**Dear Editorial Board,**

**We are grateful for the editorial and reviewers’ suggestions and comments. The revised manuscript, included in this resubmission, addresses all arguments raised by the reviewers. Thanks to the reviewers’ work, our manuscript is improved and better suited for publication in JoVE. Below is a step-by-step response to the comments we received.**

1. *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

We have corrected any spelling or grammar error.

1. *Please revise the title to avoid the use of colon.*

We have changed the title to Swimming induced paralysis is a valuable behavioral assay to assess dopamine signaling in *Caenorhabditis elegans*.

3. *Keywords: Please provide at least 6 keywords or phrases*.

Behavior and thrashing have been included in the keywords section

4. *Please rephrase the Long Abstract to more clearly state the goal of the protocol.*

See lines 34-35

5. *JoVE cannot publish manuscripts containing commercial language. ...6. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). 7. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion. 8. Please revise the Protocol steps (1.7, 1.9, 2.2.1, 2.2.2, 2.2.4, 2.2.5, 3.2.1, etc.) so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. 9. Please highlight more steps for filming. The currently highlighted content is not sufficient for the video. 10. Table of Materials: Please sort the items in alphabetical order according to the Name of Material/Equipment.*

Changes indicated from point 5 to 10 have been addressed throughout the manuscript

**Reviewer #1:**  
Minor Concerns:  
*There were a few minor typographical errors in the text.*

Errors have been corrected throughout the manuscript  
  
  
**Reviewer #2:**   
Minor Concerns:  
*Line 109 - shouldn't this be "to a final volume of 100ml", not "dissolved in 100ml"…..*

Corrected (see line 111)

*Line 219: Two-way ANOVA should be "to test for effects of either time or drug", and post-hoc analyses should be included.*

Corrected (see lines 235-236)   
  
**Reviewer #3:**  
Major  
1-*Pitfalls. …how specific the effect is to dopamine, and could other phenomena also result in SWIP effect?*

The reviewer is right, other phenomena could produce SWIP. The specificity of the dopaminergic signaling is ensured by the use of drugs known to act through dopaminergic proteins and mutants lacking expression of genes directly involved in the dopaminergic signaling. We thank the reviewer who suggested including this valuable information in the manuscript (see lines 374-400)

2-*Quantification of the results. The results provided (Fig2) show a constant decrease in number of animals showing SWIP when measured every minute. These data do not provide enough information to assess the robustness of the method….*

We agree with the reviewer that rate of progression per minute as a function of drug concentration and maximal number of affected animals at the steady state as a function of drug concentration are important parameters that can be collected from our assay and used to compare the efficiency among different drugs. We discuss these important aspects of our assay in the revised manuscript (Lines 359-362).

3-*Expected results for C. elegans mutant. I can imagine that the idea of this method is to study gene products that would be involved in regulation of dopamine homeostasis. What is missing are the possible outcomes of positive mutants. Please describe which could be the expected phenotypes for positive mutants. …*

We thank the reviewer who suggested discussing this valuable point (lines 374-400).

4-*In the introduction, Imipramine is mentioned as a blocker of DAT-1, this is different than what happens in mammalian DAT in which the imipramine effect is weak. …*

In the revised version of the manuscript, we discuss this limitation (see lines 364-372).  
  
Minor  
*Almost all solutions described in the protocol are described in grams, but the final molar composition of the solutes must be provided when appropriate.*

We have included concentrations molars (see Protocol section).  
  
  
**Reviewer #4:**  
Major Concerns:  
1) *SWIP can only be reliably observed in L4 stage animals. Even though it is mentioned in the discussion part, it should be stressed more clearly in both introduction and protocol.*

We thank the reviewer for this valuable suggestion. Accordingly, we have included more discussion on this argument in the revised version of the manuscript. In this new paragraph, we discuss the work of Masoudi et al. (2014) to inform the reader that SWIP can be observed in both L1 and L4 staged animals. But, the rate of paralysis between the 2 larval stages slightly changes. (see line 335-340)

2) *A lot of drugs paralyze worms in liquid, among which are for example sodium azide, levamisol, aldicarb. However, these drugs are not specifically targeting the dopaminergic pathway. How can paralysis by other causes such as toxicity be distinguished from a paralysis based on specific effects onto the dopaminergic system, especially, if dat-1 mutants at L4 stage paralyze in water already without any drug? It is therefore important to include both negative and positive controls into the example assay, e.g. L4 dat-1 mutants and cat-2 mutants.*

We agree with the reviewer both positive and negative controls need to be included during the assay to confirm dopamine as the only mediator of SWIP. We have discussed this important point in the revised version of the manuscript (see lines 374-400).

3) *The introduction and abstract mention "liquid media" as basis for the assay. Liquid media is a vague terminology and can mean anything from water, to M9 buffer….*

We have replaced liquid media with water throughout the manuscript and discussed that salty solutions prevent SWIP (see lines 332-333).

4) *Is the time span of the assay critical? ….*

The reviewer is correct. The time-span of the assay is critical. In the revised manuscript, we discuss this important point (see lines 335-345).   
  
Minor Concerns:  
1) *Can an alternative to the Pyrex spot well plate be used e.g. using hydrophobic pen circles on a cover slip? Any transparent container works.*

We chose to use glass Pyrex spot well plates because they can be thoroughly washed, autoclaved and re-used when different types of drugs are tested (see lines 324-325)   
  
3) *Any type of statistical program can be used e.g Prism6 Graphpad.*

The reviewer is right, and we have corrected the manuscript accordingly (see lines 231-232).

4) *Why is a two-way ANOVA test used for the analysis if only one parameter is assessed (percentage of animals swimming) in 2 or more groups, shouldn't it be one-way ANOVA test instead?*

We agree with reviewer that when only one parameter is assessed in two or more groups 1-way ANOVA should be used to perform statistical analysis. However, for data containing 2 independent variables, time and treated/control animals (figure 2) or time and N2/mutant animals, the two-way ANOVA test needs to be used. We thank the reviewer for suggesting to clarify this point. (see lines 235-236).

5) *Figure 2: "percentage of animals exhibiting SWIP was calculated by dividing the number of paralyzed worms by total number of worms.." missing: and multiplied by 100.*

We have corrected the text (lines 231 and 292).