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Pharmacological validation of the prepulse inhibition of startle response in larval zebrafish using a commercial automated system and software

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Corresponding Author:	Camila Esguerra NORWAY
Corresponding Author's Institution:	
Corresponding Author E-Mail:	c.v.esguerra@ncmm.uio.no
Order of Authors:	Camila V Esguerra
	Nancy Saana Banono
Additional Information:	
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UiO : University of Oslo
Faculty of Medicine/Centre for Molecular Medicine Norway (NCMM)

To the Editor

Journal of Visualized Experiments

March 03, 2020

Dear Dr. Aaron Berard,

Please find enclosed a replication manuscript entitled, **"Pharmacological validation of the pre-pulse inhibition of startle response in larval zebrafish using a commercial automated system and software"** by N. S. Banono & C. V. Esguerra, 2020. In this article, we aimed to provide a comprehensive validation of pre-pulse inhibition of the acoustic startle response in zebrafish larvae using the recently launched ZebraBox Revo (ViewPoint, France) in combination with EthoVision (Noldus, Netherlands) software as previously characterized by Burgess & Granato, 2007. To our knowledge, such a study is necessary to improve, refine and standardize protocols used for neurobehavioral research. Due to a growing interest in zebrafish as an animal model for central nervous system diseases, we believe it will serve as a valuable tool for those using zebrafish in neuropsychiatry research, in particular, in the area of high-throughput screening or for those trying to elucidate the contribution of neuropsychiatry risk genes to disease pathologenesis.

First, we assess the ability of the ZebraBox Revo and EthoVision software to accurately deliver/capture acoustic stimuli and characterize the larval C-start response respectively, with a detailed description of the modulatory PPI effects induced by drugs targeting dopaminergic and glutamatergic signaling. Herein, we highlight both the advantages and limitations of the two systems and provide recommendations, when relevant, on how to circumvent these challenges.

We have also included supplementary materials that complements the description of the equipment set-up used and optimizations performed to reduce external interference in our experimental protocol.

Although we realize that other people may be selected, we would like to nominate the following reviewers:

1. Prof. Dr. Mehmet Fatih Yanik
(Zebrafish expert in brain imaging and high-throughput screening)
Professur für Neurotechnologie
ETH Zürich
8057 Zürich, CH
Phone: +41 44 633 31 65
Email: yanik@ethz.ch

2. **Dr. Will Norton**
(Zebrafish Behavioural Neuroscience)
Associate Professor
Department of Neuroscience, Psychology and Behaviour
University of Leicester, University Road, Leicester, LE1 7RH, UK
Phone: +44 (0)116 252 5078
Email: whjn1@le.ac.uk

3. Prof. Dr. Stephan Neuhauss
(Zebrafish expert in visual system function and behavioural assays of visual behaviours)
Institute of Molecular Life Sciences
University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, CH
Phone: +41 (0)44 635 60 40
Email: stephan.neuhauss@uzh.ch

4. Dr. Christian Tudorache
Assistant professor
Wiskunde en Natuurwetenschappen
Instituut Biologie Leiden
IBL Animal Sciences & Health
Sylviusweg 72, 2333 Leiden, Room number 6.514B, NL
Phone: +31 71 527 4759
Email: c.tudorache@biology.leidenuniv.nl

5. Prof. Teresa Nicolson
(Zebrafish expert in hearing and balance)
300 Pasteur Drive
Edwards R139 Stanford University
Stanford, CA 94305, USA
Phone: 650-725-3708
Email: tnicolso@stanford.edu

6. **Prof. Kari Espolin Fladmark**
(Expert in behavioural testing of neurotoxicants linked to human neurodegenative diseases using the zebrafish)
Department of Biological Sciences (BIO)
University of Bergen
Phone: +47 55 58 45 14
Email: Kari.Fladmark@uib.no

We hope that you will find our study interesting and relevant for publication in the *Journal of Visualized Experiments*, and hope that it fulfills all requirements necessary for its publication. This manuscript has not been and will not be published in whole or in part in any other journal. **We, the authors, have no competing interests, have read and have abided by the statement of ethical standards for manuscripts submitted to the *Journal of Visualized Experiments*.**

We thank you for considering our manuscript and look forward to hearing from you!

Best regards,

Camila V. Esguerra, PhD
Group Leader, Chemical Neuroscience Group, Centre for Molecular Medicine Norway (NCMM)
Adjunct Associate Professor, School of Pharmacy, University of Oslo
Oslo Innovation Centre (Forskningsparken), Gaustadalléen 21, 0349 Oslo, Norway
Office: +47 228 40534; Mobile: +47 950 40178
E-mail: c.v.esguerra@ncmm.uio.no
Website: http://www.med.uio.no/ncmm/english/groups/esguerra-group/

TITLE:

Pharmacological Validation of the Prepulse Inhibition of Startle Response in Larval Zebrafish Using a Commercial Automated System and Software

AUTHORS:

Nancy Saana Banono[1, 2] and Camila V. Esguerra[1, 2]

[1]Chemical Neuroscience Group, Centre for Molecular Medicine Norway (NCMM), Faculty of Medicine, University of Oslo, Gaustadalléen 21, 0349, Oslo, Norway

[2]Section for Pharmacology and Pharmaceutical Biosciences, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Oslo, Norway

Corresponding Author:

Camila V. Esguerra (c.v.esguerra@ncmm.uio.no)

Email Addresses of Co-Authors:

Nancy Saana Banono (n.s.banono@ncmm.uio.no)

KEYWORDS:

Zebrafish, acoustic startle response, prepulse inhibition, pharmacology, apomorphine, haloperidol, ketamine

SUMMARY:

Here we describe a protocol that utilizes commercially available automated systems to pharmacologically validate the prepulse inhibition (PPI) assay in larval zebrafish.

ABSTRACT:

While there is an abundance of commercial and standardized automated systems and software for performing the prepulse inhibition (PPI) assay in rodents, to the best of our knowledge, all PPI assays performed in the zebrafish have, until now, been done using custom made systems which were only available to individual groups. This has thereby presented challenges, particularly with regard to issues of data reproducibility and standardization. In the present work, we generated a protocol that utilizes commercially available automated systems to pharmacologically validate the prepulse inhibition (PPI) assay in larval zebrafish. Consistent with published findings, we were able to replicate the results of apomorphine, haloperidol and ketamine on the PPI response of 6 days post-fertilization zebrafish larvae.

INTRODUCTION:

The zebrafish (*Danio rerio*) larva is a suitable candidate for modelling psychiatric diseases such as schizophrenia (reviewed by Gawel et al.[1]) because of the numerous advantages it possesses. These include a fully sequenced genome with 70% sequence homology to human orthologues[2], existence of forward and reverse genetic tools to manipulate the genome and to identify the contribution of a given gene towards development or disease[3], and the presence of major human/rodent neurotransmitters in the zebrafish brain[4]. There is an availability of several neuro-

phenotypic domains in zebrafish, such as anxiety, learning and memory[3]. Optical transparency and sensitivity to the major classes of neurotropic drugs makes it an ideal candidate of choice for pharmacological manipulations and phenotypic drug screening[5, 6].

To perform high throughput drug screening, automation and the presence of a robust endophenotype is highly important[7]. For instance, a variety of automatic recording techniques have been developed for measuring larval zebrafish behavior such as thigmotaxis, startle response, optokinetic response, optomotor response, habituation, prey capture, sleep/wake behavior, locomotor behavior and several others[6]. While some laboratories develop custom-built systems for automated measurements and analysis of some of these behaviors, there are commercially available imaging and software systems[8–11]. Prepulse inhibition (PPI), a form of sensorimotor gating in which the startle response is reduced when a weak non-startling stimulus is presented briefly before the startling stimulus, has been used as an endophenotype for studying schizophrenia in animal models (reviewed by [12, 13]). In addition, acoustic startle response (ASR) and PPI have played useful roles in studying hearing and auditory function in animal models including the zebrafish[14, 15]. The larval zebrafish displays a characteristic C-start in response to an unexpected startling stimulus that is lessened by a weaker stimulus called the prepulse. The C-start has long been described as an escape behavior controlled by distinct neural cell populations and has been thoroughly characterized in the larval zebrafish[15–17].

There is an abundance of commercial and standardized automated systems and software for performing the PPI assay in rodents[18–20]. However, to the best of our knowledge, all the PPI assays performed in the zebrafish until now have been done using custom made systems which are only available to the individual groups[15, 16, 21, 22]. This presents challenges for achieving data reproducibility and replicability with regard to standardization[23].

Recently, a known vendor in the zebrafish community developed a set-up embedded with a fast camera and PPI generator add-ons to carry out the PPI assay in larval zebrafish[24]. The camera records at 1000 frames per second which enables the recording of fast acting behaviors such as the C-start, while the PPI generator allows for user-controlled delivery of various acoustic stimuli to evoke a startle response[24]. Here, we combine the aforementioned system with a commercially available comprehensive software package designed for the automated analysis of complex behaviors[11], to generate a protocol for performing PPI response assays in larval zebrafish. We pharmacologically validate the PPI response using 1) apomorphine, a dopamine agonist known to cause deficits in PPI; 2) haloperidol, a dopamine antagonist and antipsychotic known to enhance PPI and 3) ketamine, a NMDA receptor antagonist known to modulate PPI.

PROTOCOL:

All animal experiments were approved by the Norwegian Food Safety Authority experimental animal administration's supervisory and application system (FOTS-18/106800-1).

1. Zebrafish husbandry

1.1. Set up matings of wild type adult male and female zebrafish (*Danio rerio*) stocks, maintained under standard conditions[25] the evening before. Here, Tupfel long-fin (TL) strain is used.

1.2. Remove barriers the next morning and allow to mate through natural spawning.

1.3. Collect eggs out of the mating tanks.

1.4. Remove unfertilized eggs and other debris, then transfer eggs to Petri dishes (n = 60) and raise in an incubator at 28 °C in embryo medium: 1.5 mM HEPES, pH 7.6, 17.4 mM NaCl, 0.21 mM KCl, 0.12 mM MgSO4, and 0.18 mM $Ca(NO3)_2$.

1.5. Renew half of the embryo medium and remove dead larvae daily until 6 dpf.

NOTE: All experiments were performed on individual larvae at 6 days post-fertilization (dpf).

2. Pharmacological agents and pre-treatment of larvae

2.1. Dissolve apomorphine and ketamine in E3 medium to make 500 µM and 10 mM stock solutions respectively.

2.2. Dissolve haloperidol in 100% dimethyl sulfoxide (DMSO) to make a 10 mM stock solution. The final concentration of DMSO used was 0.1%.

2.3. Use 0.1% DMSO and E3 medium as vehicle controls.

2.4. Use the following final concentrations of drugs: 10 mg/mL of apomorphine, 1 mM ketamine and 20 µM haloperidol[16].

2.5. Pre-expose the apomorphine and ketamine groups larvae for 10 min and the haloperidol and DMSO vehicle control groups for 20 min[16].

3. Setup prior to the behavior test

3.1. On the day of the experiment, transfer larvae and all relevant materials into the experiment room. Set the experiment room to a temperature of 27 ± 1 °C.

3.2. Ensure that the background noise in the **test chamber** is as low as possible, preferably not more than 45 dB sound pressure level (**SPL**).

3.2.1. Install the sonometer microphone of the dB meter in the test chamber (the opening for installation is already bored by the manufacturer).

3.2.2. To reduce the background noise in the room, insulate the test chamber with a custom-built sound booth (see **Figure 1B** for an overview of the set-up).

3.3. Prepare a 96-well plate for the prepulse inhibition test.

NOTE: The video camera has a 2048 × 500 pixel resolution, meaning only a maximum of 3 lanes (33 wells) can be imaged at a time.

3.3.1. Use a custom-made acrylic plate of 96-well format to reduce interference from shadows.

NOTE: The measurements for the custom plate can be found at the following website: https://zenodo.org/record/3739378#.XooyLW5uKas

3.4. With the aid of a transfer pipette, transfer 310 µL of exposure solution/medium with one larva into each well.

3.5. Calibrate and measure the stimulus intensity using the volume knob of the stereo amplifier and a decibel meter respectively.

3.6. Register the maximum sound intensity in the “level reference” section.

4. Stimulus parameters and video acquisition

4.1. Turn on the computer, the amplifier system and the decibel (dB) meter (see **Figure 1A** for an overview of set-up).

4.2. Use the volume knob by turning it to minimum or maximum to adjust the sound intensity.

4.2.1. Check the sound level with the dB meter each time, the volume knob is adjusted. This is important in finding the maximum and minimum sound intensity that can be produced by the set-up.

NOTE: The dB meter computes the RMS dB output for the stimulus. The system generates the sound inside the solid components of the test-chamber, keeping the plate firm while producing a vibration in the horizontal plane of the entire plate support.

4.2.1.1. Adjust the volume knob to maximum, measure the sound intensity with the dB meter and use this value.

4.3. On the interface of the PPI generator, define the parameters: inter-stimulus interval represented as **Delay**; inter-trial interval represented as **Acquisition delta time**; duration of prepulse etc.

4.3.1. For prepulse alone trials, ensure that the “**Amplitude**” or **Duration of stimulus** for **Startle** parameters are set to zero and vice versa for startle alone trials.

4.4. To generate a trial list, select **Add** > give a name to the trial. For example, “Prepulse 50 dB alone”.

NOTE: One can generate as many trials as desired, but be careful of how long the list is since this can crash the program.

4.4.1. Interleave prepulse trials with pulse alone trials in all PPI experiments using a pseudorandom order. Where multiple stimuli are presented in an experiment, an inter-trial interval (ITI) of 30 s is used.

NOTE: In this study, a 100 ms startle stimulus (pulse) of 660 Hz, and 5 ms prepulse stimuli of 440 Hz were used. For PPI experiments, inter-stimulus interval (ISI) was 100 ms.

4.5. To save the protocol, select **File** > **Save as**.

4.6. Adjust lighting conditions in the test-chamber as follows.

4.6.1. Launch **USB measurement computing**, select **analog out** then go **to D/A OUT O** (P13) to make changes to the lighting. A value of zero means no light while increasing the D/A OUT O value, increases the intensity of light in the box. Light intensity of 100 Lux was used for all experiments.

4.7. Set-up the camera

4.7.1. Launch the software and wait for the camera to load.

4.7.2. Select **Adjustments** (found on right-hand side) and set the acquisition frame rate to 1,000 then click apply to effect the change.

4.8. Acclimate larvae to a 100 lx test chamber for 5 min before the experiments are started.

4.9. To begin an experiment, select the **Experiment** menu on the **PPI generator**, click **Run** and the select well format (e.g., 33 wells).

4.9.1. Always make sure that the camera software is launched with the right settings before running an experiment.

4.9.2. Acquire a 2 s video for each trial.

4.9.3. Make sure that the acquisition frame rate is set at 1,000.

5. **Automated tracking and analysis of acoustic startle response and PPI**

5.1. Protocol setup.

5.1.1. Launch the analysis software (see the **Table of Materials**). Choose **New from template** > **Apply a predefined template** and then go through other menus (details below).

5.1.1.1. Choose **from video file** under **Video Source**.

5.1.1.2. Browse video file. Set **subject** as **fish > zebrafish larvae > zone template** (no template). Specify **Number of arenas** under **Arenas**.

5.1.1.3. Specify **Number of subjects per arena** (set as 1) under **Subjects**.

5.1.1.4. Select **Center-point, nose-point and tail-base detection** under **Tracked Features** (see **Figure 2A,B**).

NOTE: This is important to calculate the body angle of the C-start response (see **Figure 2C**).

5.1.1.5. Click **Name** > **save as**. Units used are mm, s, deg for distance, time and rotation respectively.

NOTE: Remember to use the same unit for calibrating the scale.

5.1.2. Choose **Arena setting**.

5.1.2.1. Click **Grab background image**.

5.1.2.2. Go through the steps on the right-hand menu (if in doubt, use the **Help** menu).

5.1.2.3. Choose the circle drawing tool to draw the arenas.

5.1.3. Choose **Trial control settings** > **create new** > **name**.

5.1.4. Choose **Detection settings**, go through the steps on the right-hand menu.

5.1.4.1. Set sample rate to 25. Choose **advanced detection settings.** Under **Method**, select **dynamic subtraction**, **advanced model/adult fish**, then set **Subject color compared to background** as **Darker** and move the slider to define the larva's contrast.

5.1.4.2. Under subject contour, select erode first, then dilate and increase the contour erosion and dilation values until the animal is completely detected.

5.1.5. Save the protocol and use for subsequent analyses of PPI videos acquired.

5.2. Trial list setup.

5.2.1. Choose **trial list**, define independent variables such as larval ID, treatment, stimulus type, etc. Select the path of videos and define a list of trials for batch acquisition.

5.3. Acquisition setup.

NOTE: If a trial list has been generated, one can perform a batch acquisition of the videos.

5.3.1. If some tracks are lost, use the track editor to adjust the tracked features.

5.3.2. Exclude from analysis, the tracking errors that remain unresolved after using the track editor.

5.3.3. Set track smoothing profile to 1 mm to decrease noise from data. This can be adjusted based on the background activity of larvae.

5.4. Analysis setup.

5.4.1. To select trials to be analyzed, choose **Data profiles** and define tracks based on the independent variable of interest.

NOTE: If components are hidden, click on the eye symbol to the upper right-hand corner to display.

5.4.1.1. **Filter** parts of trials to be analyzed (e.g., based on treatment or type of stimulus group).

5.4.1.2. Select part of the tracks to be analyzed (**nesting**). For this study, data was nested for tracks between onset of stimulus and 100 ms after stimulus onset.

5.4.1.3. Remember to connect all filters and nesting boxes with arrow lines to complete the instruction.

5.4.2. Define dependent variables to be analyzed, select **Analysis profiles** and specify the variables of interest (focus on **Body** under **dependent variables**).

NOTE: If components are hidden, click on the eye symbol to the upper right-hand corner to display.

5.4.2.1. Double click **Body angle**. Select **absolute bend**. Go through **Trial settings** and select **maximum**, then click on **add.**

5.4.3. Double click **body angle state**. Set averaging interval to **5 samples**. Set bend angle threshold. To calculate statistics for **bent**, go through **Trial statistics** and select **latency to first** > **add**. Repeat steps until varying thresholds are obtained (between 20–80° was used) and name accordingly.

5.4.4. Generating **statistics and charts**.

5.4.4.1. Choose **analysis** > **results** > **statistics & charts**, then click **calculate**.

5.4.4.2. Make sure the data and analysis profiles are set to the right template since several templates can be made under each section.

5.4.5. Export trial and group statistics as spreadsheet files for processing and analysis.

6. **Data analysis**

6.1. Open the spreadsheet file containing the trial statistics.

6.2. Select the columns **Body angle Maximum deg, Bent latency** (of the various body angle thresholds).

6.3. Consider every change in body angle ≥ 30° within a cut-off latency of 50 ms after stimulus onset as a positive C-start response (i.e., a responder); those with <30° body angle are non-responders.

6.4. In a binary fashion, assign 1 to a responding larva and 0 to non-responding larvae for each plate.

6.4.1. Count the total number of responders and non-responder larvae for each plate. Calculate the responders (%) in each case calculated as (number of larvae responding/total number of larvae) × 100. Exclude larvae that respond less than 30% to the startle stimulus from the analysis[16].

NOTE: Any stimulus intensity capable of eliciting a C-start response in equal to or more than 70% of the larvae is considered a suitable startle stimulus[16].

6.5. Calculate %PPI as 100 × (percentage responding to startle stimulus − percentage responding to prepulse + startle sequence)/ (percentage responding to startle stimulus)[16].

7. Statistical analysis

7.1. Present data as the mean ± standard deviation, S.D. (see the **Table of Materials** for statistical software).

7.2. Determine the effects of varying prepulse intensities on larval response using one-way ANOVA followed by a Tukey's post-hoc test.

7.3. Use two-way ANOVA followed by Holm-Sidak's post-hoc test to determine the effects of drug treatment on % PPI response with varying prepulse intensities.

REPRESENTATIVE RESULTS:

Three experiments were performed to validate the protocol of combining multiple systems to analyze prepulse inhibition of acoustic startle response in the larval zebrafish. First, the ability to accurately deliver acoustic stimuli and to capture the response of larvae to the startle stimulus was tested. Next, was validating the ability to attenuate startle response when a prepulse stimulus is presented. Finally, the ability to detect the pharmacological modulation of prepulse inhibition of the startle response by the drugs apomorphine, haloperidol and ketamine was established.

Larval zebrafish response to acoustic startle stimuli

Previous work has demonstrated that larval zebrafish display a characteristic C-start when presented with startling acoustic stimulus[16]. The ability to incite and capture the behavior of larvae to the startle stimuli was tested. Recorded larvae were observed to display the C-start response (**Figure 2**). A stimulus of 70 dB re (100 ms, 660 Hz, **Supplementary Figure 1A**) was strong enough to elicit response in ≥70% of the larvae (**Figure 3A**). When repeatedly presented 30 times at an inter-trial interval of 30 s, the 70 dB re stimulus did not result in larval habituation (N = 3 replicates; 16 larvae/replicate), as shown in **Figure 3B**.

Prepulse decreases startle response of larval zebrafish to acoustic stimuli

A plethora of evidence shows that prepulse stimuli modulates larval response to a startle stimulus[15, 21, 22, 26]. A two-pulse paradigm was used, where a weak stimulus called the prepulse preceded the startle-inducing stimulus called the pulse. The prepulse stimuli used were either 20, 17, or 14 dB less than the pulse stimulus that was set at 70 dB re. The prepulse (5 ms, 440 Hz) was always presented 100 ms before the pulse onset (**Supplementary Figure 1B**). Each tested prepulse stimulus significantly reduced larval response to the pulse. In **Figure 4** the larval response (in %) to acoustic startle stimuli is shown for 6 dpf TL in E3 medium, N = 6 (16 larvae/group). The percentage of larvae responding to the startle stimulus (pulse) was 79.86 ± 9.772. Expectedly, when the startle stimulus was preceded by either a 50, 53 or 56 dB prepulse, the larval response decreased to 40.87% ± 11.30%, 39.58% ± 7.345% and 29.17% ± 9.350% respectively. One-way Anova analysis revealed a statistical difference in stimulus effect on larvae ($F(3, 48) = 57.23$, $P < 0.0001$) with Tukey's multiple comparisons test revealing statistical significance across groups at 95% confidence interval.

Pharmacological modulation of prepulse inhibition

Earlier studies showed that the dopaminergic drugs, apomorphine and haloperidol, as well as the glutamatergic drug, ketamine, significantly modulated prepulse inhibition in larvae just as in their mammal and rodent equivalents[16]. Concentrations for validation of the set-up were selected based on these studies. The inter-stimulus interval (ISI) for all the pharmacological experiments was 100 ms.

Effect of apomorphine on prepulse inhibition

In **Figure 5**, larvae pretreated with 10 mg/mL apomorphine for 10 min displayed an overall reduction in % PPI compared to E3 control larvae (two-way ANOVA, non RM (factors: treatment

and prepulse intensities; treatment: F (1, 34) = 16.21, p = 0.0003; prepulse intensity: F (2, 34) = 8.674, P = 0.0009, this showed a non-significant interaction: F (2, 34) = 2.514, p = 0.0959). To investigate the differences in more detail, Holm-Sidak's post-hoc test revealed significant differences in the startle response between E3 control and apomorphine treated larvae at prepulse intensities 53 (p = 0.0126) and 56 (p = 0.0044) but not at 50 dB (p = 0.5813).

Effect of haloperidol on prepulse inhibition

Figure 6 shows an overall increase in %PPI in larvae pretreated for 20 min with 20 µM haloperidol compared to those in E3 medium (two-way ANOVA, non RM (factors: treatment and prepulse intensities; treatment: F (1, 32) = 20.75, p < 0.0001; prepulse intensity: F (2, 32) = 3.147, p = 0.0565, with no significant interaction: F (2, 32) = 0.7455, p = 0.4826). Using the Holm-Sidak's post-hoc test, presence of statistical significance was observed only at prepulse intensities 53 (p = 0.00489 and 56 (p = 0.0348) but not at 50 dB (p = 0.067).

Effect of ketamine on prepulse inhibition

Figure 7 shows that at different prepulse stimulus intensities, there were differences in the startle response between E3 control larvae and those pretreated for 10 min in 1.0 mM ketamine (two-way ANOVA, non RM (factors: treatment and prepulse intensities; treatment: F (1, 35) = 25.46, p < 0.0001; prepulse intensity: F (2, 35) = 6.018, p = 0.0057, with no significant interaction: F (2, 35) = 0.8450, p = 0.4381). Holm-Sidak's post-hoc test, showed significance only at prepulse intensities of 50 (p = 0.0039) and 53 (p = 0.0027), but not at 56 dB (p = 0.0802).

FIGURE LEGENDS:

Figure 1: Testing apparatus. (**A**) Overview of equipment set-up. (**B**) In-house insulation of the set-up equipment to minimize background noise during experiments.

Figure 2: Analysis of the larval zebrafish acoustic startle response. (**A**) Characteristic C-start displayed by 6 zebrafish larvae at 6 dpf. (**B**) Representative image of the three tracked features superimposed on a 6 dpf larva: center-point (red), nose-point (cyan) and tail-base (purple). (**C**) Representative image of the absolute bend angle displayed by a 6-dpf TL wild type larvae.

Figure 3: Determination of acoustic startle threshold. (**A**) A stimulus intensity of 70 dB (represented by red dash lines) is capable of eliciting a C-start response in >70% of larvae (N = 33; 6 dpf TL). (**B**) Larvae do not habituate to 70 dB re stimulus presented 30 times (trials) at an inter-trial interval of 30 s (N = 3 replicates; 16 larvae/replicate). Data are presented as mean ± S.D.

Figure 4: Pre-pulse-induced decrease in larval response (%). Pre-pulse stimuli at 20, 17 and 14 dB lower than the 70 dB re startling stimulus cause a reduction in the number of wild type TL larvae C-start responders. All data represented as mean ± S.D., N = 5 (16 larvae/group), ****p < 0.0001, significantly different from startle stimulus by Tukey's post-hoc test after one-way ANOVA.

Figure 5. Apomorphine induced deficits in %PPI. All data are presented as mean ± S.D., N = 4–5 (16 larvae/group), statistically significant difference by Holm-Sidak's post-hoc test after two-way ANOVA. *p = 0.0126, E3 ctl/apomorphine treated group at 53 dB; **p = 0.0044, E3 ctl/apomorphine treated group at 56 dB.

Figure 6: Haloperidol induced enhancement in %PPI. All data are presented as mean ± S.D., N = 4–5 (16 larvae/group), statistically significant difference by Holm-Sidak's post-hoc test after two-way Anova. **p = 0.0048, DMSO ctl/apomorphine treated group at 53 dB; *p = 0.0348, DMSO ctl/apomorphine treated group at 56 dB.

Figure 7: Ketamine induced enhancement in %PPI. All data represented as mean ± S.D, N = 4–5 (16 larvae/group), statistically significant difference by Holm-Sidak's post-hoc test after two-way Anova **p = 0.0039, E3 ctl/apomorphine treated group at 50 dB, **p = 0.0027, E3 ctl/apomorphine treated group at 53 dB.

Supplementary Video 1: Representative video of larvae displaying a C-start in response to 70 dB acoustic startle stimulus.

Supplementary Figure 1: Representative examples of generated stimulus conditions using the PPI generator. (**A**) Stimulus alone trial, (**B**) pre-pulse inhibition trial (pre-pulse + pulse), (**C**) no stimulus trial to measure threshold baseline bend angle of unstimulated larvae.

DISCUSSION:

It is essential to validate any new behavioral assay system with the aim of improving and refining protocols for neurobehavioral research. In the current investigation, the ability of two commercially available systems and software to induce an acoustic startle response in zebrafish larvae and to detect and quantify previously described pharmacological modulation of such behaviors were assessed.

A number of modifications and troubleshooting were performed to optimize the set-up. The default software for analysis of C-start responses was such that analysis automatically proceeded after the data for every experiment was acquired (22 trials/plate constituted an experiment). This reduced the number of plates that could be run per day, thus reducing the throughput (5 plates per day). To avoid this limitation, there was a need to de-couple the analysis software from the data collection process, which increased the throughput to an average of 10 plates per day. Thus, the decision to turn to an independent analysis software for non-live analysis proved successful and more efficient. To avoid interference from shadows or other debris which introduces noise to the data, it is recommended to fill wells completely with medium, remove all bubbles and avoid food particles or similar which can be mistaken for larvae, thereby generating noise in the data. After calibration of the sound stimuli, the maximum intensity reachable by the amplifier system as captured by the dB meter was 85 dB re, while the initial background noise in the testing chamber was 60 dB re. This resulted in a narrow dB window in which to operate. Hence, it was critical to keep background noise as minimal as possible. To achieve this, parafon acoustics material (see **Table of Materials**) was used to build an additional layer of insulation around the

test-chamber and an extra layer of insulation using a vocal booth bundle (see **Table of Materials**). With these layers of insulation, the background noise inside the testing chamber was successfully reduced from the initial 60 dB to 45 dB re.

Currently, one advantage of this set-up is that all the components are commercially available and as such, not limited to only a few labs. Individuals with limited knowledge in coding language can use it, as the protocol is rather easy to understand and follow. For example, by using the PPI system, it was possible to deliver pulses and pre-pulses at varying inter-stimulus and inter-trial intervals, as well as capture larval responses to such stimuli. Once these behaviors were captured, they could be classified using the analysis software into responders and non-responders. The responder group was categorized as larvae that displayed a C-start of 30° or more at a latency of <50 ms. In addition, the PPI response is modulated by drugs that target dopaminergic and glutamatergic signaling (reviewed by Geyer and colleagues [27]). Consistent with previous studies, apomorphine, a non-selective dopamine receptor agonist, reduced the pre-pulse inhibition of startle response in larval zebrafish, while haloperidol a dopamine antagonist enhanced the response. In larval zebrafish, ketamine has been shown to modulate PPI differentially based on the duration of the ISI[16]. In the aforementioned study, larval PPI was enhanced at 30 ms but suppressed at 500 ms ISI when pre-treated with ketamine. Although this study did not use variable ISI, the observation that ketamine enhanced PPI at an ISI of 100 ms, makes it comparable with the previous study's data when an ISI of 30 ms was used. The study demonstrated that by combining these commercially available systems, it is possible to perform the PPI assay and to reliably detect pharmacologically induced changes in the zebrafish larval PPI response. A limitation of the system is that the nose-point feature tracked by the analysis software always falls on one of the eyes of the larvae, thereby creating a baseline angle. To overcome this, it is necessary to always determine the baseline bend angle of unstimulated larvae, which was found to be ~30° for larvae used in this study. Thus, forming the basis for the choice of 30° as the threshold of what was considered a positive C-start response in startled larvae. If these points are taken into account, it should be possible to perform the PPI assay in any lab with access to the set-up equipment. This paper did not focus on categorizing the kinematics of startle response into short latency and long latency as reported earlier[16], due to the scope of the variability of latency. Hence, only C-start responses <50 ms after stimulus onset were used[15].

Strain differences have been reported to influence zebrafish behavior in several assays[28–31] as well as influence hearing sensitivity[32]. Hence, it is essential to determine the baseline bend angle of each strain tested. Since hearing sensitivities may also be different, it is crucial to determine baseline startle responses, the sound intensity most suited as either prepulse or startle stimulus for each strain and at what duration the stimulus is presented. The ISI is another parameter that should be carefully considered because some drugs can either enhance or reduce PPI based on the interval between the prepulse and startle stimulus onset[16]. The expectation is that, laboratories interested in studying cognitive function, neuropsychiatric disorders and hearing (auditory function) will find this PPI set-up and protocol useful in screening their pharmacological and/or genetic models. This protocol also provides a basis for high-throughput screening of compound libraries.

ACKNOWLEDGMENTS:

We thank Ana Tavara and João Paulo R. P. Santana for excellent fish care and invaluable help with testing and setting up of the soundproof booths, and Dr. Wietske van der Ent for initial support with setting up the EthoVision software. This study was funded by the Research Council of Norway (ISP, BIOTEK2021/ DigiBrain).

DISCLOSURES:

The authors declare no competing financial interests.

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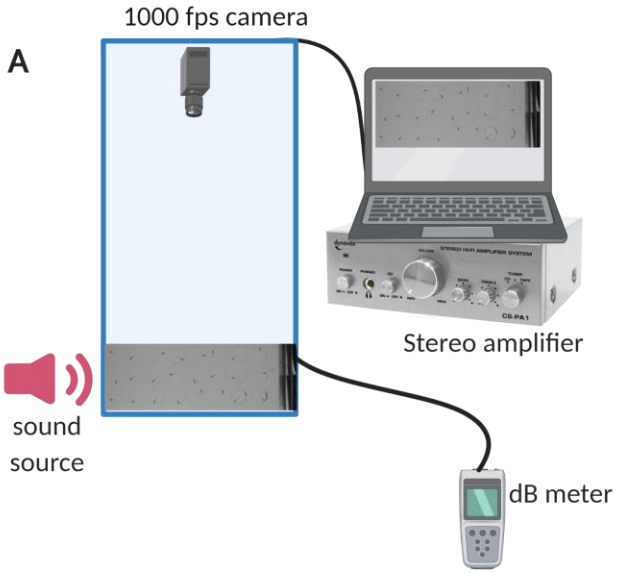
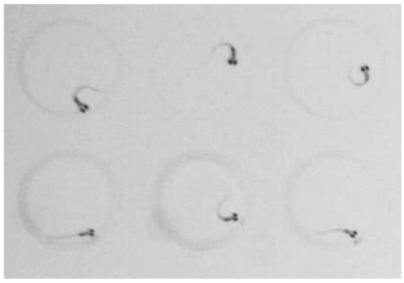
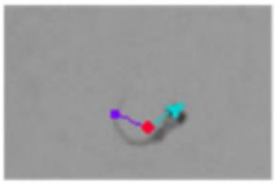
Figure



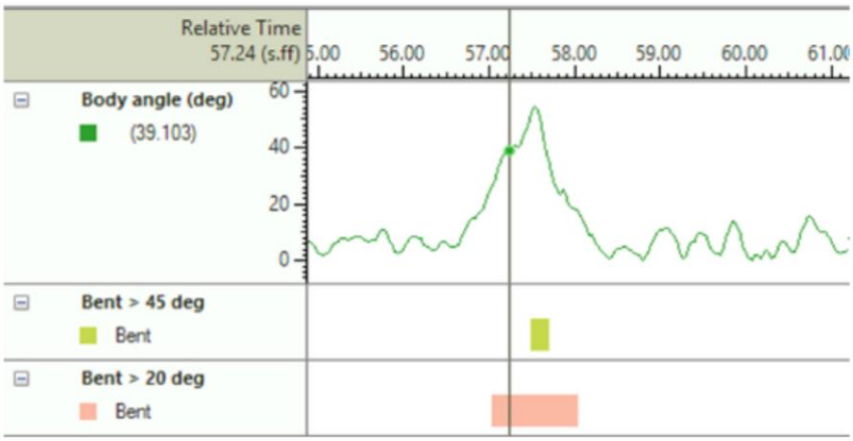
A

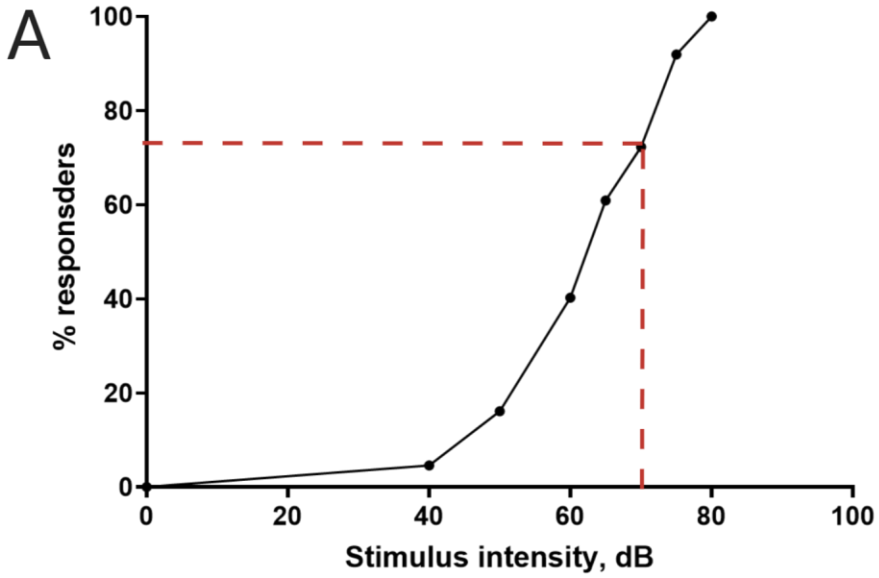


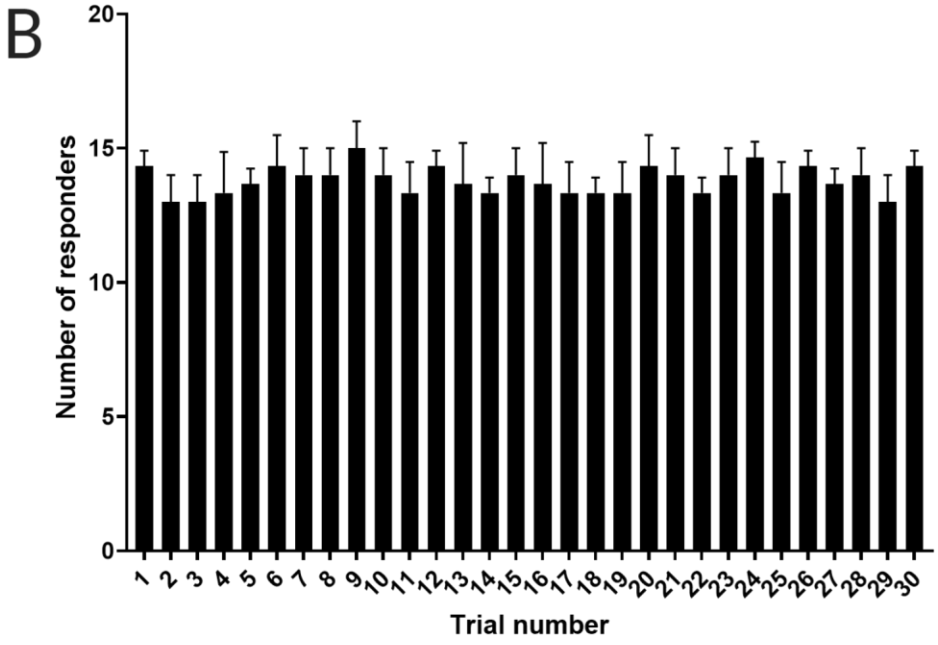
B

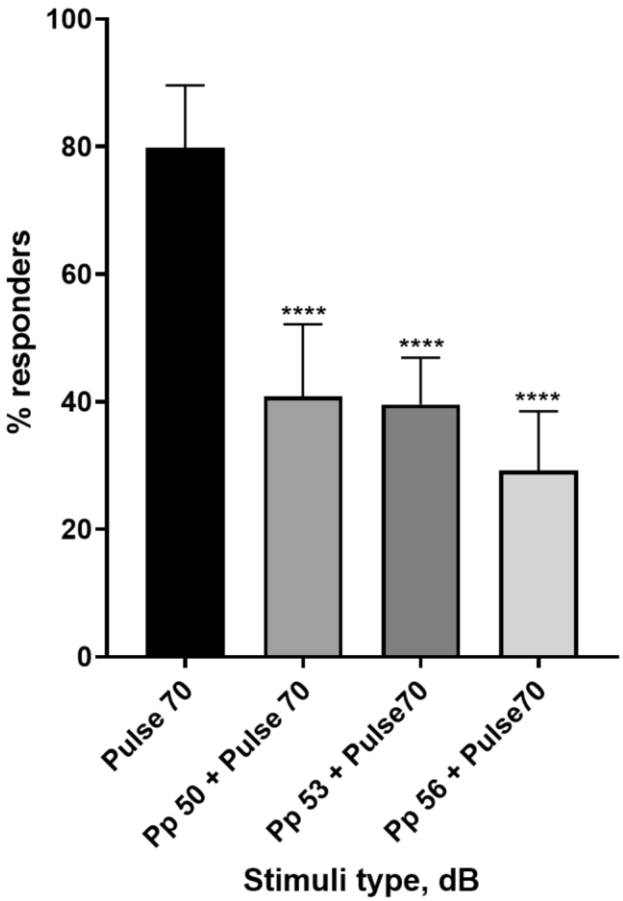


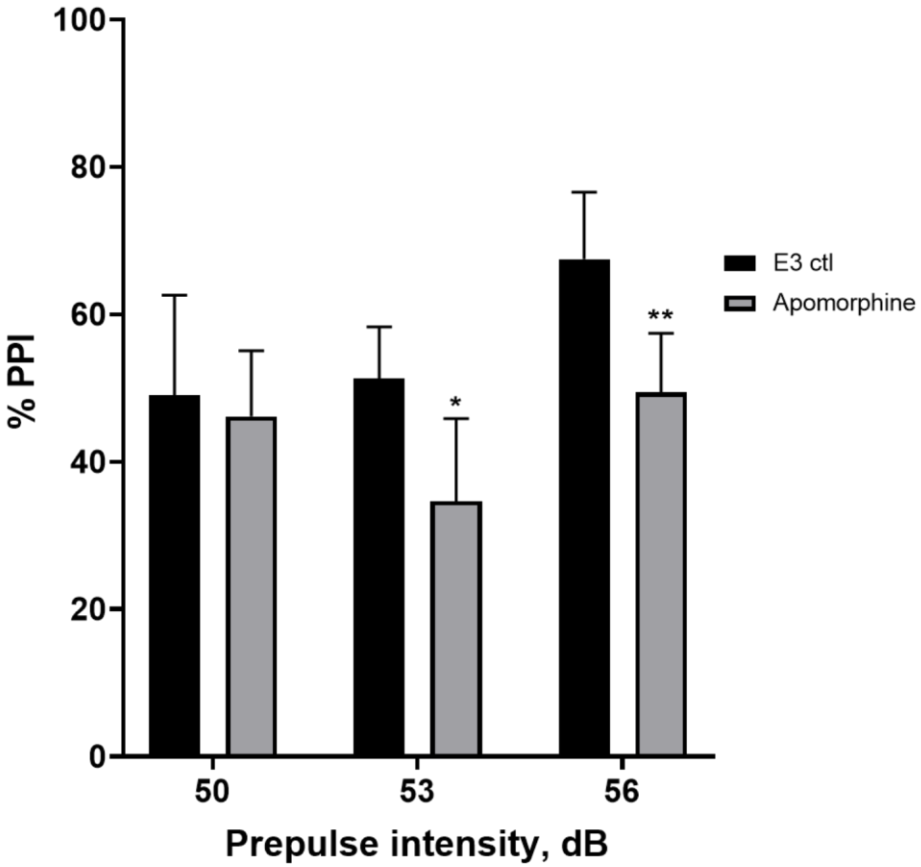
C

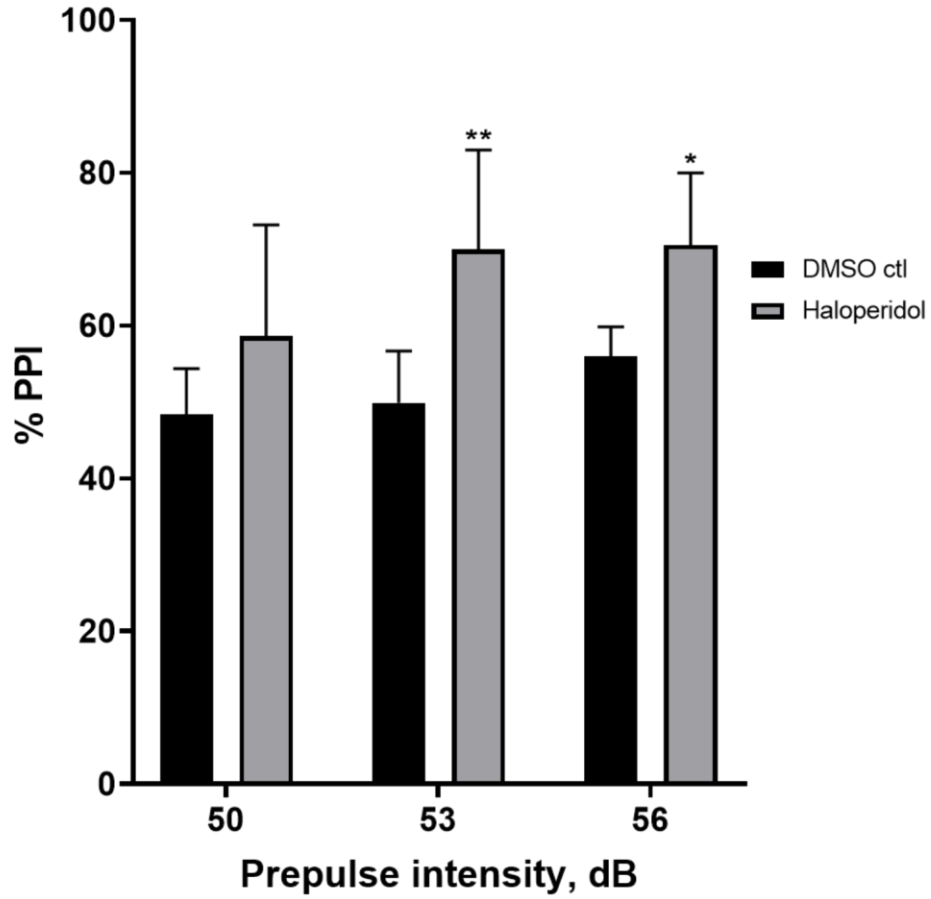

Relative Time
57.24 (s.ff)
5.00
56.00
57.00
58.00
59.00
60.00
61.00
Body angle (deg)
(39.103)
60
40
20
0
Bent > 45 deg
Bent
Bent > 20 deg
Bent

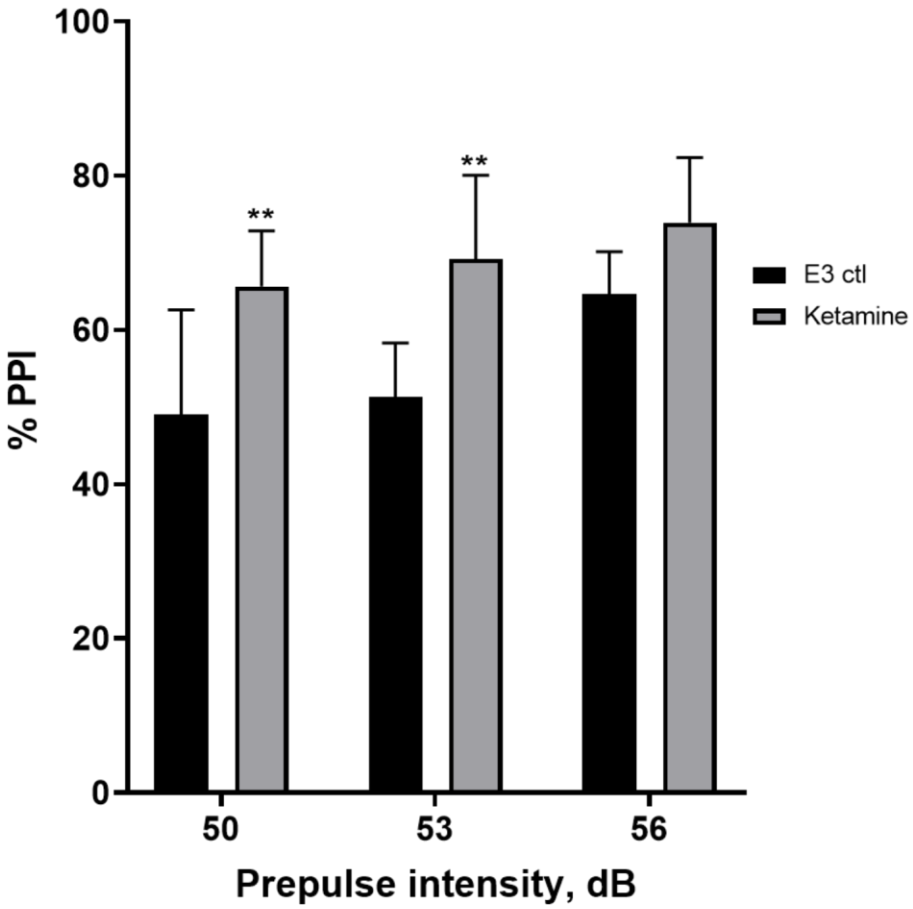

A
100
80
60
40
20
0
% responsders
0
20
40
60
80
100
Stimulus intensity, dB


B
20
15
10
5
0
Number of responders
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Trial number

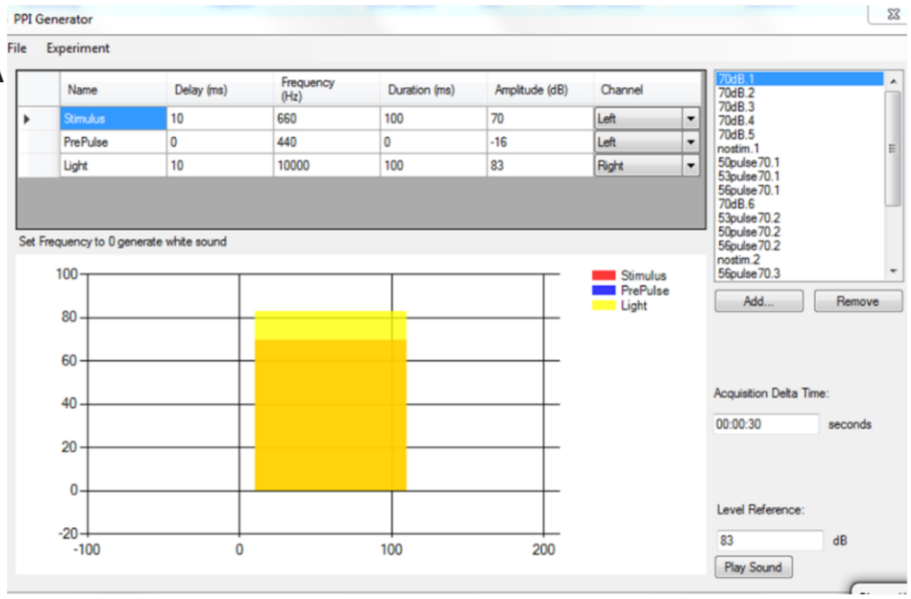

100
80
60
40
20
0
% responders
****
****
****
Pulse 70
Pp 50 + Pulse 70
Pp 53 + Pulse70
Pp 56 + Pulse70
Stimuli type, dB


100
80
60
40
20
0
% PPI
*
**
E3 ctl
Apomorphine
50
53
56
Prepulse intensity, dB

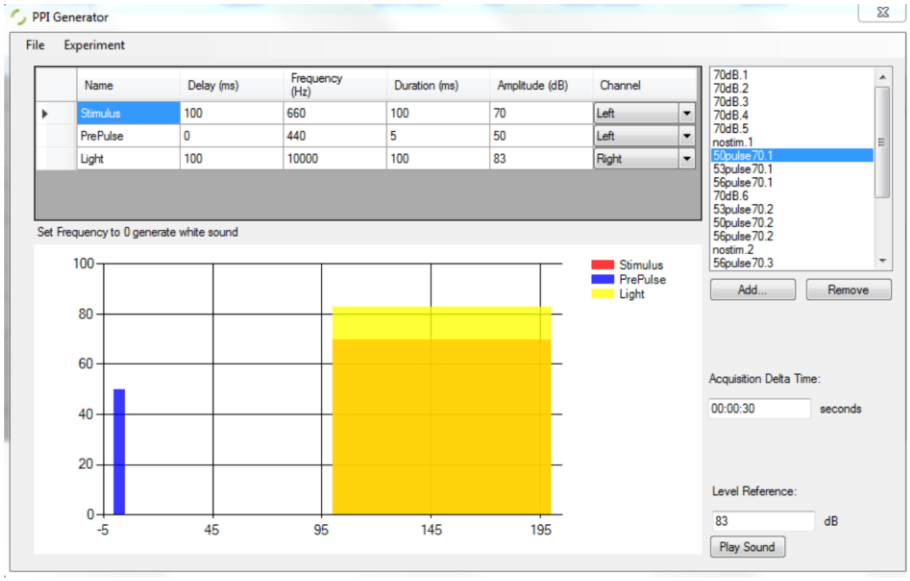

100
80
60
40
20
0
% PPI
**
*
DMSO ctl
Haloperidol
50
53
56
Prepulse intensity, dB


100
80
60
40
20
0
% PPI
**
**
50
53
56
Prepulse intensity, dB
E3 ctl
Ketamine

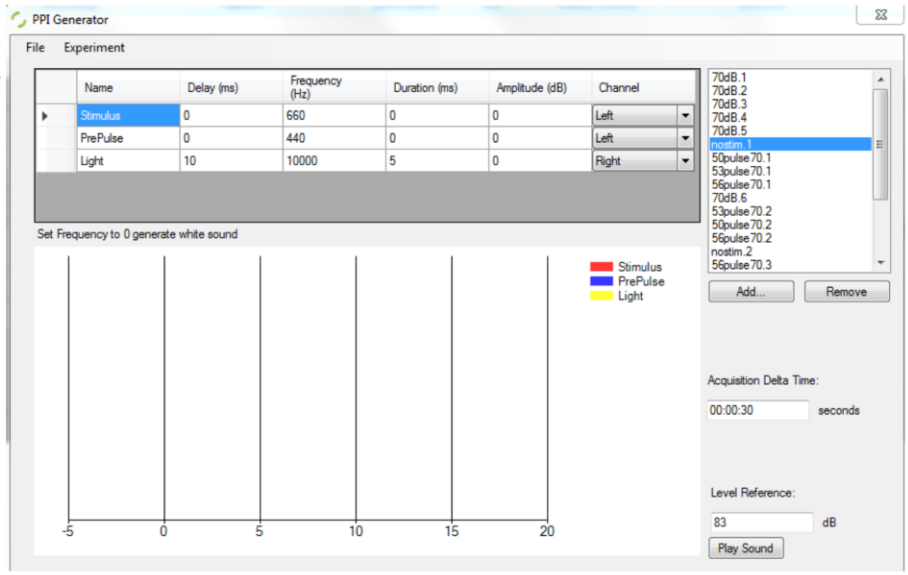
A


PPI Generator
File
Experiment
Name
Delay (ms)
Frequency (Hz)
Duration (ms)
Amplitude (dB)
Channel
Stimulus 10 660 100 70 Left
PrePulse 0 440 0 -16 Left
Light 10 10000 100 83 Right
Set Frequency to 0 generate white sound
Stimulus
PrePulse
Light
70dB.1
70dB.2
70dB.3
70dB.4
70dB.5
nostim.1
50pulse70.1
53pulse70.1
56pulse70.1
70dB.6
53pulse70.2
50pulse70.2
56pulse70.2
nostim.2
56pulse70.3
Add...
Remove
Acquisition Delta Time:
00:00:30
seconds
Level Reference:
83
dB
Play Sound

B


PPI Generator
File
Experiment
Name
Delay (ms)
Frequency (Hz)
Duration (ms)
Amplitude (dB)
Channel
Stimulus 100 660 100 70 Left
PrePulse 0 440 5 50 Left
Light 100 10000 100 83 Right
Set Frequency to 0 generate white sound
Stimulus
PrePulse
Light
70dB.1
70dB.2
70dB.3
70dB.4
70dB.5
nostim.1
50pulse70.1
53pulse70.1
56pulse70.1
70dB.6
53pulse70.2
50pulse70.2
56pulse70.2
nostim.2
56pulse70.3
Add...
Remove
Acquisition Delta Time:
00:00:30
seconds
Level Reference:
83
dB
Play Sound

C


PPI Generator
File
Experiment
Name
Delay (ms)
Frequency (Hz)
Duration (ms)
Amplitude (dB)
Channel
Stimulus 0 660 0 0 Left
PrePulse 0 440 0 0 Left
Light 10 10000 5 0 Right
Set Frequency to 0 generate white sound
Stimulus
PrePulse
Light
70dB.1
70dB.2
70dB.3
70dB.4
70dB.5
nostim.1
50pulse70.1
53pulse70.1
56pulse70.1
70dB.6
53pulse70.2
50pulse70.2
56pulse70.2
nostim.2
56pulse70.3
Add...
Remove
Acquisition Delta Time:
00:00:30
seconds
Level Reference:
83
dB
Play Sound

Figure

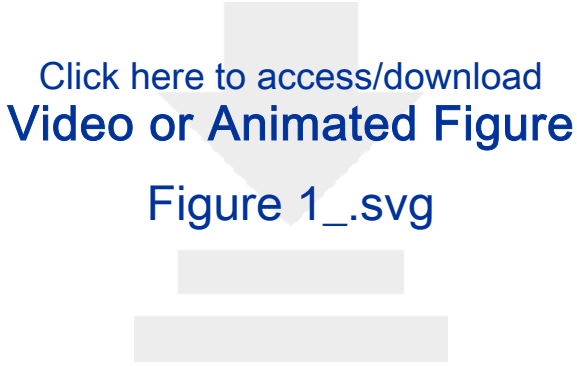

Click here to access/download
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Figure 1_.svg

Figure


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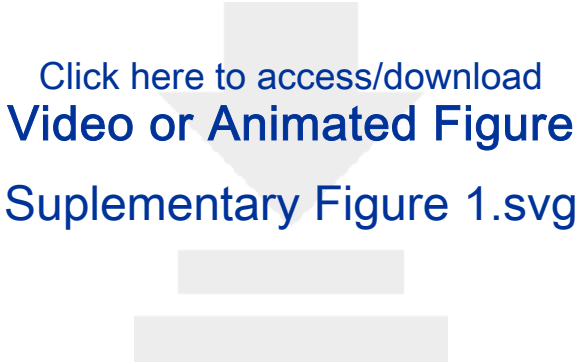
Figure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Suplementary Figure 1.svg

Supplemental video

Click here to access/download

Video or Animated Figure

Supplementary video 1_NSB_CVE.avi

Name of Material/Equipment	Company	Catalog Number
Apomorphine	Sigma Aldrich	A4393
dB meter	PCE instruments	PCE-MSM 4
DMSO	Sigma Aldrich	D8418
Dynavox Amplifier	Dynavox	CS-PA1 MK
EthoVision XT	Noldus, Netherlands	EthoVision XT, version 14
GraphPad Prism	GraphPad Software	Version 8
Haloperidol	Sigma Aldrich	H1512
Ketamine	Sigma Aldrich	Y0000450
parofon acoustics materials	Paroc	8528308
t.akustik Vocal Booth Bundle	Thormann, Germany	458543
ZebraBox Revo with PPI add-ons	ViewPoint, France	ZebraBox Revo with PPI add-ons

Comments/Description
Dopamine agonist
For measuring stimulus intensity
For dissolving organic solutes
For delivering acoustic stimuli
Automated tracking software
Statistical analysis software
Dopamine antagonist
NMDA receptor antagonist
Helps reduce background noise in the test cabinet
Helps reduce background noise in the test cabinet
Includes hardware and software

To the Editor

Journal of Visualized Experiments

April 12, 2020

Dear Dr. Dsouza,

Thank you for the feedback and for giving us the opportunity to revise our manuscript (JoVE-61423R1) titled "Pharmacological validation of the prepulse inhibition of startle response in larval zebrafish using a commercial automated system and software". We have endeavored to reformat the manuscript according to your specifications, format all new information in the manuscript in bold to make it easy for tracking and to address your comments (please see below).

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

To the best of our knowledge, spelling and/or grammatical errors are absent in the manuscript.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

To the best of our knowledge, we have provided enough detail in each step to supplement the actions in the video.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE's instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

We have adjusted the numbering of our protocol section to follow JoVE's instructions.

• **Protocol Highlight:** Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step

are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.

We have highlighted steps in the protocol that should be featured in the video.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

We have reworked the discussion to cover the forms you suggested. Additional text included is formatted in bold.

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Examples of commercial sounding language in your manuscript are Sigma Aldrich, ViewPoint, ZebraBox Revo, EthoVision (Noldus, San Diego Instruments, O'Hara & Co, med associates, (D8418, (A4393, Stereo Hi-Fi Amplifier system "dynavox", Graphpad Prism version 8, Thormann, etc.
1) Please use MS Word's find function (Ctrl+F), to locate and replace all commercial sounding language in your manuscript with generic names that are not company-specific. All commercial products should be sufficiently referenced in the table of materials/reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names.

All commercial sounding language has been removed from the manuscript.

• **Table of Materials:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials/software in separate columns in an xls/xlsx file. Please include items such as fish strains, reagents, software, drugs.

The "Table of Materials" has been revised.

• Please avoid personal pronouns "you", "your", etc.

To the best of our knowledge, we have avoided the use of personal pronouns.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

The manuscript is well written and the experimental details are spelled out in sufficient detail. The manuscript is not very original, since all the equipment is commercial and a careful reading of the manufacturer's manual would probably suffice to perform the experiments. There are a few, but very few, individual touches, like the custom made 96-well plate.

Major Concerns:

none

Minor Concerns:

Figure 1 and 2b are not mentioned in the main body of the text. These figures could anyway easily be placed to the supplementary information. The figures are of very low spatial resolution, but I guess this is only true for my reviewer's copy.

Thank you, reviewer, for your comments.

Figures 1 and 2 have now been mentioned in the main body of the text while issues regarding the quality of figures have been fixed.

Reviewer #2:

Manuscript Summary:

This is a clear description of how to conduct prepulse inhibition work in a standardized way and will be of use to those researchers that can afford the commercial systems described herein. For those researchers it will have value as a training tool for new researchers in those labs and may help standardization across labs. Just a few comments (below), mostly due to my work on acoustics, but not really fit as "major" concerns so I list all under "minor"

Minor Concerns:

1. The authors stress that PPI is used as a model in zebrafish for psychiatric disorders but it was first used as a test for hearing ability in fish so it might help this work appeal to more investigators to also make that point explicit. The authors do cite the appropriate references for this so just mention that use in the introduction.

We have mentioned the usefulness of PPI for studying hearing in fish in the introduction.

In addition, acoustic startle response (ASR) and PPI have played useful roles in studying hearing and auditory function in animal models including the zebrafish[14, 15].

2. Put in Genus and species of zebrafish at first mention

We have put in genus and species name of zebrafish at first mention in introduction.

3. The authors use the term C-bend instead of the more accepted C-start terminology through much of the manuscript, although they do use "C-start" toward the end. It would be better to use "C-start" and cite Eaton's earlier work establishing this as an escape behaviour

We have changed all C-bend to C-start throughout the manuscript and have cited the work of Eaton et al, 1977 establishing the C-start as a zebrafish escape behaviour.

The C-start has long been described as an escape behaviour controlled by distinct neural cell populations and *has been thoroughly characterized in the larval zebrafish*[15–17]*.*

4. As this is technically an acoustics paper the authors should give the reference pressure for "dB", at least at first mention (so here it would be said that the threshold stimulus was "70 dB re 1uPa"

We have inserted the reference pressure level i.e. SPL for the sound intensity measurements.

5. Unless I missed it I saw no specifications for the speaker used. In the figure it looks to be a computer speaker but these are notoriously bad for acoustics and can vary greatly from one manufacturer to the next. Please specify.

We omitted that information because the Amplifier was part of the PPI set-up received from the supplier. That information has now been specified in Table of Materials.

6. On line 95 the authors state "to reduce background white noise in the room". Please change to "background noise" as white noise means something specific in acoustics.

We have removed white from the sentence.

7. The font in the data figures is difficult to read.

We have fixed all data figures related to font size issues.

Reviewer #3:
The manuscript describes how PPI in the acoustic stimuli induced startle response in 6 dpf zebrafish larvae could be used for high throughput pharmacological screening of neuroactive compounds. In setting up this protocol, the authors have integrated two commercially available automated systems (from two different companies)in validating the protocol utilizing three known neuroactive compounds differing in their mechanisms of action and effects. The study first showed that zebrafish larvae do not show habituation at 6dpf to acoustic stimuli and providing optimum PPI before the acoustic stimulus alters the startle response in zebrafish larvae. This PPI induced alteration of startle response

behaviour was then validated for pharmacological screening by testing in three known neuroactive compounds, where the findings corroborated with other previously published studies.
Overall, in my opinion, the protocol is, on the one hand objective given that the automated systems are used, and on the other hand, comprehensive and replicable as the specifics of each steps are thoroughly described.

Major Concerns:
I do not have any major concerns.

Minor Concerns:
Lines 314: n = 5 (16 larvae/group), if n is the number of replicates, then to my knowledge, it should be written in "N", ditto with lines 318 and 322.

We have changed all “n” to “N” in the relevant places

Although it is a video article in which there will be a clear demonstration of each steps described in the text, proofreading and editing will further clarify the manuscript, especially lines 107-205.

Thank you for the suggestion. We have tried our best to edit the manuscript to improve its clarity.

It would be beneficial to the readers if the authors can mention the duration of video taken by zebrabox revo (at 1000 fps) and the total duration of video analyzed by ethovision (at 25 fps) as the change in frame rate results in the change length of the video.

The duration of video taken by zebrabox revo (at 1000 fps) was 2 s (see line 162).

> ***A 2 sec video for each trial is acquired.***

The total duration of video analysed by EthoVision (at 25 fps) was 100 ms (see lines 217-219).

> ***Select part of the tracks to be analyzed (nesting). For this study, data was nested for tracks between onset of stimulus and 100 ms after stimulus onset.***

Line 86- Emphasizing that it is a protocol paper, it would be better to use same unit for the concentration of drugs (Either mg/l or molar concentration).

All the concentrations chosen were based on published data by Burgess & Granato, 2007, hence, the reason for maintaining the units used in the original paper.

Line 153- ethovision XT the version of the software

The version of Ethovision is mentioned in the “Table of Materials” according to journals instructions

Line 314: Writing %PPI could cause confusion. The authors have to mention that its the % larvae with PPI. And please make the necessary correction in the corresponding figures in figure 5, 6 and 7.

Thank you for the comment. We are of the view that reporting "% larvae with PPI" instead of % PPI is rather confusing and may not represent the fact(s). % PPI reflect the extent of reduction in startle response in percentage terms while "% larvae with PPI" suggest something else in the following example, one can have 100% of larvae in a group (say A) exhibiting 30% PPI while 100% of larvae in another group (say B) exhibits 50% PPI.

Also, the pictures seem to be blurred in the PDF file I downloaded.

Issues regarding the quality of figures have been fixed.

Reviewer #4:

Manuscript Summary:

Larval zebrafish have become a great preparation for studying cognitive and psychiatric disorders because of their optical transparency, conserved genetics, and ease of use in high throughput screening. Labs that study these questions often build custom experimental setups, which leads to a problem of reproducibility. In this manuscript, the authors tackle this important problem admirably by developing a standardized protocol for conducting startle and prepulse inhibition (PPI) experiments using commercially available hardware and software. They replicate earlier findings and demonstrate pharmacologically that the observed behavior is likely PPI. However, there are some limitations, including in behavioral categorization and stimulus output (see comments). Overall, this is a well-written manuscript and has the potential to be a useful tool for the zebrafish community.

Major Concerns:

Fig 1 and lines 93 and 108: It's unclear from the figure and text as to where the sound source and decibel meter are placed relative to the plate. These need to be coupled to the platform in order to get precise stimulus control and measurement.

Thank you, reviewer, for such useful comments. Indeed, the figure is a simplistic overview of the set-up albeit with limitations. Additional text has been provided to address these concerns. Also, a slight modification to the figure 1A has been made, hopefully, these together make things clearer.

> ***The sound source is coupled to the platform holding the plate and the sonometer microphone of the dB meter is installed in the test chamber for sound calibration and measurement of stimulus intensity.***

Line 129 and 216: How were the frequencies and stimulus duration for startle and PPI chosen? Why are they different?

We did not have any control of frequency settings because the 440 Hz for prepulse and 660 Hz for startle stimulus are quite stringent settings by the manufacturer (ViewPoint, France).
Guided by information in the literature, we tested the duration of prepulse and startle stimulus used by Burgess & Granato, 2007 and Bhandiwad et al, 2013 while taking into consideration the maximum stimulus intensity our set-up could reach. Eventually settling for 100 ms startle stimulus.

Line 216 and 367: Because the present analysis is unable to disambiguate between short and long-latency startle responses, this remains a significant challenge in implementing the protocol, as short latency startle responses are specifically inhibited by prepulse stimuli. There are a few possible solutions to this, including (1) reducing the startle stimulus to 5 ms, which would make latency measurements more viable for short latency classification or (2) measurement of angular velocity (ie. the slope of the onset curve in Fig. 3C). In either case, it would be ideal to have a blinded validation of the startle categorization by visual classification.

Thank you, for such detailed and useful comments. Indeed, there were some mistakes in the draft, and we have now changed them in the current version of the manuscript to be more precise. The 100 ms cut off was meant to be the total duration of video analyzed by ethoVision (at 25 fps). Hence, that was an honest error. Only "short latency" startle response was considered in this study with a 50 ms cut-off (i.e. half the 100 ms portion of the 1000 fps video considered for analysis). This means larvae that displayed a C-start after 50 ms were not included in the analysis. The decision was arrived at based on the paper by Bhandiwad et al, 2013. The necessary changes have been made.

Line 291 and 347: As the authors note, the pharmacological effects of ketamine on PPI as described by Burgess and Granato (2007) show a slight increase in PPI at 30 ms ISI and a strong decrease at 500 ms ISI. The latter has been replicated in other studies, and therefore it would be useful to replicate these experiments at 500 ms ISI to demonstrate that ketamine administration is specifically affecting PPI at these longer ISIs.

We appreciate your comment regarding demonstrating the modulatory effect of ketamine on PPI at longer ISIs. However, as a result of the current lockdown in Norway due to the novel coronavirus outbreak, we have no access to the lab and as such are unable to perform any experiments until the situation improves. However, since the focus of the study was to demonstrate the ability to perform the PPI assay using the commercial set-up and software herein described, it is our view that the currently available results should suffice.

Minor Concerns:

Line 93-94 and elsewhere: dB is a relative measure of sound pressure and should be reported with the appropriate reference level. With a commercial meter, I suspect it's the standard pressure level for human hearing, so "dB SPL" would be appropriate here.

We have inserted the reference pressure level for the background noise measurements.

Line 100: It would be useful to publish the measurements and/or blueprints for the custom plate.

Information on the measurements have been made openly available on https://zenodo.org/record/3739378#.XooyLW5uKas

Line 111: This is a critical step and I'm happy to see it mentioned here. Are these sound levels taken for 100 ms stimuli or for the shorter 5 ms prepulse tones? If they are from 100 ms stimuli, are they measured for peak-to-peak maximum dB output or RMS dB output for the entire 100 ms stimulus?

The dB meter used computes the RMS dB output for the entire 100 ms stimulus. **Additional text added have been provided to convey the above message.**

Fig 2B: How are tracking errors and missing data managed in the software or in data analysis? That is, are they categorized as non-responders or are they not included in the analysis?

Details of how tracking errors are managed can be found under protocol steps 5.3.2, 5.3.3. and 5.3.4.

> ***Tracking errors are resolved by adjusting tracked features with the track editor. Tracking errors that remain unresolved after using the track editor are excluded from the analysis.***

We look forward to hearing from you regarding our submission and to respond to any further concerns you may have.

Best regards,

Camila V. Esguerra, PhD

Short Biography: Camila V. Esguerra

Dr. Camila V. Esguerra started as Group leader in Chemical Neuroscience at the Biotechnology Centre of Oslo (now merged with the Centre for Molecular Medicine Norway, NCMM), University of Oslo, in December 2014. Her research focuses on chemical biology and disease modeling of neurodevelopmental disorders. She obtained her PhD in Medical Sciences in 2007 from the University of Leuven, Belgium. Dr. Esguerra's research team seeks to elucidate the underlying mechanisms involved in the etiology of pharmacoresistant epilepsies and neuropsychiatric disorders, by probing the function of novel disease-associated gene variants. The group uses genetically engineered zebrafish mutants and transgenic reporter lines as well as pharmacological models for carrying out phenotypic analysis and chemical modifier screens to identify novel pathways, pharmacological tools and drug leads. These models and neuroactive small molecules will serve as valuable tools towards understanding the development, function, and diseases of the brain. The group collaborates closely with clinicians to investigate important hot topics in the field such as deciphering the molecular and pathological mechanisms underlying epileptogenesis, severe mental disorders and their associated comorbidities.

Short Biography: Nancy Saana Banono

Nancy graduated with a BSc. Human Biology (Hons) degree from the University of Cape Coast (UCC), Ghana. Soon after graduation, she worked as a teaching assistant for a year before moving to Norway to pursue a MSc. Neuroscience degree at the Norwegian University of Science and Technology (NTNU). As part of her master thesis, Nancy used the CRISPR-CAS9 system to generate zebrafish genetic models of rare forms of epilepsy. She is currently a Doctoral Research Fellow at the Chemical Neuroscience Group, Centre for Molecular Medicine Norway, University of Oslo.

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Author(s):	Nancy Saana Banono and Camila V. Esguerra

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