# Recovery of Burrowing Behavior After Spinal Cord Injury in the Larval Sea Lamprey

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**Abstract.** Following traumatic spinal cord injury, most mammalian species are unable to achieve substantial neuronal regeneration and often experience loss of locomotor function. In contrast, larval sea lampreys (*Petromyzon marinus*) spontaneously recover normal swimming behaviors by 10–12 weeks post-injury, which is supported by robust regeneration of spinal axons. While recovery of swimming behavior is well established, the lamprey's ability to recover more complex behaviors, such as burrowing, is unknown. Here we evaluated the lamprey's ability to burrow into a sand substrate over the typical time course of functional recovery (1–11 weeks post-injury). Compared to uninjured control lampreys, which burrow rapidly and completely, spinal-transected animals did not attempt burrowing until 2 weeks post-injury; and they often did not succeed in fully covering their entire body in the sand. Burrowing behavior gradually improved over post-injury time, with most animals burrowing partially or completely by 9–11 weeks post-injury. Burrowing behavior has two components: the initial component that resembles swimming with propagated body undulations and the final component that pulls the tail under the sand. While the duration of the initial component did not differ between control and spinal-transected animals across the entire recovery period, the duration of the final component in spinal-transected animals was significantly longer at all time points measured. These data indicate that, after spinal cord injury, lampreys are able to recover burrowing behaviors, though some deficits persist.

## Introduction

After traumatic injury to the central nervous system (CNS; brain or spinal cord), most mammals cannot regenerate and have little to no functional recovery. In contrast, many non-mammalian vertebrates regenerate well and recover after CNS injury (Kaslin *et al.*, 2008; Tanaka and Ferretti, 2009; Lee-Liu *et al.*, 2013; Morgan and Shifman, 2014). As an example, after a complete spinal transection, the larval sea lamprey (*Petromyzon marinus*), a species that is a member of the sister group to the remainder of the vertebrates, is able to re-establish swimming behavior comparable to that of uninjured animals (Rovainen, 1976; Oliphint *et al.*, 2010). This functional recovery of swimming behavior undergoes a well-characterized progression, reaching nearly normal levels by 11 weeks post-injury (WPI) (Davis *et al.*, 1993; Oliphint *et al.*, 2010; Hanslik *et al.*, 2019). Functional recovery of sinusoidal swimming behavior in lampreys is accompanied by the return of coordinated ventral root and muscle activity (Cohen *et al.*, 1986, 1988; Davis *et al.*, 1993), due at least in part to long-distance regeneration of descending reticulospinal axons (Selzer, 1978; Yin and Selzer, 1983; Davis and McClellan, 1994; Oliphint *et al.*, 2010) and formation of new synapses (Wood and Cohen, 1979; Mackler and Selzer, 1987; Oliphint *et al.*, 2010). Remarkably, lampreys can also recover swimming behaviors to the same degree after a second, repeated spinal cord transection in the same lesion plane (Hanslik *et al.*, 2019). Such robustness and tractability have set the lamprey apart as an ideal model for studying functional recovery of behaviors after spinal cord injury (SCI).

Previous work examining post-injury behavioral recovery in lampreys has predominantly focused on forward-swimming behavior in an open tank with shallow water. This assay represents the simplest form of free swimming because the animal is confined to an effectively two-dimensional environment and

does not have to accommodate any obstructions or resistance. Here we ask whether lampreys are able to recover a more complex behavior—burrowing—and if so, to what degree.

Compared to swimming, burrowing is a more complex and commonly observed behavior for larval lampreys, because these animals spend most of their larval stage filter feeding while burrowed in the substrate (Moore, 1980). Burrowing animals must move between two environments with very different physical properties (Gidmark *et al.*, 2011). In the case of aquatic burrowers, the transition is between water and a substrate, such as sand or gravel (Quintella *et al.*, 2007). Previous studies using kinematic analyses and X-ray videography have shown that different species of burrowing fish, including lampreys, sand lances, eels, and wrasses, use very similar locomotor strategies to burrow into substrate (Paggett *et al.*, 1998; Gidmark *et al.*, 2011; Herrel *et al.*, 2011). Most of these studies have described burrowing behavior in two or three distinct stages that reflect modulation of locomotor strategy as the animal transitions between substrates (Paggett *et al.*, 1998; Gidmark *et al.*, 2011; Tatom-Naecker and Westneat, 2018).

In the larval lamprey, these distinct locomotor phases are referred to as the initial and final components (Paggett *et al.*, 1998). The initial component resembles forward swimming, with rapid, propagating undulations that drive the head into the sand; and it is characterized by oscillatory waves of muscle contractions (Paggett *et al.*, 1998). The final component involves long-duration, large-amplitude body flexions; these flexions are arrhythmic and are generated by a unilateral contraction or staggered bilateral muscle contractions, thus creating a C or S shape in the submerged portion of the body that serves to pull the tail into the sand (Paggett *et al.*, 1998). In this way, compared to swimming, burrowing represents a more complex locomotor behavior to examine after SCI. We therefore hypothesized that burrowing behavior might not recover to the same degree as swimming behavior.

Here we test this hypothesis and provide what we believe to be the first measurements of the recovery of burrowing behavior after SCI in larval lampreys. As with swimming behavior, burrowing behavior gradually recovered by 11 WPI. However, unlike swimming, recovery of burrowing was incomplete; and the final component was significantly slower, indicating some persistent deficits. These findings provide an independent measure of lampreys' robust regenerative capabilities and also present an opportunity to explore the neural basis of burrowing behavior and the mechanisms underlying post-injury recovery.

## Materials and Methods

### Spinal cord surgeries

Late larval stage (11–13 cm; 5–7 years; male or female) sea lampreys (*Petromyzon marinus* Linnaeus, 1758) were acquired from Lamprey Services (Ludington, MI) or Acme Lamprey Company (Bowdoin, ME). Spinal cord transec-

tions were performed as described in Oliphint *et al.* (2010). Briefly, late larval sea lampreys were anesthetized in 0.2 g/L tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, WA). When the lampreys were fully anesthetized, a horizontal incision was performed at the level of the fifth gill slit on the dorsal side of the animal to expose the spinal cord. Fine iridectomy scissors were used to completely transect the spinal cord. After spinal transection, two sutures were used to close the incision site (Ethilon 6-0 monofilament; Ethicon, Somerville, NJ). Spinal-transected lampreys were housed in small isolated breeder tanks (6.75 × 3 × 3 inches), which were placed within 10-gallon tanks, at room temperature (RT; 20–25 °C) for up to 11 WPI. Animals that were transected on the same day were kept together as a cohort to keep track of the post-injury time points. For this study, 8 uninjured control and 45 spinal cord-transected animals were used. All animal procedures were approved by the Institutional Animal Care and Use Committee at the Marine Biological Laboratory in Woods Hole, Massachusetts, and in accordance with standards set by the National Institutes of Health.

### Behavioral assays and video analysis

Burrowing and swimming behaviors in lampreys were assessed weekly after spinal cord transections for up to 11 WPI, which is when swimming behavior has reached a steady state of recovery at nearly normal levels (Rovainen, 1976; Oliphint *et al.*, 2010; Hanslik *et al.*, 2019). Because we performed spinal transections biweekly, animals at several post-injury time points were available for behavioral testing on any given day. We therefore did not perform repeated measurements on the same animals throughout the recovery period but instead made independent measurements of behavioral recovery at multiple post-injury time points on testing days, which produced the same results as in prior studies (Oliphint *et al.*, 2010; Herman *et al.*, 2018; Hanslik *et al.*, 2019). We recorded one swimming behavior trial and one burrowing behavior trial per animal each week. This was done to ensure that the animals did not exhibit fatigue (evidenced by reduced swimming movements), which can occur in lampreys after several swimming trials in rapid succession.

Swimming behavior trials were performed in a 15-gallon tank (50.5 cm length × 26 cm width × 30 cm height) filled to a depth of 2 cm with oxygenated tank water at RT. For each trial, a lamprey was placed in the behavioral tank for about one minute and allowed to free swim and, if necessary, stimulated with a light tail pinch. As in our prior studies (Oliphint *et al.*, 2010; Herman *et al.*, 2018; Hanslik *et al.*, 2019), swimming behavior movements were scored from 0 to 4, with the following criteria: 0—lamprey was paralyzed below injury site and did not respond to light touch; 1—responded to touch, head movements above the lesion site, and no forward swimming; 2—brief and abnormal swimming movements, such as rapid head oscillations, atypical body contractions, and start-stop

movements; 3—more regular swimming undulations over longer time periods, but with some atypical contractions; 4—persistent sinusoidal undulations that were similar to uninjured controls (see Fig. 1D, inset).

For each animal, the burrowing behavior trial was performed about one minute after the swimming behavior trial in a different arena. A 5-gallon tank (39 cm length × 21 cm width × 25.5 cm height) was filled to a depth of 3.5 cm with sand and 10.5 cm of oxygenated tank water at RT. Prior studies indicate that larval lampreys burrow at depths ranging from 1.2 to 15.0 cm, and so this depth of substrate was sufficient for complete burrowing (Applegate, 1950; Paggett *et al.*, 1998). One burrowing trial consisted of placing an animal in the tank, allowing it to burrow, and then removing it after five minutes, regardless of whether it completely burrowed. Burrowing behaviors were recorded in each trial with a Sony HDR-CX455 at 60 frames per second.

To determine whether there was recovery in burrowing behavior, we developed a scoring system to describe burrowing performance before and after SCI, based on how much of the lamprey's body was submerged beneath the substrate at the end of the five-minute trial period. The burrowing scores were as follows: 0—no burrowing, no body coverage; 1—the body is covered rostral to the seventh gill slit; 2—the body is covered past the seventh gill slit but still has a substantial portion of the body uncovered; 3—most of the body is covered, but the tip of the tail (*i.e.*, the caudal fin) is exposed; 4—full body coverage (Fig. 1A). The burrowing behavior scores were recorded once the body was fully covered by sand or at the end of the five-minute trial period. If the animal fully burrowed before the end of the five-minute trial, it was noted whether any part of its body subsequently became uncovered after completing the burrow.

Larval lamprey burrowing behavior can be broken down into an initial component and a final component, as described by Paggett *et al.* (1998). During the initial component, the animal exhibits rapid undulations, which drive the head into the substrate. In the final component, the visible portion of the body ceases the rapid undulations and is pulled into the substrate by the animal performing a deep C- or S-shaped bend below the substrate (Paggett *et al.*, 1998). The initial component was analyzed for all animals that attempted burrowing, while the final component was measured only from animals that burrowed completely (score of 4). The initial component was measured from video recordings, and its duration was determined from the frame where the snout touched the substrate to when the rapid undulations ceased. Tail beat frequency was also determined from the initial component by dividing the total number of complete tail beat cycles, defined as the tail tip moving from one extreme side to the opposite and back, by the time from the start of the first complete tail beat cycle to the end of the last. The final component was determined to be the frame from where the rapid undulations ended until the tail was no longer visible.
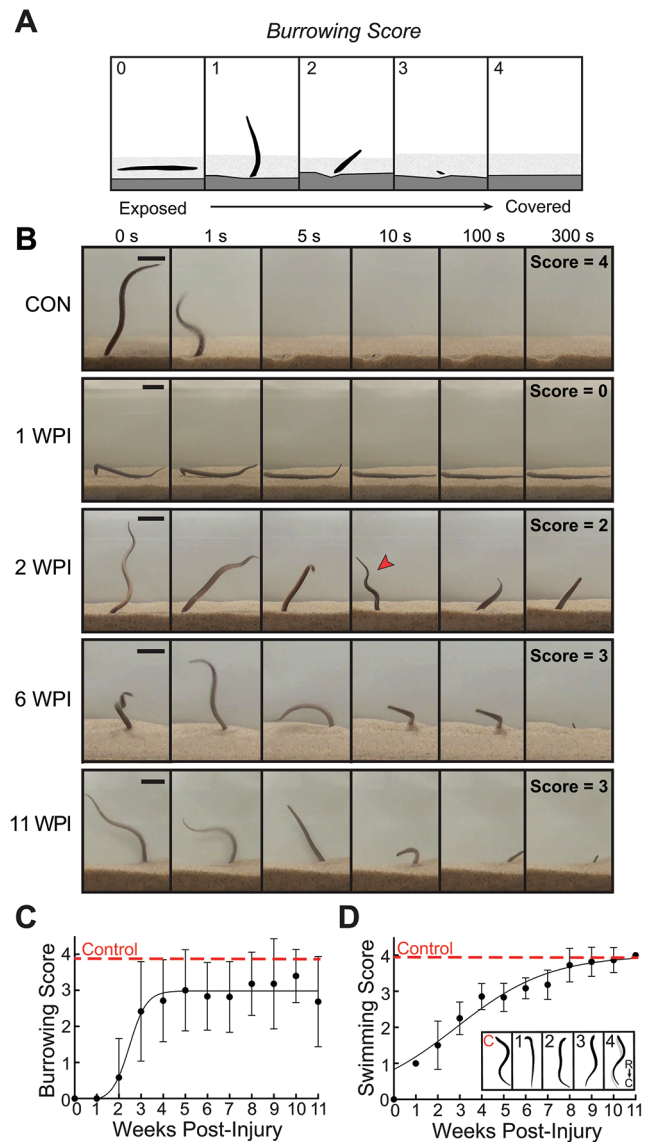


**Figure 1.** Sea lampreys (*Petromyzon marinus*) recover burrowing behavior after spinal cord injury. (A) Silhouettes of lampreys showing body coverage corresponding to each burrowing score. (B) Representative images of uninjured control (CON) animals and those at 1, 2, 6, and 11 weeks post-injury (WPI) showing post-injury recovery of burrowing based on improved burrowing scores. Red arrowhead indicates body waves in the uncovered portion of the body. Scale bar = 2 cm. (C) Recovery of burrowing behavior after spinal transection. Red dashed line indicates the score of control animals, which is 3.94. Data points represent mean ± SD from $n = 8$–16 animals. Data were fit with a Boltzmann sigmoidal curve ($R^2 = 0.46$, $t_{1/2} = 2.50$ wk, $y_{max} = 2.98$). (D) Recovery of swimming behavior in the same animals reaches control levels by 11 WPI. Inset indicates swimming scores, as described in Oliphint *et al.* (2010). Red dashed line indicates the score of control animals, which is 4.00. Data points represent mean ± SD from $n = 8$–16 animals. Data were best fit by a Boltzmann sigmoidal curve ($R^2 = 0.83$, $t_{1/2} = 2.85$ wk, $y_{max} = 4.00$).

*Statistical analyses*

GraphPad Prism version 8.0.0 (GraphPad Software; La Jolla, CA) was used to calculate mean swimming and burrowing scores, perform statistical analyses, and generate behavior score graphs. The mean burrowing and swimming scores were fit with a Boltzmann sigmoidal curve, and the $R^2$ value was used to assess the goodness of fit. Mean and standard deviation calculations, statistical analyses, and graphs of the initial and final component measurements were made in R (R Core Team, 2019). Kruskal-Wallis and Wilcoxon rank-sum tests were run on both datasets to test for significant differences across groups. A chi-squared test was used to compare proportions of burrowing behavior performance categories (*i.e.*, complete, partial, failure) across recovery time points. An ANOVA test was used to compare differences in tail beat frequency across time points.

## Results

*Recovery of burrowing performance in lampreys after spinal cord injury*

To determine whether burrowing behavior recovers after SCI in lampreys, we developed a scoring system from 0 to 4 to assess performance based on how much of the animal is covered by the substrate at the end of a 5-minute trial period (Fig. 1A; see *Materials and Methods*). The vast majority ($n = 15/16$ animals) of uninjured control lampreys were able to fully burrow, achieving full body coverage and a burrowing score of 4, in $22.92 \pm 16.21$ seconds ($n = 15$ animals) (Fig. 1B). At 1 WPI, lampreys made no attempt to burrow, and all remained at a score of 0 (Fig. 1B). At 2 WPI, animals burrowed portions of their body, ranging from just past their head to half of their body, achieving burrowing scores of 1 or 2 (Fig. 1B). Some lampreys recovering at time points after 4 WPI could almost fully burrow their body, but the tip of their tail remained uncovered at the end of the trial period (Fig. 1B). However, some animals were able to burrow completely by 11 WPI. We observed that a subset of spinal-transected lampreys would sometimes generate atypical body waves that appeared to slowly back-propagate from the tail region toward the head (Fig. 1B, red arrowhead), unlike uninjured control animals, which demonstrated little to no bending along the visible portion of their body during the final component of burrowing behavior (Fig. A1, red arrowheads). Average burrowing scores improved after spinal transection, reaching a maximum at 76% of control levels by 5 WPI, with a half time to maximum recovery (at steady state) of 2.50 weeks (Fig. 1C) ($n = 16$ animals; Boltzmann sigmoidal curve, $R^2 = 0.46$, $t_{1/2} = 2.50$ wk, $y_{max} = 2.98$). These data show that lampreys undergo improvement of burrowing behavior performance within a few weeks after spinal transection, though they never fully recovered to the levels observed in uninjured control animals.

To confirm that lampreys were exhibiting behavioral recovery from spinal cord transections consistent with previous work (Oliphint *et al.*, 2010; Herman *et al.*, 2018; Hanslik *et al.*, 2019), we also measured the recovery of sinusoidal swimming behaviors for the same animals and found that the swimming scores all recovered to control levels along the same time course as previously reported (Fig. 1D; Oliphint *et al.*, 2010; Herman *et al.*, 2018; Hanslik *et al.*, 2019). By 11 WPI, lampreys achieved swimming scores comparable to uninjured controls, with the half time of recovery at 2.85 weeks (Fig. 1D; $n = 16$ animals; Boltzmann sigmoidal curve, $R^2 = 0.83$, $t_{1/2} = 2.85$ wk, $y_{max} = 4.00$). These data show that while spinal-transected lampreys completely recovered their ability to swim, the same animals still had deficits in their burrowing, as evidenced by the inability of many individuals to burrow completely.

As a second measure of recovery of burrowing behavior, we determined the proportion of animals that burrowed completely (*i.e.*, full body coverage) after spinal cord transection. For these and later measurements, we binned animals into three post-injury time points: during peak change in performance (1–4 weeks, or 2–4 weeks when examining animals with a burrowing score greater than 0), once maximum burrowing behavior performance is reached (5–8 weeks), and late recovery (9–11 weeks), based on the burrowing behavior recovery curve (Fig. 1C). Of the uninjured control animals, 93.75% ($n = 15/16$) completely burrowed, while only 6.25% ($n = 1/16$) partially burrowed, leaving a portion of their tail uncovered (Fig. 2A). Compared to controls, the relative proportion of animals that burrowed completely, burrowed partially, or failed to burrow was significantly different at all post-injury time points ($\chi^2$ test, $P < 0.01$). At 1–4 WPI, only a small percentage of lampreys were able to burrow completely (Fig. 2A, white bars), and this steadily increased during the post-injury time course (1–4 WPI = 17.39% [$n = 8/46$]; 5–8 WPI = 41.30% [$n = 19/46$]; 9–11 WPI = 50.00% [$n = 21/42$]). Compared to 1–4 WPI, the later time points from 5–11 WPI had significantly different proportions of burrowing behavior performance, with fewer failures and more partial or complete burrowing behaviors (Fig. 2A; $\chi^2$ test, $P < 0.01$).

In some instances, after the animal burrowed completely (burrowing score of 4), a small portion of the tail would re-emerge from the substrate and become uncovered. We therefore quantified the proportion of animals in which this occurred. Of the uninjured control animals, 93.33% ($n = 14/15$) remained covered after burrowing (Fig. 2B). In contrast, only 25.00% ($n = 2/8$) of 2–4 WPI animals that burrowed completely remained covered, while the remaining 75% ($n = 6/8$) became uncovered. Over recovery time, from 1 to 11 WPI, this uncovering behavior was observed less; and those that remained completely covered consequently increased (Fig. 2B; 2–4 WPI = 25.00% [$n = 2/8$]; 5–8 WPI = 57.89% [$n = 11/19$]; 9–11 WPI = 66.67% [$n = 14/21$]). The
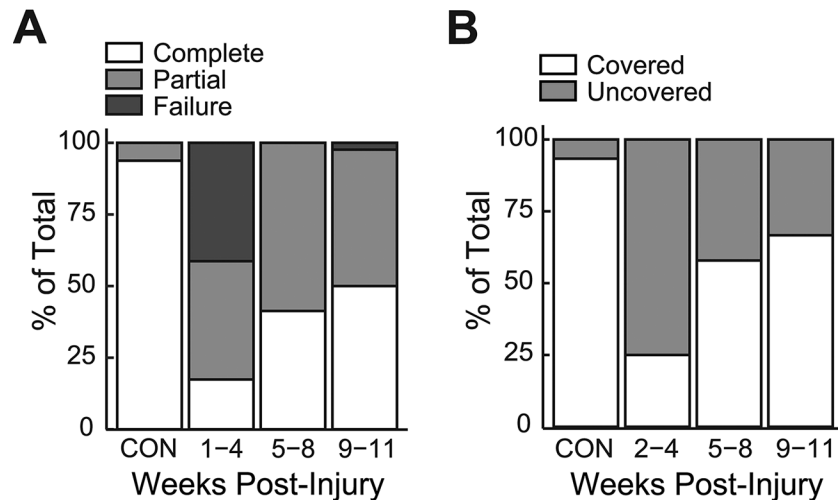
**Figure 2.** Completeness of burrowing behavior improved over time after spinal cord injury. (A) Graph showing percentage of lampreys within a given time bin that completely burrowed (*i.e.*, score of 4), partially burrowed (*i.e.*, score of 1–3), or failed to burrow (*i.e.*, score of 0). Data represent percent of total from $n = 16$–45 animals per time point. (B) Graph showing percentage of completely burrowed lampreys that either remained covered or became partially uncovered during the recording period. Data represent percent of total from $n = 8$–21 animals per time point. In (A) and (B), proportions were significantly different across all pairs of groups ($\chi^2$ test, $P < 0.01$), with the exception of the 5–8 WPI and 9–11 WPI bins, which showed no significant difference.

distributions at all post-injury time points were significantly different from each other ($\chi^2$, $P < 0.01$) except for the 5–8 WPI and 9–11 WPI time periods, which had similar distributions. These findings show that lampreys gradually improved their ability to burrow completely and to remain burrowed after SCI, and they further suggest that burrowing performance is largely stable after 5 WPI (see also Fig. 1C).

*Persistent deficits in the final component of burrowing after spinal cord injury*

Next we wanted to quantify whether there were any changes in the duration of burrowing behavior during the recovery from SCI. We therefore measured the duration of the initial and final components from video recordings. While the initial component was measured from all trials where the animal attempted to burrow (burrowing behavior score of 1 or higher), the final component could only be measured from trials where the animals burrowed completely within the five-minute recording period (burrowing behavior score of 4). As previously mentioned, the initial and final components of burrowing behavior in uninjured control lampreys were completed in less than 30 seconds (Fig. 3A). Across all post-injury time points, lampreys performed the initial component similar to control animals, but the final component appeared to last for a longer period of time (Fig. 3A). Measurements of the initial and final components of burrowing behavior from videos revealed that the duration of the initial component was around 2 seconds in uninjured control animals and that this was not significantly different in the spinal-transected animals up to 11 WPI (Fig. 3B; Kruskal-Wallis test and Wilcoxon rank-sum test, $P > 0.05$). To further examine the initial component, we deter-

mined the tail beat frequency in uninjured controls and spinal-transected lampreys between 2 and 11 WPI. Similar to the duration, this kinematic analysis of the initial component revealed no significant differences in tail beat frequency between uninjured controls and spinal-transected lampreys at any post-injury time point (Fig. 3C; ANOVA, $P > 0.05$). In contrast, the duration of the final component was significantly longer in spinal-transected animals at all post-injury time points compared to controls, on average a fivefold difference (Fig. 3D; Kruskal-Wallis test and Wilcoxon rank-sum test, $P < 0.001$). Thus, while lampreys robustly recovered burrowing behaviors after spinal transection, there were persistent deficits in the final component that delayed burrowing.

**Discussion**

The lamprey has set itself apart as a model for studying functional recovery after SCI. Even among other regenerative vertebrate models for SCI, the larval lamprey is distinctive in regard to both the robustness and the characterization of functional recovery after spinal injury. Compared to the zebrafish SCI model, in which functional recovery is often measured in total distance moved (Vajn *et al.*, 2014; Sasagawa *et al.*, 2016; Wang *et al.*, 2017), functional recovery of swimming behavior in the larval lamprey has been examined in depth by using kinematics, providing additional information on body shape and the quality of movements (Davis *et al.*, 1993; Oliphint *et al.*, 2010; Hanslik *et al.*, 2019). Furthermore, the lamprey has a higher probability with which it successfully recovers swimming behavior compared to, for example, the eastern newt, *Notophthalmus viridescens* (Davis
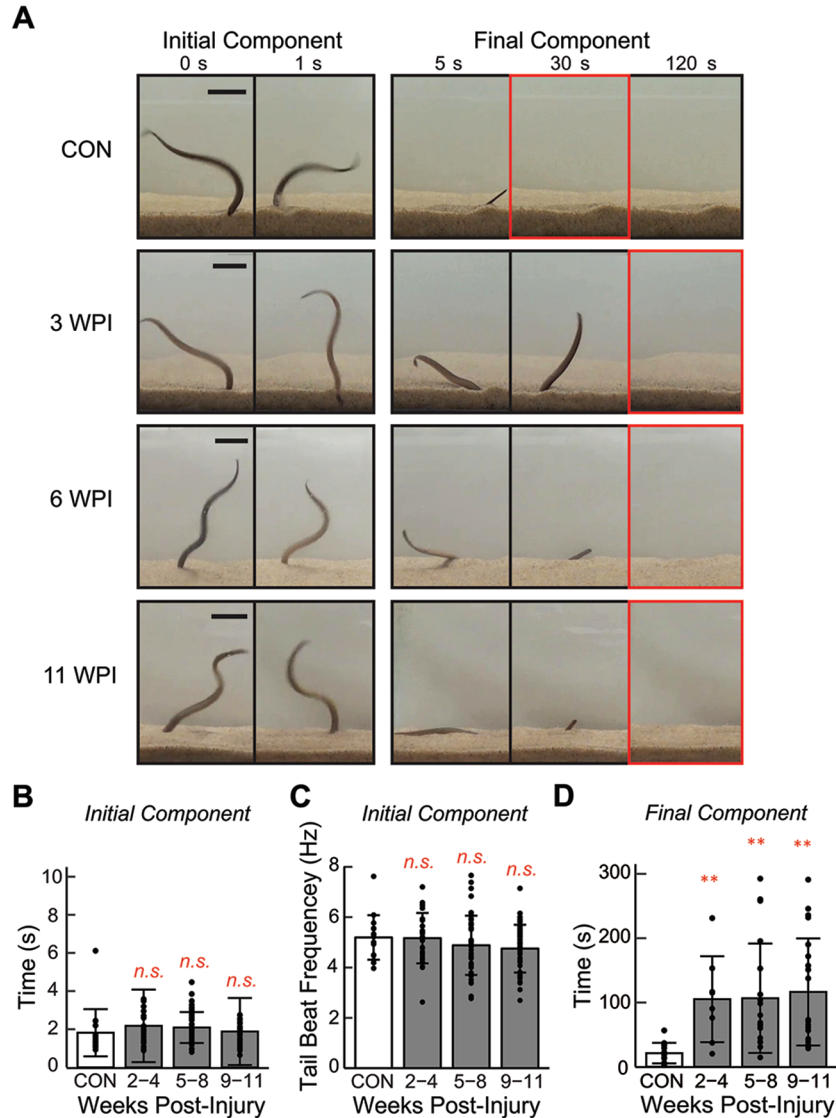
**Figure 3.** Despite substantial recovery, the duration of burrowing behavior increased after spinal cord injury. (A) Representative still images of uninjured control (CON), 3 weeks post-injury (WPI), 6 WPI, and 11 WPI showing the initial and final components of burrowing behavior. Red boxes indicate the time point at which the animal is completely burrowed. Scale bar = 2 cm. (B) The initial component of burrowing behavior was unaltered between controls and all recovery time points. Bars represent mean ± SD. Control = $1.82 \pm 1.24$ s, $n = 16$ animals; 2–4 WPI = $2.18 \pm 1.90$ s, $n = 27$ animals; 5–8 WPI = $2.09 \pm 0.81$ s, $n = 46$ animals; 9–11 WPI = $1.88 \pm 1.76$ s, $n = 42$ animals (Kruskal-Wallis test and Wilcoxon rank-sum test, $P > 0.05$). (C) Tail beat frequency remained unchanged between control and all post-injury time points. Bars represent mean ± SD. Control = $5.18 \pm 0.89$ Hz, $n = 16$ animals; 2–4 WPI = $5.17 \pm 1.00$ Hz, $n = 27$ animals; 5–8 WPI = $4.88 \pm 1.18$ Hz, $n = 46$ animals; 9–11 WPI = $4.75 \pm 0.96$ Hz, $n = 41$ animals (ANOVA, $P > 0.05$). (D) The duration of the final component of burrowing behavior significantly increased after spinal cord injury at all time points tested. Bars represent mean ± SD. Control: $21.13 \pm 15.95$ s, $n = 15$ animals; 2–4 WPI: $105.00 \pm 66.87$ s, $n = 8$ animals; 5–8 WPI: $106.60 \pm 85.01$ s, $n = 19$ animals; 9–11 WPI: $116.32 \pm 83.30$ s, $n = 21$ animals ($**P < 0.001$ by Kruskal-Wallis test and Wilcoxon rank-sum test). n.s., not significant.

*et al.*, 1990). In this study we sought to explore the limits of functional recovery in the lamprey by examining a more complex behavior, burrowing. We showed that, like swimming behavior, burrowing behavior in lampreys undergoes recovery and improvement over time after SCI (Figs. 1, 2). The onset recovery of burrowing behavior coincided with the recovery of swimming behavior, which was around 2–3 weeks post-injury,

as may be expected, given that self-initiated forward movement is necessary for the animal to make a burrowing attempt. Interestingly, between control and spinal-transected animals, we observed no difference in the initial component of burrowing (Fig. 3), which, similar to swimming, is driven by thrust-generating, propagating sinusoidal waves along the body (Gidmark *et al.*, 2011; Tatom-Naecker and Westneat, 2018).

In other studies in lampreys, it has been shown that functional recovery of swimming behavior after SCI is nearly complete in spite of the fact that there are persistent anomalies in the spinal cord tissue, such as an irregular hourglass shape at the lesion site, a swollen central canal, and an altered cytoskeleton (Hanslik *et al.*, 2019). Furthermore, only 30%–50% of descending reticulospinal axons regenerate across the lesion site, forming only a few small synaptic connections (Rovainen, 1976; Yin and Selzer, 1983; McClellan, 1990; Davis and McClellan, 1994; Jacobs *et al.*, 1997; Oliphint *et al.*, 2010; Hanslik *et al.*, 2019). Thus, incomplete axon regeneration appears to be sufficient for supporting the sinusoidal movements that are observed during recovery of both swimming behavior and burrowing behavior. The large variation that we observe in more complex locomotor behaviors, such as burrowing behavior, could be driven by individual differences in the new spinal circuits that form during regeneration. Other mechanisms that likely contribute to behavioral recovery after SCI in lampreys include alterations in neuronal excitability and synaptic plasticity of intraspinal neurons (McClellan *et al.*, 2008; Cooke and Parker, 2009; Parker, 2017), as well as changes in expression of neurotransmitter receptors, ion channels, and axon guidance molecules (Shifman and Selzer, 2000, 2007; McClellan *et al.*, 2008; Shifman *et al.*, 2009; Fernandez-Lopez *et al.*, 2016; Herman *et al.*, 2018; Romaus-Sanjurjo *et al.*, 2018).

Unlike swimming behaviors, there were clear deficits in burrowing behaviors that persisted throughout the recovery period (Figs. 1–3). For example, only half of the spinal-transected animals were able to burrow completely by 9–11 weeks post-injury (Fig. 2A), and the animals that completely burrowed had a significantly longer final component (Fig. 3). Because these animals exhibited apparently normal swimming behavior (Fig. 1D), and because the initial component of burrowing behavior was unaltered (Fig. 3C, D), this would suggest that the observed deficits in burrowing are not due to impairments in motor output driven by a central pattern generator, though we acknowledge this is difficult to assess in the absence of a full kinematic analysis. One possible explanation could be a loss in muscle strength post-SCI. Injured lampreys may appear to swim normally; but when faced with greater resistance when entering a denser substrate, they may not have the muscle strength to burrow as well as control animals.

Alternatively, the observed deficits in the final component of burrowing could be due to post-injury alterations in the ability to localize motor output. Electromyographic recordings have previously shown that the final component is produced by motor output in the portion of the body submerged in the substrate, which results in little to no visible bending in the uncovered portion of the body (Paggett *et al.*, 1998). This localized motor activation pattern likely requires sensory feedback from the region of the body in contact with the substrate in order to initiate bending in this specific body region. Therefore, it is possible that the observed delay in the final component of burrowing behavior could be due to a lack of sensory feedback, which may also lead to an inability to distinguish between covered and uncovered regions of the body. This might explain why some animals become uncovered again after burrowing completely.

At least three populations of sensory neurons have been identified in the lamprey spinal cord: edge cells, dorsal cells, and cerebrospinal fluid-contacting neurons (Rovainen, 1967; Grillner *et al.*, 1984; Di Prisco *et al.*, 1990; Jalalvand *et al.*, 2018). Regeneration of edge cells and dorsal cells across the transection site has been reported (Yin and Selzer, 1983), and edge cells exhibit post-injury physiological changes (Hoffman and Parker, 2011; Becker and Parker, 2019). However, the precise contribution of sensory neuron regeneration to functional recovery of locomotor behaviors, including burrowing behavior, remains unclear. Another interesting possibility is that the photoreceptors in the tail, which ensure the withdrawal of the tail into the burrow, may also be impaired after SCI (Young, 1935; Francis and Horton, 1936).

Whatever the mechanism, the inability to burrow normally after spinal injury may have important impact on the lamprey's fitness, because remaining uncovered for longer periods of time could leave the animal susceptible to further injury or predation. Although it would be interesting to follow the lampreys beyond 11 WPI, we think it is unlikely that they would recover more normal burrowing behaviors, given that burrowing behavior was fairly stable at all time points measured after 5 WPI (Fig. 1B, C).

In summary, this study further highlights the robustness of the lamprey as a model for functional recovery after SCI by revealing that lampreys are capable of recovering burrowing behaviors in addition to swimming behaviors. Additionally, this work emphasizes the value of examining multiple behavioral paradigms in assessing functional recovery in SCI models. The post-injury deficits observed in lamprey burrowing behaviors suggest that in order to truly link regeneration and functional recovery, it will be necessary to examine how sensorimotor integration is achieved in the regenerated spinal cord.

## Ethical Care Considerations

All vertebrate animal procedures were approved by the Institutional Animal Care and Use Committee at the Marine

Biological Laboratory, as covered under Animal Welfare Assurance no. D16-00039.

## Data Accessibility

Raw data values are available in a supplementary spreadsheet (Table S1, available online).

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# Appendix
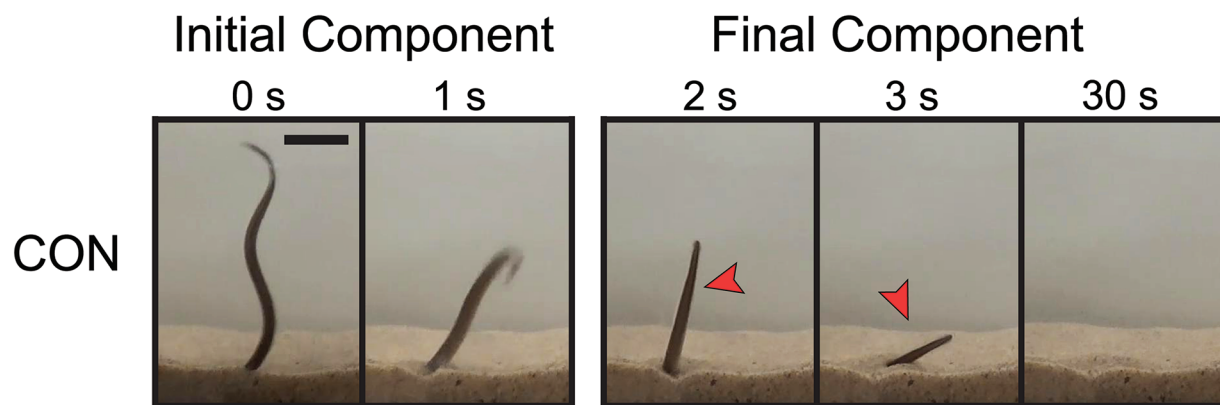


**Figure A1.** Uninjured control animals do not exhibit standing tail bends or waves during burrowing. Representative still images of an uninjured control (CON) animal burrowing. Red arrowheads indicate the straight tail observed during the final component of burrowing.