their gait is only affected for a single step cycle (Jindrich and Full, 2002). This gait correction is too quick for neuronal feedback and is likely mediated by the biomechanics of the legs and body. Even in mammals such as mice, rats, and cats, partial spinal lesions often result in only transient changes in locomotion with some functional recovery and coordination returning over several weeks to months (Rossignol *et al.*, 2009; Gorska *et al.*, 2013). Acute spinalized cats treated with the noradrenergic receptor agonist Clonidine can resume treadmill walking within hours post-lesion (Forssberg and Grillner, 1973). Thus, upon acute perturbations, the neural networks and body mechanics supporting locomotion rapidly compensate in order to persist the orchestration of this essential behavior, and this phenomenon appears to be broadly conserved across many species.

Despite the numerous examples of fault tolerance in both invertebrates and vertebrates, there is surprisingly little known about the underlying neural and biomechanical mechanisms that support this type of acute resilience. Given all the different locomotor modalities and body plans, it is entirely possible that multiple, disparate mechanisms are deployed. It is generally assumed that fault tolerance within neural systems can emerge from a redundancy of elements with similar or overlapping functions, so that paralysis will occur only when all redundant elements are lost. For example, in larval zebrafish two morphologically and genetically distinct classes of excitatory interneurons are both recruited during slow speeds of locomotion (McLean *et al.*, 2008; Menelaou and McLean, 2019); this circuit redundancy may be what allows any persistence of the slow locomotor network if one class is damaged. In theory, paralysis could occur with or without gradual degradation of the behavior, though the nature of degradation has not been rigorously tested. Another hypothesis, is that there may be rapid cellular and synaptic compensatory changes in the locomotor network, producing alternative activity patterns that allow the behaviors to persist despite the loss of select inputs. For example, such compensation could be driven by uninjured CPGs or local and global sensory feedback. Broadly speaking, control theory provides insights into how appropriate feedback can compensate for the effects of damage to a mechanical or electrical control system (Ashby, 1956; Cowan *et al.*, 2014), e.g. by maintaining robust (homeostatic) functionality within a dynamic range. In computational models, the bistability of *C. elegans* motoneurons (Boyle *et al.*, 2012) is consistent with such enhancement of dynamic range. Moreover, proprioceptive sensing may be able to produce or maintain appropriate movements, even in the absence of neural coupling along the body, or across the two sides of the body. For example, immediately after spinal cord transection, which completely disconnects descending input, eels produce appropriately synchronized muscle activity below the lesion, suggesting that proprioceptors can activate the local CPG and synchronize it to passively propagated mechanical inputs (Wallen, 1982). Leeches also use the mechanical wave to synchronize body segments when the nerve cord is transected (Yu *et al.*, 1999). While the roles for proprioception in normal locomotion are still being investigated, even for the well-characterized lamprey, zebrafish and *C. elegans* nervous systems (Daghfous *et al.*, 2016; Fouad *et al.*, 2018; Knafo and Wyart, 2018), it is likely that sensory activation plays a significant role in overcoming acute inactivation of descending input. Computational models have helped elucidate the conditions under which proprioceptive control suffices to generate undulations across a wide range of environmental and internal parameters, even with all inhibitory neurons ablated *in silico* (Boyle *et al.*, 2012; Denham *et al.*, 2018; Deng *et al.*, 2021).

Locomotor Networks Undergo Long-Term Reorganization to Restore Function

Other forms of locomotor resilience are observed with longer-term neural circuit reorganization that occurs after injury, which includes regenerative mechanisms. In response to severe lesions that cause paralysis, neural networks in many non-mammalian species spontaneously reorganize both anatomically and functionally (Morgan and Shifman, 2014; Rasmussen and Sagasti, 2016; Morgan, 2017), ultimately restoring locomotor behaviors ranging from mildly dysfunctional to functional and indistinguishable from control.

Undulatory swimmers have provided foundational studies on long-term functional reorganization of locomotor networks. In lampreys, complete transection of the rostral spinal cord leads to immediate paralysis, after which locomotor behaviors like swimming and burrowing gradually return over the course of a few months **(Figure 3A, top)** (Rovainen, 1976; Selzer, 1978; Cohen *et al.*, 1986; Oliphint *et al.*, 2010; Katz *et al.*, 2020). Remarkably, lampreys can recover nearly normal swimming after one or two complete spinal transections **(Figure 3A-B)** (Hanslik *et al.*, 2019), though with slower swim speed and mildly altered body kinematics (Oliphint *et al.*, 2010; Fies *et al.*, 2021). Functional recovery occurs even when only 30-50% of the descending reticulospinal axons regenerate across the lesion site, re-grow in atypical paths, and terminate prematurely **(Figure 4A)**, making only a few, small synapses (Yin and Selzer, 1983; Davis and McClellan, 1994; Oliphint *et al.*, 2010). In addition to regeneration of descending inputs, altered intrinsic and synaptic properties within local spinal circuits contribute to locomotor recovery in lampreys (Cooke and Parker, 2009; Becker and Parker, 2019). Such physiological changes occur both above and below the lesion site and are dynamic over time (Parker, 2017). One crucial shift, in our view, is the increase in sensitivity of local proprioceptive sensors (Hoffman and Parker, 2011), which increases the importance of mechanical interactions that maintain function. Despite detailed knowledge of how some of the neural connections in the lamprey CPG reorganize after injury through axon regeneration and physiological compensation, the understanding of functional recovery at the network level is lacking.

Zebrafish and *Xenopus laevis* tadpoles also show remarkable resilience in response to removal of descending inputs, particularly when disrupted at early developmental stages. Both larval and adult zebrafish demonstrate robust regeneration and functional recovery after complete spinal transection, aided by glia and a dynamic immune response (Goldshmit *et al.*, 2012; Becker and Becker, 2014; Briona and Dorsky, 2014; Tsarouchas *et al.*, 2018). *Xenopus* can recover from complete transection with restored locomotion as a tadpole, but not as an adult frog, due to metamorphosis-induced changes in the transcriptional program that subsequently limits axon regeneration (Gibbs and Szaro, 2006; Gibbs *et al.*, 2011; Belrose *et al.*, 2020). In adult zebrafish, transection of the caudal spinal cord does not halt swimming, due to intact rostral CPGs, but results in full paralysis past the lesion site that gradually recovers over 4-6 weeks until the animals swim indistinguishably from controls (van Raamsdonk *et al.*, 1998; Dias *et al.*, 2012). Supporting locomotor recovery, regeneration of descending axons past the lesion is robust in both fish and amphibia, but with sparse connections relative to the uninjured spinal cord (Gibbs and Szaro, 2006; Goldshmit *et al.*, 2012; Becker and Becker, 2014). In larval zebrafish, the M-cells do not easily regenerate upon spinal lesion, unless treated with a cAMP analog (Bhatt *et al.*, 2004), highlighting one of the few known pathways that promotes axon regeneration from invertebrates to mammals (Hannila and Filbin, 2008; Ghosh-Roy *et al.*, 2010). Interestingly, M-cells regenerate more robustly when lesioned closer to the soma, and short latency startle responses are restored **(Figure 3C-D)**, even when the M-cell axon regrowth is aberrant **(Figure 4B)** (Hecker *et al.*, 2020a).

Similarly in *C. elegans,* behavioral recovery occurs after only partial cellular regeneration of ventral cord neurons (Yanik *et al.*, 2004) or aberrant regeneration **(Figure 4C)**. Following laser microsurgery on ventral cord neurons, the vast majority of proximal commissures (>80%) regrow towards the dorsal cord within 24 hours (Yanik *et al.*, 2004; Hammarlund *et al.*, 2009), while the distal portion survives microsurgery and sometimes reconnects (Ohnmacht *et al.*, 2016), thus restoring avoidance

behavior (Yanik *et al.*, 2004). *A priori*, the recovery of normal locomotion suggests that some ventral cord neurons regain function. The likelihood of partial or complete regeneration seems to depend on neuronal classes (Gabel *et al.*, 2008; Harreguy *et al.*, 2020). Moreover, functional but uncoordinated locomotion is one of the most prevalent phenotypes following unbiased backward genetics screens, ever since *C. elegans* was established as a prominent neurogenetic model (Brenner, 1974). With a large variety of underlying causes, from impairments in neural development and synaptic transmission to cuticular defects and body shape, the 132 so-called '*unc*' (uncoordinated) mutant strains of animals exhibit abnormal locomotion phenotypes ranging from very subtle changes in the locomotion pattern to full paralysis, which occurs in only a few mutant strains. This variety demonstrates a remarkable ability to overcome severe perturbations by mechanisms that are likely a combination of developmental reorganization and compensatory proprioception.

Beyond undulatory locomotors, long-term functional reorganization and behavioral recovery of locomotion occur widely across vertebrate taxa. For example, many fishes, amphibians and reptiles achieve functional recovery of locomotion after spinal lesion, supported by regeneration of descending axons (Tanaka and Ferretti, 2009; Morgan and Shifman, 2014; Rasmussen and Sagasti, 2016). After spinal cord crush injuries in adult goldfish, startle responses recover but often with lower probability and longer latency, even under conditions of aberrant M-cell regeneration, suggesting compensatory mechanisms (Zottoli *et al.*, 1994; Zottoli and Freemer, 2003). Adult salamanders recover undulatory swimming after spinal transection, supported by descending axon regeneration, but with altered swimming kinematics (Davis *et al.*, 1990; Chevallier *et al.*, 2004; Zukor *et al.*, 2011). Similarly, coordinated overground stepping is also restored after complete spinal transection in salamanders and turtles, but with long-term changes in stepping kinematics (Chevallier *et al.*, 2004; Rehermann *et al.*, 2009). Interestingly, in salamanders, the long-term deficits in locomotor kinematics are more pronounced for swimming recovery than for stepping, indicating differences in the adaptive plasticity mechanisms between the two locomotor modalities (Chevallier *et al.*, 2004). Multiple studies in salamanders indicate lack of sensory axon regeneration (Stensaas, 1983; Chevallier *et al.*, 2004; Zukor *et al.*, 2011), suggesting a lack of mechano-sensory coupling across the lesion that may occur in lampreys and other anguilliform fishes and therefore distinct mechanisms (Wallen, 1982). Even in spinal transected neonatal rats (but not adults), stepping is restored, and this occurs in the absence of axon regeneration (Tillakaratne *et al.*, 2010).

In all animals studied thus far, locomotor networks that restore behaviors are both anatomically and functionally reorganized, often dramatically, lending support for the notion that the regenerated spinal cord is a "new" locomotor circuit (Bradbury and McMahon, 2006; Blesch and Tuszynski, 2009; Parker, 2017). Conserved molecular pathways that promote axon regeneration across both invertebrate and vertebrate species are emerging, including cAMP and regeneration-associated genes (which are transcription factors) (Bhatt *et al.*, 2004; Ghosh-Roy *et al.*, 2010; Lau *et al.*, 2013; Chandran *et al.*, 2016; Herman *et al.*, 2018). However, with the exception of the lamprey model, the neurophysiological mechanisms underlying functional recovery of locomotor behaviors in most other species remain vastly underexplored, leaving a significant gap in our understanding of resilience mechanisms.

Future Directions

Despite decades of research, we still have only a limited and rudimentary understanding of the neural circuit and network mechanisms that underlie both short-term fault tolerance and longer-term functional reorganization in locomotor systems. Even in undulatory locomotors where these phenomena are fairly well described, it is not understood how central pattern generation, circuit function, and active wave propagation are restored after injury at the neural network level. While excellent foundational work has been done on plasticity of individual cell types within lamprey and zebrafish spinal circuits (Yin and Selzer, 1983; Becker and Becker, 2014; Becker and Parker, 2019; Hecker *et al.*, 2020a), much less is known about network-level plasticity across neuronal populations or contributions of other local circuit components in any of our models. In *C. elegans*, no studies have recorded network activity, functional reorganization, and behavior in the same animals during regeneration, nor has regeneration of premotor interneurons been tested. To move the field forward will therefore require revisiting these phenomena with new methods that permit precise neuronal lesion and simultaneous assessment of neural network activity and behavioral output.

We therefore suggest a synergistic and comparative approach that begins with lamprey, larval zebrafish, and *C. elegans*, leveraging the foundational work on locomotion in these undulatory swimmers. To achieve a better understanding of the underlying neural circuit mechanisms, both shared and distinct, will require experimenters to perform similar types of ablations to analogous neural circuit elements, and observe the physiological and behavioral consequences both acutely and over time as the neural circuits functionally reorganize. A variety of optogenetic inactivators and cell-ablation tools will reduce experimental barriers across models when targeted to analogous neuronal classes via gene editing technologies such as CRISPR (Sternberg *et al.*, 2016; Kimura and Higashijima, 2019; Liu *et al.*, 2019; Antinucci *et al.*, 2020). Techniques like GRASP (GFP Reconstitution Across Synaptic Partners) are becoming more widespread and will provide new ways to determine connectivity within networks (Feinberg *et al.*, 2008; Kishore *et al.*, 2020). To measure circuit dynamics and reorganization in real time, the rise of all-optical approaches and whole nervous system imaging in zebrafish and *C. elegans* combined with microscopy advances to visualize large volumes and with moving animals lend the ability to see near simultaneous pan-neuronal activity (Ahrens *et al.*, 2013; Kim *et al.*, 2017). Although lagging behind *C. elegans* and zebrafish, genetic advances in lamprey will facilitate comparable studies (Kusakabe *et al.*, 2003; York and McCauley, 2020), ideally when combined with new optical approaches to visualize neural activity within larger tissue volumes (Abrahamsson *et al.*, 2013). Voltage imaging will complement calcium imaging with higher temporal resolution and recording of membrane hyperpolarization following constant improvements in sensors and optics (Mollinedo-Gajate *et al.*, 2019). Such advances in new imaging technologies will foster more synergy between model systems.

Computational modeling also presents a powerful approach where the interplay of neural network activity, functional reorganization and behavioral output of undulatory swimmers can be explored and then used for predictive testing. Early models of lamprey elegantly captured fictive traveling wave in terms of weakly coupled neural oscillators (Cohen *et al.*, 1982), suggesting that the traveling wave is formed by coordinating the patterns along a chain of oscillators. Later experimental work showed that coupling is strong (Kiemel *et al.*, 2003), but models based on the weak coupling assumption have proven accurate nevertheless (Varkonyi *et al.*, 2008). Each oscillator is highly

nonlinear, producing stable oscillations with frequencies and patterns that can be tuned and dynamically modulated, and combined to produce a rich repertoire of behaviors. Two principles — local neurons or neural circuits acting as relaxation oscillators, and weak coupling between these oscillators—have generalized to other locomotor systems and have been pivotal in developing mathematical models of undulations, from fish (Kopell, 1987) to worms (Ji *et al.*, 2020). They have also inspired a generation of biologically inspired robots of undulators, crawlers and legged locomotors (Ijspeert *et al.*, 2007; Ding *et al.*, 2013; Dutta *et al.*, 2019), and have provided key insights into possible mechanisms of resilience (Sproewitz *et al.*, 2008).

There have been recent advances in integrative models that couple different aspects of neural signaling, muscle mechanics, material properties of the animal's body with external fluid mechanics and sensory feedback in lampreys (Hamlet *et al.*, 2018; Tytell *et al.*, 2018), other fishes (Gazzola *et al.*, 2015), and *C. elegans* (Boyle *et al.*, 2012; Denham *et al.*, 2018; Izquierdo, 2019; Deng *et al.*, 2021). In these models, as in the animals, waveform of the swimmer is not pre-set, but emerges from the coupled neuromechanical system, providing a direct comparison with experimental measurements of undulatory kinematics. *In silico* injuries to the neural network may be simulated by adjusting the topology and strengths of the connections, resulting in altered body kinematics **(see Figure 2B)**. This computational testbed may then be used to probe numerous neural circuit reorganization strategies that could restore locomotor behavior, in ways not possible in a laboratory, either due to limitations in our ability to target the biological system or due to the labor-intensive nature of physiology and imaging experiments. The results of the computational experiments, comparing hypotheses, and sweeping over synaptic strengths and connectivities, proprioceptive mechanisms, and material properties, in both intact and injured models, will continue to provide insight and guide further lab experiments. Comparing models of the different organisms, particularly by reduction to approximate models such as phase-oscillator models of CPGs, embedded within a physical body, can illuminate shared (perhaps conserved) and distinct principles of locomotor resilience across scales and evolutionary history.

Summary

Resilience of motor systems is not only ubiquitous; it is a defining characteristic of animal behavior and their nervous systems. Identifying conserved and distinct mechanisms that underlie resilience hold promise and insight for design of autonomous vehicles, robots, and therapeutic approaches (Boyle *et al.*, 2013; Ijspeert, 2014; Iosa *et al.*, 2016; Courtine and Sofroniew, 2019).

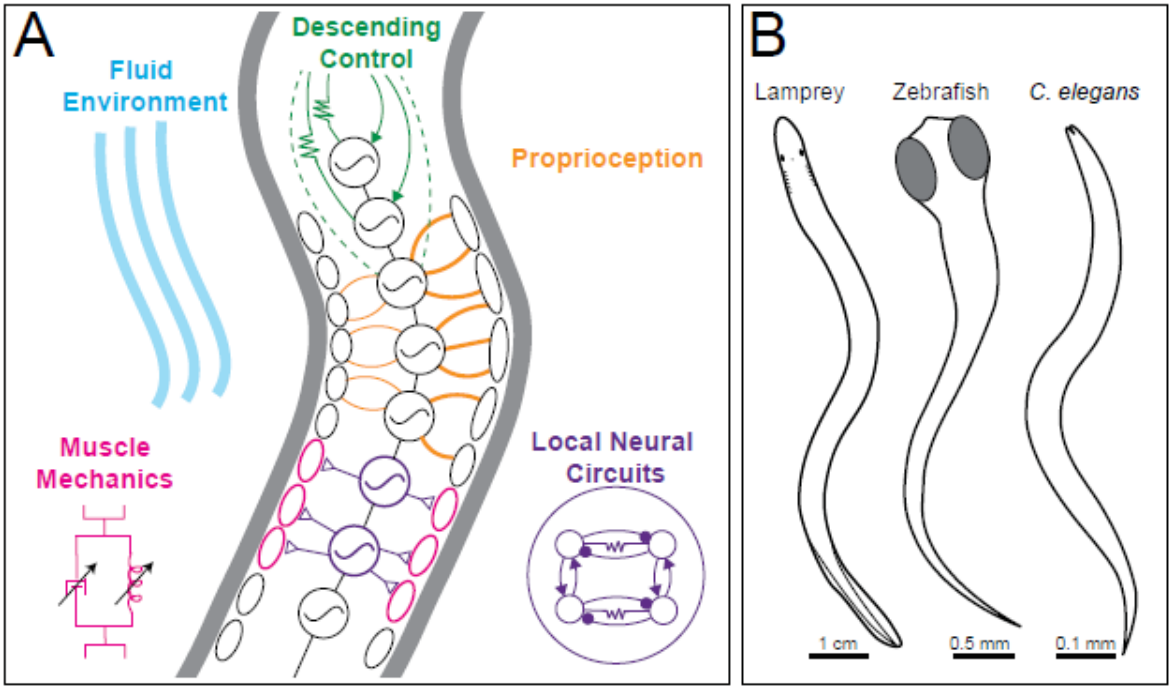


Figure 1. Undulatory locomotion. A. Neural circuits that control locomotor behaviors. Descending neurons activate rostro-caudally coupled central pattern generators (CPGs), resulting in propagation of contralaterally-alternating muscle contractions that is tuned by proprioceptive feedback. **B.** Lampreys, larval zebrafish, and *C. elegans* use similar axial undulations to move in their environment, despite significant differences in size, overall nervous system organization, and fluid dynamics.

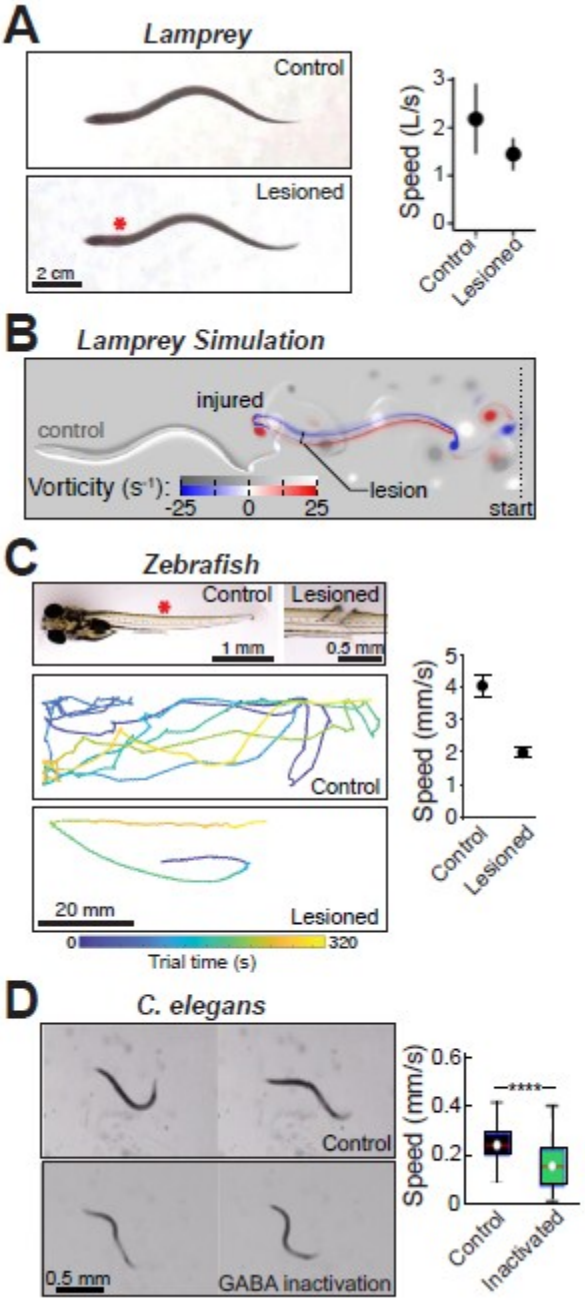


Figure 2. Fault tolerance in lampreys, larval zebrafish, and *C. elegans*. A. *(Left)* Uninjured Control lamprey before and 2 hours after partial lesioning of the medial spinal cord. Asterisk = lesion site. *(Right)* Lampreys continue to swim robustly, but with reduced swim speed (ANOVA $p<0.005$). L=body length. (Morgan, Tytell labs) **B.** Simulation of a swimming lamprey. The injured lamprey, which has purely passive mechanical wave propagation below the lesion, swims slower. Vorticity, a measure of fluid motion, is shown in red and blue or shades of gray. (Hamlet, Fauci, Tytell). Rostral is to the left in panels A-B. **C.** Similarly, larval zebrafish lesioned at age 5 days post-fertilization are able to swim 24 hours after a caudal spinal cord lesion, albeit with longer stops (paths over 320 seconds) and reduced mean swim speed over the trial period. (Gowda and Mahajan, Severi lab; FishTracker2 software provided by Michael Orger lab) **D.** *C. elegans* also swim robustly but more slowly immediately after optogenetic inactivation of all GABAergic inhibitory neurons (Adapted from Deng *et al.,* 2021).

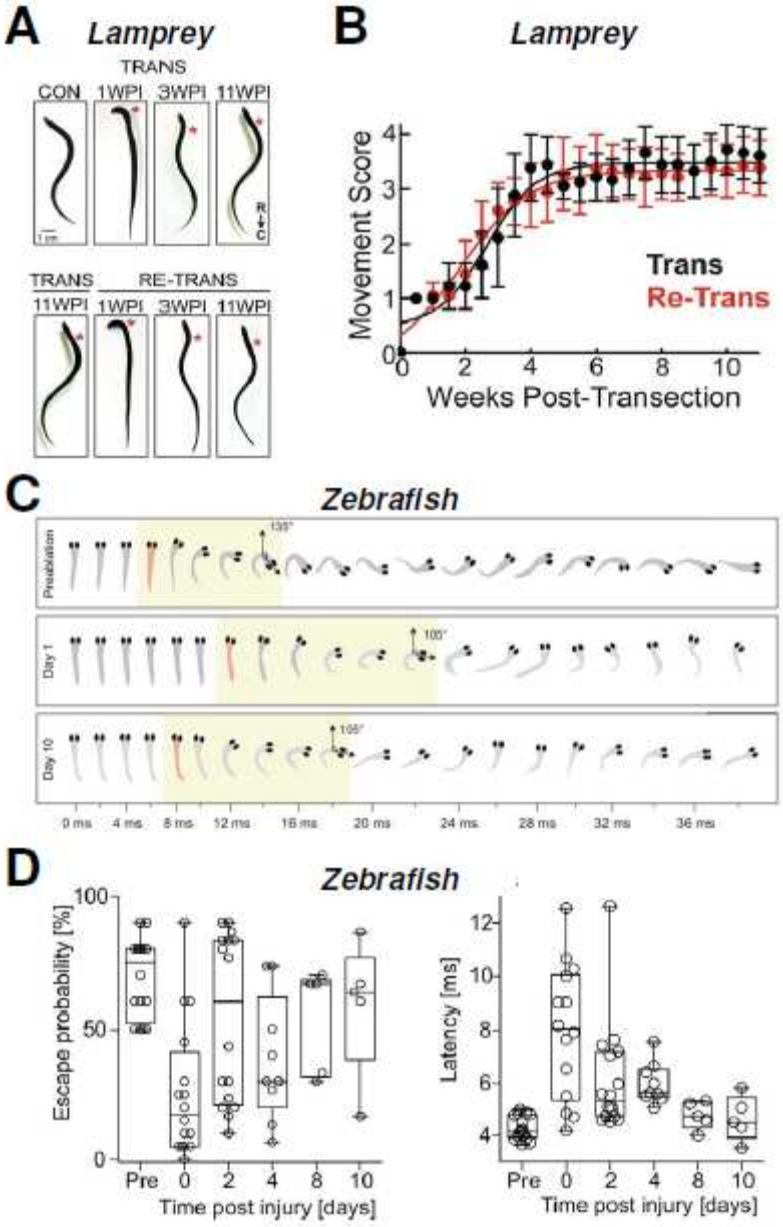


Figure 3. Recovery of locomotion in lampreys and larval zebrafish. A. *(Top)* After spinal transection, which initially results in paralysis, lampreys recover swimming behaviors within 11 weeks post-injury (WPI). Asterisks = lesion site. *(Bottom)* Upon spinal re-transection, lampreys undergo functional recovery a second time. **B.** Movement scores showing that lampreys recover swimming behaviors equally well after spinal transection and a second re-transection. A score of 0 indicates paralysis, while a score of 4 indicates normal swimming. Panels A and B adapted from Hanslik *et al.*, 2019. **C.** Sequences showing C-starts in larval zebrafish before and after proximal injury to an M-cell axon. While latency (orange fish) and time to maximal bend (yellow bar) are longer at 1 day post-injury, the C-start partially recovers by Day 10. **D.** Recovery of escape probability and latency in larval zebrafish after M-cell axotomy. Panels C and D adapted from Hecker *et al.*, 2020a, as stated under Creative Commons license https://creativecommons.org/licenses/by/4.0/.

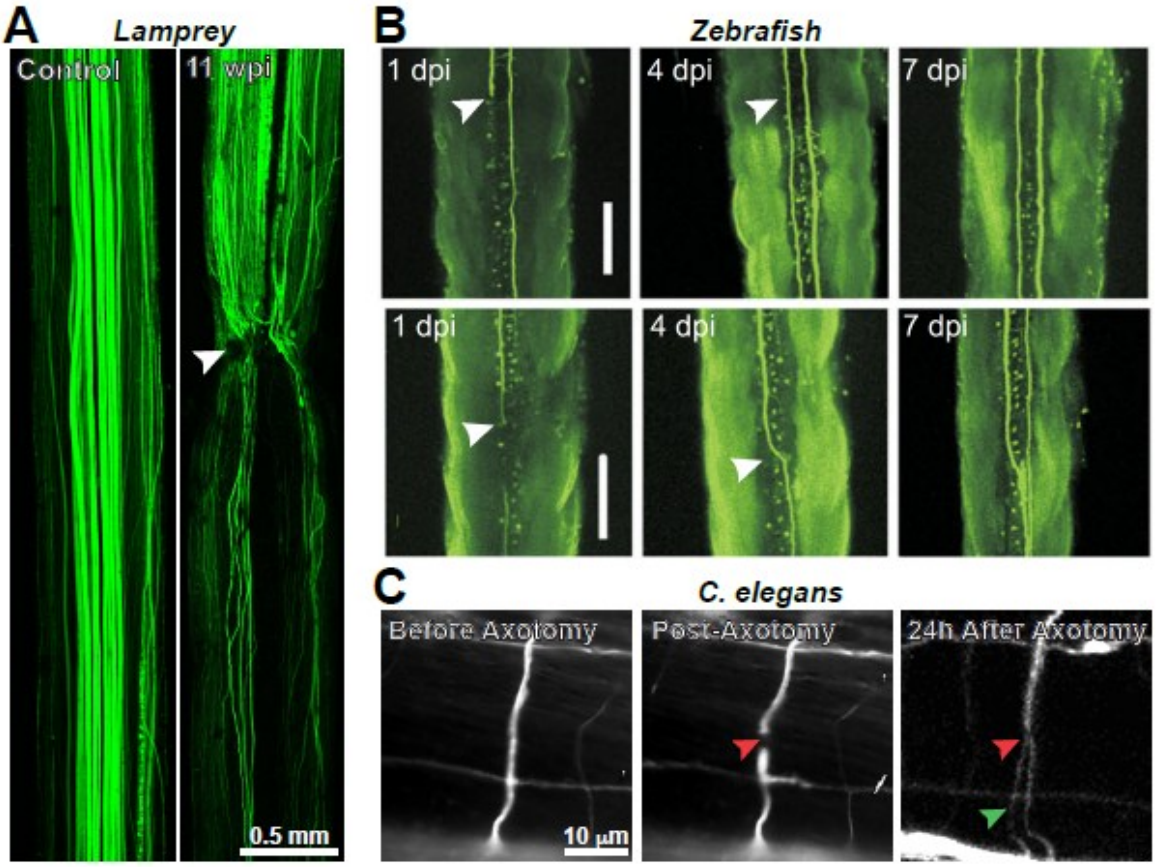
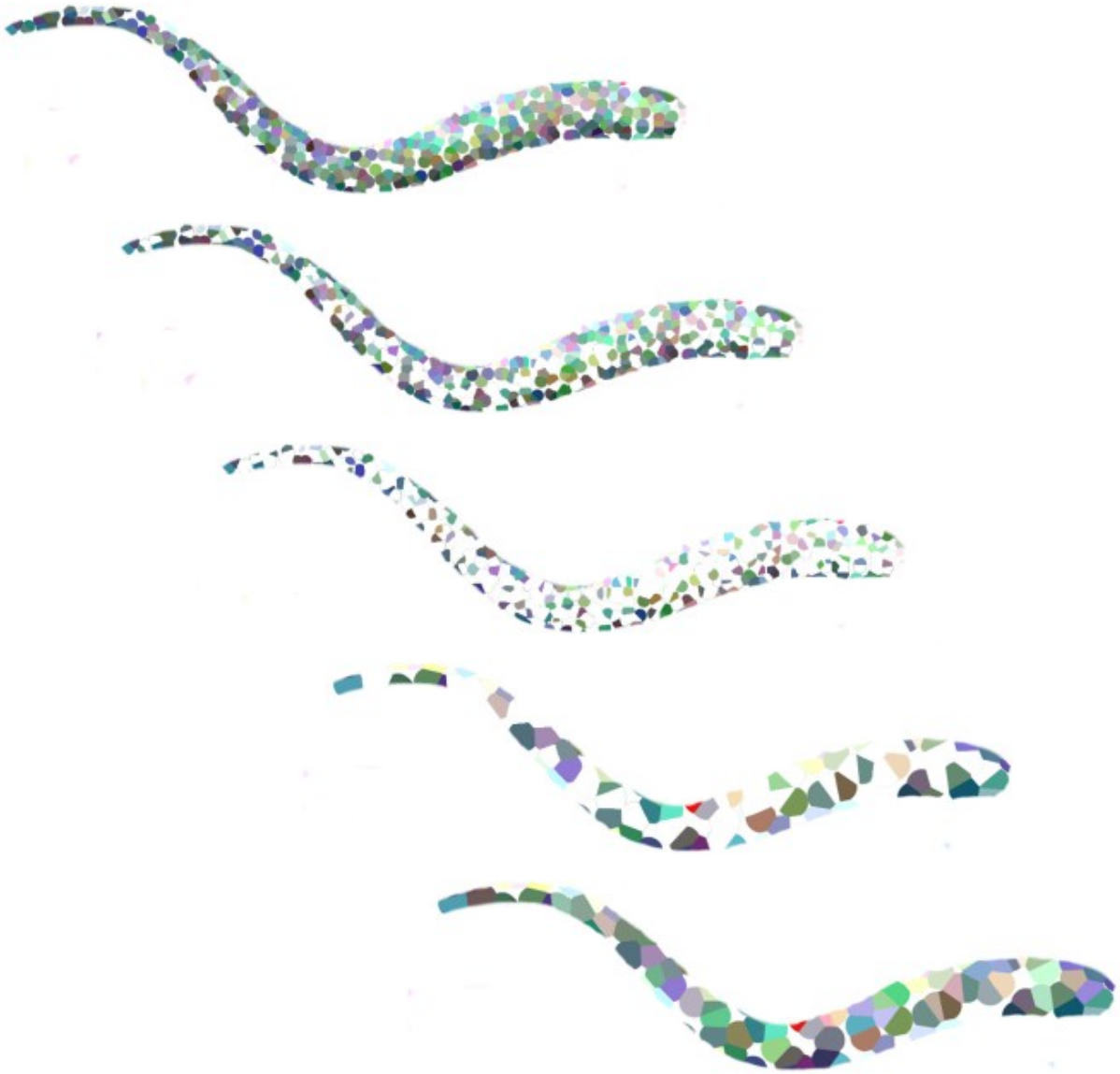


Figure 4. Long-term anatomical reorganization of descending locomotor circuits. A. AlexaFluor™ 488-labeled reticulospinal (RS) axons within the lamprey spinal cord. In the control spinal cord, RS axons project straight along the rostro-caudal axis. At 11 weeks post-injury (wpi) following complete spinal transection, only a subset of RS axons regenerate, often along atypical projection patterns. Despite this, the animal exhibited nearly normal swimming as in Figure 3A-B. Arrow indicates lesion site. (Katz, Morgan lab). **B.** Proximal injury of an M-cell axon within the spinal cord of larval zebrafish leads to accurate (*Top*) and in some cases aberrant (*Bottom*) regeneration within 4 days post-injury (dpi). Arrows indicate ablation sites. Scale bars = 100 µm. (Adapted from Hecker *et al.*, 2020a, as stated under Creative Commons license https://creativecommons.org/licenses/by/4.0/). **C.** In *C elegans*, 24 hours after axotomy of a DD motoneuron (red arrow), two axon branches regenerated (green arrow). Scale bar 10 µm. (Harreguy, Haspel lab). Rostral is up in panels A-B and left in panel C.



Abstract Figure. Undulatory locomotion is an ideal behavior for exploring principles of circuit organization, neural control and resilience of locomotion. The neural circuitry underlying locomotion is highly robust: on the short term, fault tolerance allows locomotion to persist immediately; while on the longer term neural reorganization can restore locomotion after severe perturbations.

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Additional information section

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