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Spinal cord transection in Xenopus laevis tadpoles

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Corresponding Author:	Juan Larraín CHILE
Corresponding Author's Institution:	
Corresponding Author E-Mail:	jlarrain@bio.puc.cl
Order of Authors:	Juan Larraín
	Paula G Slater
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TITLE:

Spinal Cord Transection in *Xenopus laevis* Tadpoles

AUTHORS AND AFFILIATIONS:

Paula G. Slater, Juan Larraín

Center for Aging and Regeneration, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago de Chile, Chile 7820436

Email address of co-author:

Paula G. Slater (pslaterg@gmail.com)

Corresponding author:

Juan Larraín (jlarrain@bio.puc.cl)

SUMMARY:

Xenopus laevis tadpole spinal cord transection is a relevant injury method to study spinal cord injury and regeneration by making a transverse cut that completely severs the spinal cord at the thoracic level.

ABSTRACT:

Spinal cord injury (SCI) is a permanent affliction, which affects the central nervous system (CNS) motor and sensory nerves, resulting in paralysis beneath the injury site. To date, there is no functional recovery therapy for SCI, and there is a lack of clarity regarding the many complexes and dynamic events occurring after SCI. Many non-mammalian organisms can regenerate after severe SCI, such as teleost fishes, urodele amphibians, and larval stages of anuran amphibians, including *Xenopus laevis* tadpoles. These are bona fide model organisms to study and understand the response to SCI and the mechanisms underlying successful regenerative processes. This type of research can lead to the identification of potential targets for SCI therapeutic intervention. This article describes how to perform *Xenopus laevis* tadpole spinal cord transection, including husbandry, surgery, postsurgery care, and functional test evaluation. This injury method can be applied for elucidating the different steps of spinal cord regeneration by studying the cellular, molecular, and genetic mechanisms, as well as histological and functional evolution after SCI and during spinal cord regeneration.

INTRODUCTION:

Spinal cord injury (SCI) is an affliction that affects approximately 250,000–500,000 people worldwide every year[1]. In addition to this high prevalence, SCI affects sensory and motor nerves, generating paralysis beneath the injury site and disconnection of some internal organs from the control of the CNS. The spinal cord, a part of the CNS, cannot regenerate, and due to the complexity of the affliction and the lack of complete understanding of all the involved processes, there are still no efficient therapies allowing functional recovery.

Non-mammalian organisms, such as teleost fishes, urodele amphibians, and larval stages of anuran amphibians, which can regenerate the spinal cord after severe SCI[2–4], are excellent model organisms for studying the processes that govern a successful regenerative event and understanding the failure of mammalian regeneration. This understanding is of great interest as it could provide original insights to develop new therapeutic targets and possible therapies for SCI.

The anuran frog, *Xenopus laevis,* is an excellent model organism to study SCI. It has excellent regenerative capacities during the tadpole stages, which are progressively lost during metamorphosis, allowing experimentation in the regenerative and nonregenerative stages[3,5]. The established injury method for studying SCI in *Xenopus laevis* tadpoles consists of tail amputation, where the entire tail is removed, including tissues such as muscle, notochord, and spinal cord[6]. This approach has been instrumental in the understanding of general mechanisms of regenerative processes[4,7–10].

As tail amputation involves multiple tissues in addition to the spinal cord, which is different from what happens after human SCI, a more relevant injury paradigm is needed for the study of SCI. We have relied on studies used in the past[11] for generating comprehensive descriptions of injury paradigms[5,12–14] and different methods for the study of SCI[12–18]. After spinal cord transection, the caudal portion of the spinal cord can be isolated for RNA and protein expression and high-throughput analyses[14,19–21]. Additionally, intracelomic injections of drugs and small molecules, as well as electroporation of cDNA, RNA, or morpholinos, before or after spinal cord transection, allow the study of the effects of these molecules in the prevention or treatment of SCI or of specific events occurring after SCI and spinal cord regeneration[13,14]. Further, injury evolution and the regenerative processes can be studied at different timings after injury using biochemical, molecular, histological, and functional approaches[12–14,17,19–23].

Finally, all the aforementioned techniques can be used in non-regenerative stages, highlighting one of the most important advantages of using *Xenopus laevis* as a model organism to study SCI—the comparative studies of regenerative and non-regenerative mechanisms in the same species[13,19–22]. This paper presents a protocol for *Xenopus laevis* tadpole spinal cord transection, starting with the staging and selection of regenerative Nieuwkoop and Faber (NF) stage 50 tadpoles. This is followed by the description of the procedures for spinal cord surgery to produce sham and transected animals, postsurgical care, and finally the analysis of functional recovery by the measurement of free tadpole swimming distance.

PROTOCOL:

This protocol provides enough information to successfully perform SCI and evaluate swimming recovery in *Xenopus laevis* tadpoles. Of note, there are excellent detailed protocols of these techniques published elsewhere[14], which can complement the one presented here. All animal procedures have been approved by the Committee on Bioethics and Biosafety from the Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

1. Natural mating of frogs

1.1. Three to five days prior to mating, subcutaneously preinject male and female frogs with 50 units of human chorionic gonadotropin (hCG). Use the “iron claw” technique for restraining the frogs; as the frogs are slippery, use a net to surround the frog if necessary. Insert the tip of a 26 G x ½” needle posterior to the lateral line, pushing it dorsally to a depth of 1 cm, between the skin and the muscle.

1.2. Before mating, inject the male with 300 units and the female with 700 units of HCG.

1.3. For mating to occur, place the male and the female in 2 L of 0.1x Barth solution immediately after chilling the solution at 4 °C for 15 min to resemble spring conditions and leave overnight at 18 °C.

1.4. Sixteen hours later, carefully collect the embryos with the help of a plastic Pasteur pipette, with the tip cut off, and place them in 10 cm diameter Petri dishes. Remove the embryonic jelly coat by incubating the embryos with 25 mL of 2% cysteine in distilled water (pH 7.8; ensure the solution covers the embryos) for 5 min with slight agitation. Wash 3 times with distilled water and 3 times with 0.1x Barth solution (8.9 mM NaCl; 102 µM KCl; 238.1 µM $NaHCO_3$; 1 mM 4-(2- hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES); 81.14 µM $MgSO_4$; 33.88 µM $Ca(NO_3)_2$; 40.81 µM $CaCl_2$, pH 7.6).

1.5. Select healthy embryos that have a brownish color and symmetrically dividing blastomeres. Place the embryos in 10 cm diameter Petri dishes with 50 mL of 0.1x Barth solution at a density of no more than 100 embryos per dish.

2. Husbandry

2.1. During the first week, maintain the embryos at 18 °C until they get off the vitelline sac. During this time, change the Barth solution every day, and remove whitish dead embryos, tadpoles presenting any visible anatomical alteration or tadpoles without any swimming movement.

2.2. After the first week, transfer tadpoles to chlorine-free water in plastic tanks at a density of 10 animals per liter. Grow tadpoles at 20–21 °C with a 12-h light/12-h dark cycle, with oxygen stones available in each tank to aerate the water and fed once a day with 0.5 mg per animal. Replace water once a week and check for accumulated waste and dead animals daily[24].

3. Staging

3.1. Three to four weeks after fertilization, place the animals in a Petri dish; then, one by one, check the morphology and appearance of forelimbs and hindlimbs. If necessary, anesthetize the animals by placing the animals in a Petri dish with 50 mL of 0.02% tricaine mesylate in 0.1x Barth

solution for better manipulation. After no more than 2 min, place the animals in 0.1x Barth solution for recovery from the anesthesia.

3.2. Look for the following anatomical characteristics of stage 50 animals[25]: forelimbs that are just appearing and are spherical (**Figure 1**); hindlimbs that are protruding and are spherical (**Figure 1**).

NOTE: Animals from stages 49 to 51 can be used for this procedure (**Figure 1**); for more information about stages, refer to Nieuwkoop and Faber's Normal Table of *Xenopus laevis*[25].

4. Surgery: spinal cord transection and sham-operated animals

4.1. Anesthetize stage 50 tadpoles by placing them in a Petri dish with 50 mL of 0.02% tricaine mesylate in 0.1x Barth solution for 2 min.

4.2. With the help of a tablespoon and forceps, place the tadpole, dorsal side-up, on a wet piece of gauze in the upper half of a glass Petri dish.

4.3 Perform an incision of the skin and dorsal muscles at the mid-thoracic level (**Figure 2A, B**) using microdissection spring scissors.

4.3.1. For control sham animals, ensure that the incision size is only ~0.2 mm (**Figure 2C**); do not damage the spinal cord (**Figure 2D,D´**).

4.3.2. For transected animals, perform a second incision of ~0.2 mm (**Figure 2C**) to fully transect the spinal cord (**Figure 2E,E´**).

5. **Postsurgery care**

5.1. After surgery, transfer the tadpoles to a tank containing 0.5 L of 0.1x Barth solution with 1x Penicillin–streptomycin, at a density of 10–12 animals per tank. Maintain the transected and control sham animals in separate tanks.

NOTE: Tadpoles will recover from the anesthesia in a couple of minutes.

5.2. Maintain the tadpoles with aeration at a temperature of 20–21 °C.

5.3. Change the Barth solution with antibiotics every other day until the end of the experiment.

5.4. Start feeding the animals one day after surgery, once per day.

5.5. Eliminate dead animals.

6. **Swimming assay**

6.1. Obtain a box with LED illumination from the inside, covered with a transparent polystyrene sheet, which allows the light to pass through.

6.2. Install a camera over the LED box.

6.3. Place a 15 cm diameter Petri dish on top of the box, filled with 100 mL of 0.1x Barth solution.

6.4. One day post transection, place a tadpole in the Petri dish and leave for a 5 min adaptation period.

6.5. After adaptation, start video-tracking the free-swimming behavior using the referenced software (see the **Table of Materials**) for 5 min.

6.6. After the video is completed, transfer the tadpole back to its tank.

6.7. Repeat the video tracking 5, 10, 15, and 20 days post transection (**Figure 3**).

7. **Bioethical considerations**

NOTE: The mortality of animals after sham surgery and transection is 13% and 30%, respectively. Additionally, a minimum of 15–20 animals per group is necessary for statistical analysis. Therefore, start with 23 sham and 26 transected animals.

7.1. Anesthetize the animals with 0.02% tricaine mesylate for 2 min to assure reduction in neuronal and motor activity and pain before surgery.

7.2. After surgery, check the animals for recovery from anesthesia. Additionally, feed and check the animals daily.

7.3. After finishing the swimming assay, sacrifice the animals with an overdose of tricaine mesylate (1% tricaine mesylate prepared in 30 mM sodium bicarbonate solution).

REPRESENTATIVE RESULTS:

The protocol described herein allows the study of spinal cord regeneration in *Xenopus laevis*. The effects of specific pharmacological treatments and the contribution of specific gene expression in spinal cord regeneration can be evaluated by measuring their effects on swimming recovery. The total swimming distance is plotted against the days after injury to compare control and treated animals at a specific time point or over a specified period. The recovery of motor function through time is exemplified in **Figure 3**, showing the swimming distance at 5, 10, 15, and 20 days post transection. At 5 days post transection, animals swam an average of 0.7 m in 5 min, showing a reduced swimming capacity. This capacity increased with the passing days, as an average of 2.1

and 3.1 m/5 min was observed after 10 and 15 days post transection, respectively, and complete recovery of swimming capacities was observed at 20 days post transection, with an average of 5.7 m/5 min.

FIGURE AND TABLE LEGENDS:

Figure 1: Xenopus tadpole staging. Representative images of stages 49–51, showing fore- and hindlimbs for animal staging reference. Scale bars = 2 mm. Magnification of the boxed region shown in the lower-right of each image. Scale bars = 1 mm. In stage 49, forelimbs are not observed, while hindlimbs are just appearing, showing a spherical shape. Stage 50 presents forelimbs that are just appearing, showing a spherical shape and hindlimbs protruding with a spherical shape. In stage 51, forelimbs present a protruding spherical shape and hindlimbs a protruding elongated shape. Dashed outlines show fore- and hindlimbs.

Figure 2: Spinal cord transection. (**A**) Representative image showing the correct positioning of the animal, dorsal side up, for performing the surgery. Scale bar = 2 mm. (**B**) Magnification of **A** shows the location and extent of injury. The red cross shows the exact location of the injury site at the thoracic level of the spinal cord, and the dashed line shows the extent of the injury. Scale bar = 1 mm. (**C)** Representative image showing a lateral view of the thoracic level of the spinal cord. The extension of the sham incision and transection are shown. Dashed lines delineate the limits of the spinal cord. Scale bar = 1 mm. (**D**) Representative image showing a sham animal with an intact spinal cord. Scale bars = 1 mm. (**E)** Representative image showing a transected animal with an interrupted spinal cord. Scale bars = 1 mm. Magnification of the boxed region shown in the lower-right of each image (D´and E´). Scale bars = 1 mm. Abbreviations: S = sham incision; T = transection.

Figure 3: Swimming function recovery over time. Representative dot plot of the swimming distance covered by transected animals in 5 min at 5, 10, 15, and 20 days post transection. Samples of swimming trajectories are shown on top. Data presented as mean ± SEM from 10 tadpoles. Abbreviations: dpT = days post transection; SEM = standard error of the mean.

DISCUSSION:

The protocol described herein is an excellent method to perform SCI and evaluate functional recovery. For reproducibility, it is essential to grow healthy tadpoles and choose animals that are similar in size. Lack of proper feeding generates nutrient stress, which results in poor regenerative capacities[26]; therefore, special attention should be paid to tadpole feeding. As tadpoles reach stage 50 after 3–4 weeks, they can be reared at higher temperatures to accelerate the growth process, 18–25 °C being optimal[27]. Water quality is important, as animals are sensitive to water conditions and chemical products. The optimal water conditions include using carbon filtered, chlorine-free water with the following parameters: pH (6.5–7.5), chloride (<0.02 mg/L), conductivity of water (1.0 mS/cm ± 0.1 units), copper (<0.3 mg/L); carbonate hardness (KH: 5–10 dKH); general hardness (GH: 6–16 dGH); nitrate (NO_3: <20 mg/L); and nitrite (NO_2: <0.1 mg/L)[14,27,28]. Additionally, to avoid contamination, plastic tanks should be cleaned once a week for rearing animals or every other day after surgery by washing thoroughly with chloride-free water and a sponge; detergent must be avoided.

For a better survival rate after surgery, tadpoles must not be exposed to anesthesia for long periods (no longer than 2 min). Moreover, it is recommended to anesthetize one tadpole at a time. As the animals need to stay hydrated, keep the animals immersed in solution all the time before and after surgery, and pour the solution with a spoon on top of the tadpole before beginning the surgery. Ensure that the damage is extensive enough to cover the whole spinal cord but not too extensive as it can induce poor functional recovery or death. If the notochord is damaged, the animal will be bent, and the functional recovery will be affected. If the damage extends beyond the notochord, the probability of death increases[14]. During the swimming assay, recording is considered correct if the software identifies each animal with a blue shadow; otherwise, the recording should be repeated. It is important to avoid movement and air or light changes during the recording process to prevent recording mistakes.

There are still many open questions about the cellular and molecular mechanisms underlying spinal cord damage and regeneration. The protocol described in this work can be used to study the contribution of different cellular events, gene expression, and treatments to functional recovery, determined by measuring swimming capacities. Additionally, many other techniques can be applied to the operated animals. The spinal cord can be isolated to perform protein and/or mRNA extraction[14] to study protein and gene expression profiles after damage and treatment[19,20]. This surgery has also been the basis for studying the spinal cord cellular response[22] and the behavior of neural stem progenitor cells[12,13,22] after spinal cord injury. Signaling cascades involved in spinal cord regeneration have also been studied using the spinal cord damage paradigm described herein[23]. In summary, the protocol described here is an excellent model to study spinal cord injury and regeneration and has been used for many studies that have contributed to the existing knowledge about the subject.

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DISCLOSURES:

The authors have no conflicts of interest to declare.

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Figure_1

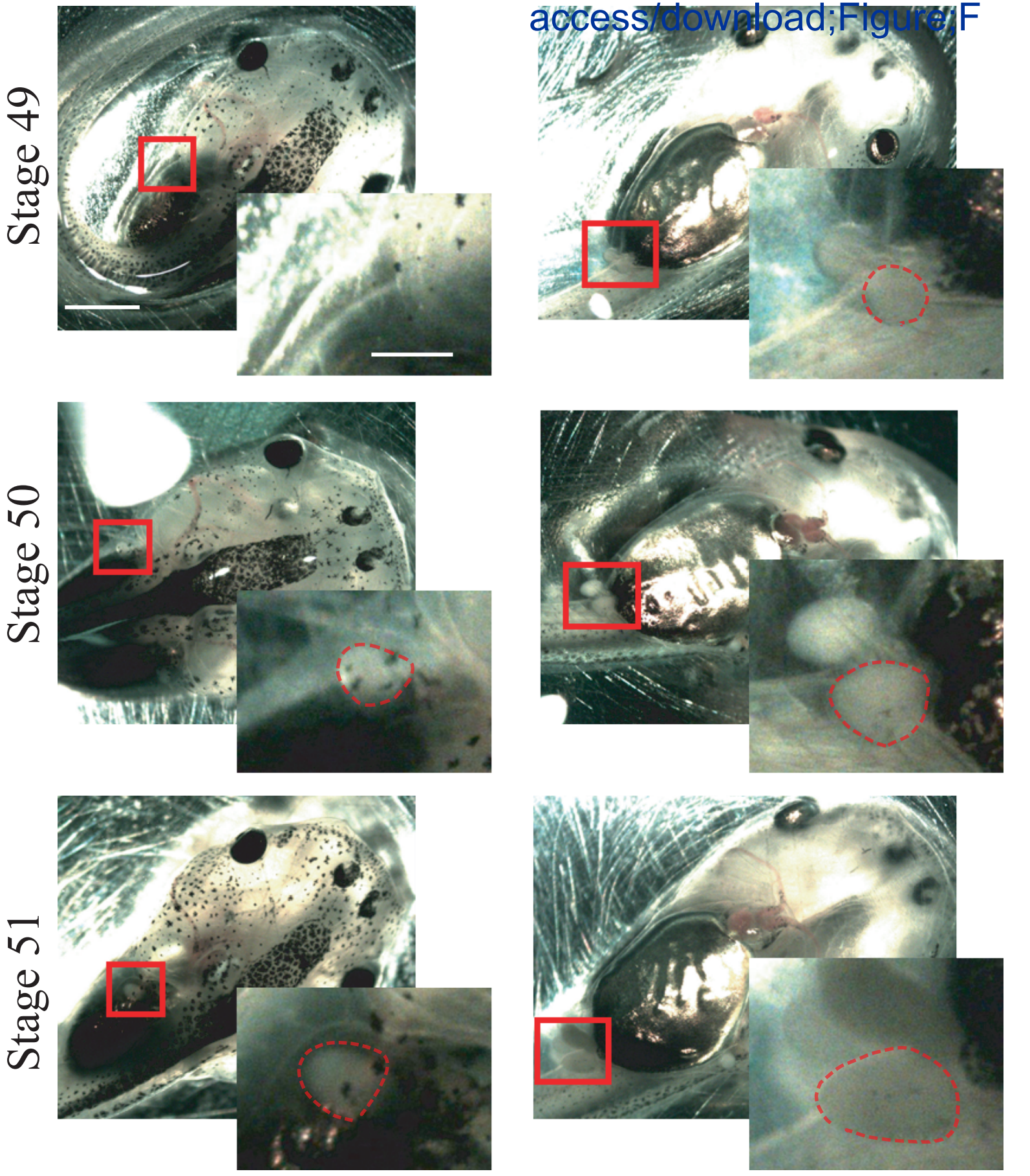

Figure_2

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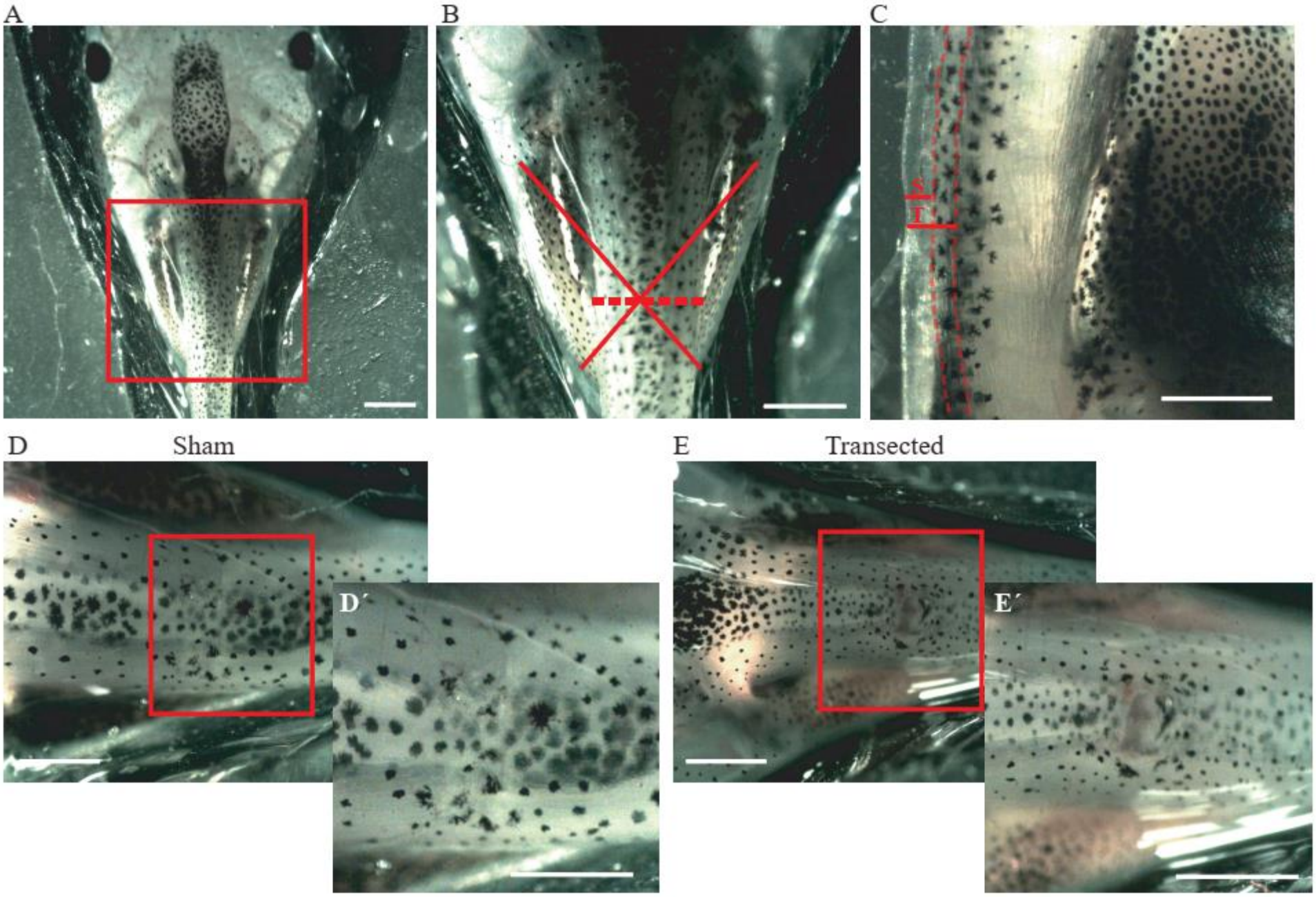

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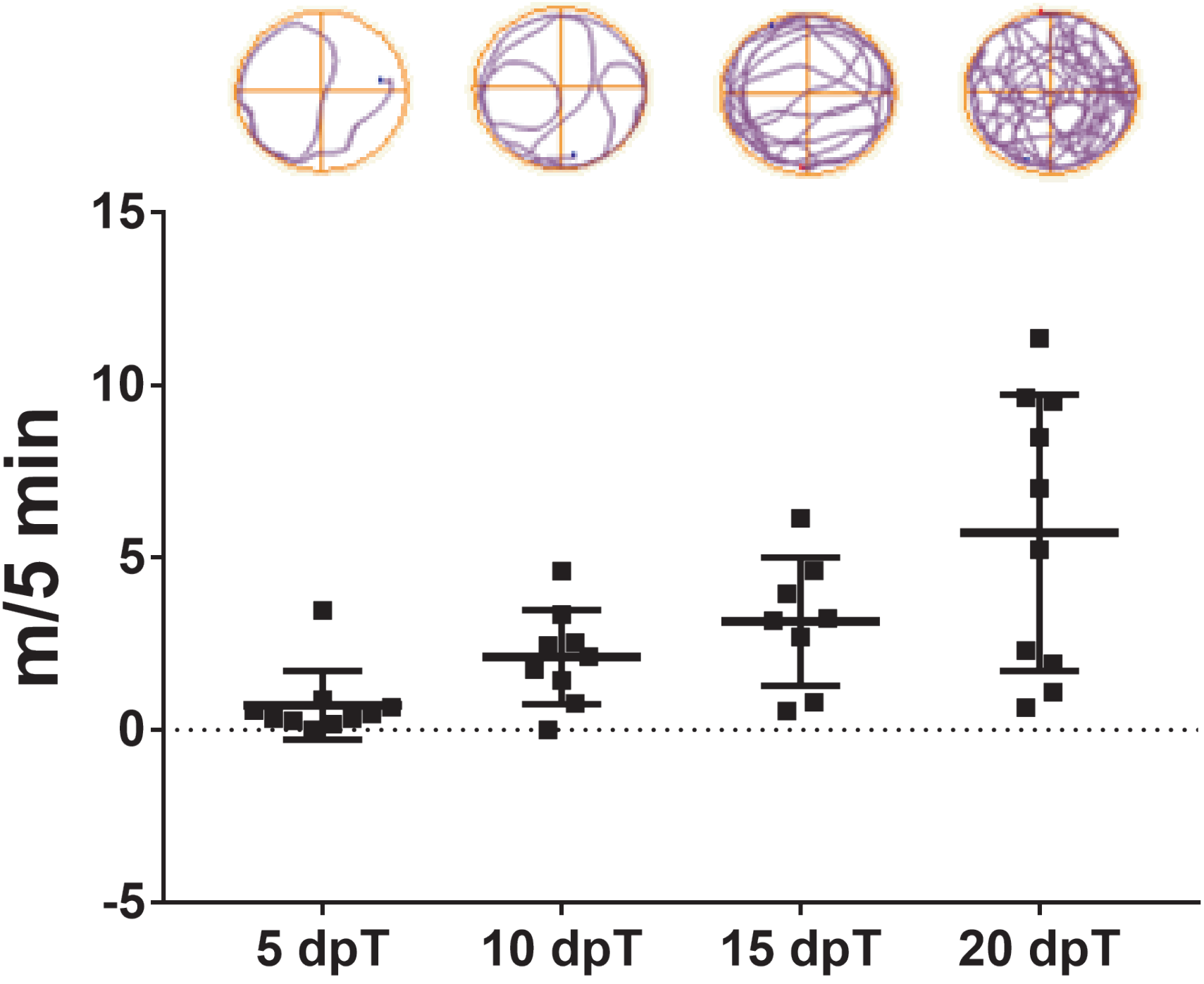

Response to Editor and Reviewers

We thank all reviewers for their very helpful suggestions to improve the manuscript. We have addressed all of their specific comments. Below are the reviews and our response to them (in blue).

Editor

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. **We read the manuscript thoroughly and made the necessary changes.**

2. Please revise the title to focus on the technique being presented. The title should directly reflect the steps being presented in the video. **We changed the title to "Spinal cord transection in Xenopus laevis tadpoles", for more precision.**

3. Please move the acknowledgment just after the Discussion and before the Disclosure. **Changed, and the disclosure was added.**

4. Please provide a Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words. **We added a summary before the abstract section.**

5. Please provide an Abstract between 150-300 words more clearly stating the goal of the protocol. **We changed the abstract including the suggestion.**

6. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s) but before the punctuation. **Changed.**

7. Please also include all the following in the Introduction along with citations:

a) The rationale behind the development and/or use of this technique

b) The advantages over alternative techniques with applicable references to previous studies

c) Information to help readers to determine whether the method is appropriate for their application

We changed the introduction and included the three suggested points.

8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.

For example: Nasco, Stoelting, Wood Dale, IL, USA, etc.

All commercial language was removed.

9. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. **An ethics statement was included, "All animal procedures have been approved by the Committee on Bioethics and Biosafety from the Faculty of Biological Sciences, Pontificia Universidad Católica de Chile".**

10. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes. **Changed.**

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. **We changed all the protocol section to imperative tense.**

12. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

The discussions and most of the notes were moved to the Discussion section.

13. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Readers with all levels of experience and expertise should be able to follow the protocol.

Line 64-66: How were the frogs injected? Please provide all the details associated including anesthesia used, where the injection was given (specific body part, if any), needle size used, etc. **Added.**

Line 67-68: The solution was chilled for 15 min and the frogs were then immediately transferred to the solution? If not, how long after the chilling were the frogs transferred? Does ON stand for overnight? If yes, please correct. **Changed.**

Line 69: How were the embryos identified and collected? **Added.**

Line 77-79: What was the volume of Barth solution? How were the embryos counted? How were the dead embryos identified? **Added.**

Line 89-90, 99: How was anesthetization done? Please provide all the associated steps. **Added.**

Line 102: Incision size? Was it made using a scalpel, scissors, etc.? Please specify. **We added a sentence about the damage extent in the discussion section. The incision size and instruments used were added in the protocol section.**

Line 119: Was the swimming assay performed immediately after performing spinal cord injury? If not, after how many days were the tadpoles subjected to swimming assay? **Added.**

14. Please include a ONE LINE SPACE between each protocol step, sub-step, and note. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. **Added.**

15. Please ensure that the Representative Results show the effectiveness of your technique backed up with data e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. **Added.**

16. As we are a methods journal, please also include the following in the Discussion in detail with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

We added important considerations and troubleshooting in the discussion section.

17. Please shift the figure legends at the end of the Representative Results in the manuscript text. **Changed.**

18. Figure legends: Please provide a short description of the data presented in the Figures. **Added.**

19. Figure 1: Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate Figure Legend. **Added.**

20. Figure 2: What does the error bar represent: standard error or deviation? What is the n number of the samples? What does dpT stand for please expand? Please include a SINGLE space between the unit and the numeral in the y-axis labeling (m/ 5 min). **Added.**

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22. Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included. **Added.**

23. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. **Checked.**

24. Please ensure that the table of materials includes all the essential supplies, reagents, and equipment. **All the essential supplies, reagents and equipment were added to the table of material.**

Reviewer 1

Manuscript Summary:
The authors detail the basic steps of birth, rearing, staging, spinal cord transection, and subsequent recovery and behavioral analysis in Xenopus laevis tadpoles. While similar processes have been detailed elsewhere, a video-based procedure is very welcome, and the exact specification of the equipment used at each step is commendable.

Major Concerns:
None. The manuscript seems quite straight-forward and well written. I look forward to seeing the accompanying video.

Minor Concerns: A few very minor suggestions for the manuscript:
1) Authors should state size of petri dish for rearing 100 embryos, as embryo density strongly affects health and survivorship of the embryos. Also, I recommend less than 100 embryos per dish, as this number tends to have high mortality in my experience.

We added the size of the petri dish. I our experience, if healthy embryos are carefully selected, and dead animals are removed daily, the survival rate is almost 100%.

2) I suggest mentioning that tadpoles can be safely reared at higher than 18C (we prefer 22C, and up to 25C) to shorten the 3-4 week lead time between birth and Stage 50.

We added that information in the discussion section.

3) Figure 1, stage 49 fore limb picture could use improvement; the lighting makes the enlarged inset somewhat washed out and difficult to interpret. All other images fine.

In stage 49 forelimbs are not distinguished, as this information may confuse the reader, we changed the inset and added an explanation in the Fig. 1 legend: "In stage 49 fore limbs are not observed, while hind limbs are just appearing, showing a spherical shape. Stage 50 present fore limbs just appearing, showing a spherical shape and hind limbs protruding, with spherical shape. In stage 51 fore limbs present a protruding spherical shape and hind limbs a protruding elongated shape."

4) I suggest stating where frogs can be obtained, or at least a referral to Xenbase for beginners.

We added the information in the table of material.

5) I recommend emphasizing the importance of using very clean water for rearing tadpoles, as even de-chlorinated water can have seasonal pollutants in it that harm egg and tadpole quality. We have had best success using reverse-osmosis purified, carbon filtered, pH and salinity conditioned water, but our area does suffer from agricultural runoff in the water supply, so this may not be necessary in all areas. **We added a paragraph in the discussion section, highlighting the importance of optimal water condition and how to reach it.**

6) Does the initial incision for the transection group also sever the spinal cord, as well as the overlying muscle, or is a second, targeted incision used to transect the spinal cord? Current wording is slightly ambiguous. **For sham animals is one incision and for transected animals two incisions. We changed the wording in the protocol for a better understanding.**

7) How quickly does the surgery need to be performed before the tadpoles begin to suffer from

being out of solution? **We added a paragraph in the discussion section highlighting the importance of maintaining animals hydrated and how to do it.**

8) In addition to the video, a figure showing the exact location of the incision would be useful. **A figure showing the exact location of the incision was added, new figure 2.**

Reviewer 2

Manuscript Summary:
The submitted protocol describes the procedures for performing spinal cord transection in Xenopus leaves tadpoles and frogs. The protocol is written in simply and in a manner that procedurally, should be straight forward for other researchers to follow. There a few concerns that should be clarified before publication but other that those, the protocol provides an accurate description of the procedure used by Dr. Larrain for spinal cord injury in Xenopus.

Major Concerns:
I don't have major concerns with the submitted protocol.

Minor Concerns:
The protocol lacks detailed information in some areas that other scientists would need to perform the protocol. For example, the type of surgical instruments used and there manufacturers. Additionally, when cutting the spinal cord, the authors do not mention the major blood vessels above and below the spinal cord in Xenopus, and whether or not they are cut during the transection procedure.

We added surgical instruments used in the protocol, and as JoVE cannot publish manuscripts containing commercial language (trademark symbols (™), registered symbols (®), and company names), we included the manufacturers only in the table of materials.

As the major blood vessels are located to close to the spinal cord they are cut during the procedure. We added a sentence about the damage extent in the discussion section.

Reviewer 3

Manuscript Summary:
The manuscript is generally well-written, only some minor details need change.

Major Concerns:
None

Minor Concerns:
I suggest it would be nice to include a line art image of Stage 50 X. laevis tadpole from the Normal table of Xenopus laevis by PD Nieuwkoop and J Faber (Plate VIII) for easy stage identification

The manuscript includes a figure showing examples of the stages needed, therefore we consider that is not necessary to include a line art image from the Normal table of

Xenopus laevis by PD Nieuwkoop and J Faber. Nonetheless, we included a better description of the characteristic of stages 49-51 in the figure legend, and we refer the reader to the Normal table of Xenopus laevis by PD Nieuwkoop and J Faber, for more information.

Line 35: change 250.000 to 250,000; **Changed**

Line 65: HCG -> hCG; **Changed**

Line 72: NaHCO3 -> $NaHCO_3$ and similar chemical symbol inconsistencies should be changed through the manuscript; **Changed.**

Line 84: death -> dead; **Changed.**

Line 105-106: Author may want to describe (both in the description and in the associated video) the difference of incision depth (e.g., in 0.2 mm vs 0.4 mm) in sham vs transected animals in more details for the user to practise. **We added this on the protocol section, additionally we included a new figure (Fig. 2) showing more details of the injury location and size.**

Reviewer 4

Manuscript Summary:

In this manuscript, the authors Paula G Slater & Juan Larraín describe a simple yet important method to study spinal cord transection and regeneration in Xenopus laevis. The spinal cord is the tight bundle of nerves that carries nerve impulses to and from the brain to the rest of the body. Spinal cord injury (SCI) can result in temporary or permanent loss to sensation, movement, and body functions with no efficient treatment available for complete functional recovery. Xenopus laevis is an excellent model organism to study SCI due to its regenerative capacities during tadpole stages. Here, the authors show a method to perform spinal cord transection and recovery in Xenopus laevis. Overall, the method protocol is properly thought and presented but needs to address the following points.

Major Points:

1) The method describes spinal cord injury in Xenopus, but the authors fail show to any images or videos of the sham and transected tadpoles, rather Figure 1 shows budding fore- and hindlimb images. How should the readers understand the procedure that were performed without any pictorial or graphical representation of the method described here?

We added a new image showing examples of sham and transected tadpoles (Fig.2). Nevertheless, the video will include all the important steps to be able to perform the surgery.

2) Similar to the above point, JoVE being a video journal, the authors do not provide any videos of the transection procedures and the swimming tadpole assay.

The video will include both, the transection procedures and swimming assay.

3) Section 6. Swimming assay description looks very complicated. Shouldn't it be possible to simply image/track tadpoles in a petri dish with a camera mounted on a dissecting microscope?

We comprehend this concern, but herein we are describing the method and method settings that we have characterized and use extensively in our research. We cannot describe a setting that we have never used.

4) Section 7. Bioethical considerations: the authors mention 30% mortality in transected animals and to use only 26 embryos for transection which does reduce the overall statistical significance in analyzing the recovery and movement compare to the initial 50 embryos mentioned in Section 4, please clarify this?

We do not talk about the number of embryos in section 4, must be a confusion because we talk about stage 50 animals. A minimum of 15-20 animals is needed for statistical analysis, thus considering the 30 % mortality, staring with 26 transected animals is sufficient.

5) The authors should justify the difference and advantage of this method over published protocols they mention as excellent including the very similar Edwards-Faret et al., 2017 from this group. Is the submitted protocol redundant with this? Is there a reason publishing again this very similar protocol?

We included the importance of the technique presented herein and a comparison with other method largely used, tail amputation, in the introduction section. Indeed, this protocol is based on Edwards-Faret et al., 2017, however this journal allows to complement the published protocol with a video-based procedure.