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Long-term sensory conflict in freely-behaving mice

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Filipa Franca de Barros

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Dear Dr. Ronald Myers,

The title of our manuscript is *Protocol for a long-term sensory conflict in freely-behaving mice* and the authors are Filipa França de Barros, Julie Carcaud and Mathieu Beraneck.

The described protocol produces a persistent sensory conflict for experiments aimed at studying long-term learning. By permanently wearing a fixed device on their heads, mice are continuously exposed to a sensory mismatch between their visual and vestibular inputs while freely moving in their homecage.

This methodology was validated by a recent publication (Carcaud et al 2017) to which we refer extensively; however the present paper gives the most important details which couldn't fit the original article. We focus on the critical steps that need to be followed so that other experimenters can reproduce similar type of experiments. We believe that such a protocol can inspire many groups who could adapt this to study long term learning both in vivo and in vitro.

After receiving an invitation from you on the 11th July 2018, we are submitting this manuscript today 20th September 2018. We believe that this manuscript is a good fit for JoVe since it will allow other scientists to have the detailed steps of this novel protocol. Thus, enabling the reproduction and re-invention of this protocol throughout the neuroscientific community.

This manuscript is original and no part of the manuscript has been published before nor is any part of it under consideration in another journal.

Thank you for your consideration,

Filipa Barros

TITLE:

Long-term Sensory Conflict in Freely Behaving Mice

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KEYWORDS:

neuroscience, mouse, freely behaving mice, vestibular, VOR, adaptation, visual, sensory conflict, video-oculography, gaze stabilization, motor learning

SUMMARY:

The presented protocol produces a persistent sensory conflict for experiments aimed at studying long-term learning. By permanently wearing a fixed device on their heads, mice are continuously exposed to a sensory mismatch between visual and vestibular inputs while freely moving in home cages.

ABSTRACT:

Long-term sensory conflict protocols are a valuable means of studying motor learning. The presented protocol produces a persistent sensory conflict for experiments aimed at studying long-term learning in mice. By permanently wearing a device fixed on their heads, mice are continuously exposed to a sensory mismatch between visual and vestibular inputs while freely moving in home cages. Therefore, this protocol readily enables the study of the visual system and multisensory interactions over an extended timeframe that would not be accessible otherwise. In addition to lowering the experimental costs of long-term sensory learning in naturally behaving mice, this approach accommodates the combination of *in vivo* and *in vitro* experiments. In the reported example, video-oculography is performed to quantify the vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) before and after learning. Mice exposed to this long-term sensory conflict between visual and vestibular inputs presented a strong VOR gain decrease but exhibited few OKR changes. Detailed steps of device assembly, animal care, and reflex measurements are hereby reported.

INTRODUCTION:

Sensory conflicts, such as visual ones, are present in daily life, for instance, when one wears glasses or during an entire lifespan (developmental growth, changes in sensory acuity, etc.). Due to a well-described circuit anatomy, easily controlled sensory inputs, quantifiable motor outputs, and precise quantification methods¹, gaze stabilization reflexes have been used as models of motor learning in many species. In humans and monkeys, the vestibulo-ocular

reflex (VOR) adaptation is studied through the use of prisms that the subject wears for several days²⁻⁵. Since the rodent model allows the combination of behavioral and cellular experiments, we developed a new method to create long-term sensory conflict in freely behaving mice with a helmet-like device. Inspired by the methodology used in humans and monkeys, the protocol generates a mismatch between the vestibular and visual inputs (i.e., visuo-vestibular mismatch, VVM) that leads to a decrease in VOR gain.

Classical protocols triggering a VOR gain-down adaptation in rodents consist of rotating the head-fixed animal on a turntable while rotating the visual field in phase. This paradigm creates a visuo-vestibular conflict, which makes the VOR counter-productive. Long-term adaptation protocols consist of an iteration of this procedure over the course of several consecutive days⁶⁻⁸. As a result, when a large group of animals needs to be tested, classical methodology requires a great amount of time. In addition, because the animal is head-fixed, the learning is mostly limited to a discrete frequency/velocity and consist of discontinuous trainings interrupted by intertrial intervals of variable duration⁶. Finally, classical protocols use passive learning, as the vestibular stimulation is not actively generated by the animal's voluntary movements, a situation that greatly shapes vestibular processing^{9,10}.

The aforementioned experimental constraints are surpassed by the presented innovative methodology. The required surgical approach is straightforward, and the materials used are readily available commercially. The sole part that relies on more expensive material is the quantification of the behavior; nonetheless, the fundamentals of the protocol may be used for any experiment, from *in vitro* investigations to other behavioral studies of learning. Overall, by generating a temporary visual impairment and a visuo-vestibular conflict over several days, this methodology can easily be transposed to any study concerned with sensory perturbation or motor learning.

PROTOCOL:

All animal procedures followed the Paris Descartes University animal regulations.

1. Device assembly

NOTE: The device used in this protocol is a helmet-like structure fixed on mice skulls by means of an implanted headpost.

1.1.1. Using a 3D printer and white opaque poly (lactic acid) (PLA) plastic, print using the design and specification files provided here (see **Table of Materials**) for both the device and headpost.

NOTE: The dimensions of the device are shown in **Figure 1** and dimensions of the headpost shown in **Figure 2**.

1.1.2. A striped as well as sham device are to be tested (Figure 2A¹¹). To obtain the striped model, using black nail polish, draw 3 mm large vertical stripes on the external surface of the device. The sham condition does not require any modification to the printed device.

2. Headpost implantation surgery

All the materials used in this protocol are detailed in the materials list in the supplementary information. Steps 2.7–2.9 use the biomaterials provided in the implantation kit (see **Table of Materials**). Ensure the use of sterile instruments and arrange surgery and recovery in different zones. Once mastered, the implantation procedure lasts about 30 min.

2.1. For analgesia, 30 min before the beginning of the surgery, subcutaneously inject buprenorphine (0.05 mg/kg) and put back the animal in its home cage.

NOTE: Buprenorphine's analgesic effects last approximately 12 h, long after the end of the procedure. In our experience, mice do not show any signs of distress related to this intervention.

2.2. Anesthetize the animal in a chamber with 2.5%–3% isoflurane gas. Wait 3 min and check if the mouse is properly anesthetized by observing respiration and lack of movement inside the chamber. Pass the mouse to a nose cone on a surgical table with a heating pad and, by interdigital pinching, verify that there is no withdrawal reflex and lower the isoflurane to 1.5%.

2.3. Shave the head of the mouse using an electric razor. To obtain a sterile environment, rub the shaved area with iodine solution and after with 70% alcohol. Repeat this procedure two more times.

2.4. Inject lidocaine hydrochloride (2%, 2 mg/kg) under the skin of the head for local anesthesia and wait 5 min for the effects to begin. To avoid eye damage due to dryness, cover the mouse's eyes with topical ophthalmic vet ointment.

2.5. With a pair of blunt forceps, grab the skin at the back of the head, and with a pair of blunt scissors (or scalpel), make a longitudinal incision of about 1.5 cm to expose the skull.

2.6. With the help of a scalpel, scratch the periosteum. Be careful not to scratch too hard, as the fixation of the headpost can be compromised if the skull starts to bleed slightly.

2.7. Apply a drop of the green activator on the middle of the skull. This will improve the fixation of the cement by increasing bone permeability.

2.8. Prepare the cement: mix one spoon (provided in the implantation kit) of polymer with five drops of monomer and one drop of catalyzer. With the help of a brush, apply a generous amount of the cement mix between the lambda and bregma skull landmarks;

2.9. Quickly place the headpost on the cement with a swiping motion going from lambda to bregma. After the headpost has been placed, reapply more cement around the inferior part to ensure that the headpost properly sticks to the skull. To guarantee proper fixation, make sure the cement is applied abundantly and that it dries before continuing to the next step.

NOTE: With this fixation procedure, the headpost will not come off and allow for long-term, repeated tests; in our hands, headpost removal is <10%.

2.10. Prepare the resin mix by applying a powder-to-liquid ratio that enables a smooth consistency of the mixture. Apply the resin where the cement was applied as well as around the headpost in order to protect its surface.

2.11. Wait 3 min for the resin to dry and close the skin at the back of the ears with stitch (vicryl 4-0). With a cotton swab, apply diluted (10%–20%) iodine solution to the operated area.

NOTE: Make sure the skin does not get stuck to the resin.

2.12. Turn off the anesthesia and place the animal under a red warm light to avoid hypothermia. Place moistened food on cage's floor. Do not leave the mouse unattended until it regains consciousness. As soon as the animal fully recovers from the procedure (usually, 30 min to 1 h after), place it in a cage with groups of three or four to stimulate social interactions.

3. Device fixation

3.1. 48 h following the surgery, secure the custom-built head device onto the headpost.

3.1.1. Using a pair of 1.2 mm screws and a screwdriver (1.3 mm hex), align the holes in the striped device with the holes in the headpost, place the screws and secure them. To fix the sham condition, turn the device upside down and, with the back part (**Figure 1A**) of the device facing the rostral direction, align the holes in the device with the holes in the headpost.

NOTE: It is recommended that this step be done by two operators, one holding the mouse with a one-handed mouse restraint, while the other securing the device to the headpost. If the fixation is done by a single operator, the device can be placed while the mouse is under gas anesthesia.

3.1.2. Check that the device is well-secured and cannot be removed by the animal and that the device does not apply pressure directly on the mouse's nose, which could potentially cause pain, difficulty to breath, or skin injury.

NOTE: It is also important to ensure the device is symmetrically inserted on the mouse face, so that eyes are completely covered by the head device. Check that the animal does not show any signs of abnormal pain or distress.

3.2. Leave the device on the mouse for 14 days.

4. Animal care and surveillance

4.1. Once back in their cages, mice will exhibit certain abnormalities in behavior. At first, the animal may stay prostrated and try to remove the device using its forepaws, but this should stop after the first hour. During the next following hours, the animal will usually display

difficulties orienting itself inside the cage and reaching for food and water. Therefore, during the 48 h following implantation, monitor the mice and provide easy access to water and food, by placing both directly on the cage floor, for example.

4.2. Keep track of mice's weights during the duration of the protocol. Weigh the mice right after implantation and again every 24 h. Special attention should be given to animals wearing the striped device, as they normally experience body weight loss (1-2 g) during the first 48 h, but start gaining weight again at a normal pace following that initial period (see Figure 2B¹¹).

4.3. After 2 days, mice are expected to return to their regular faculties. Depending on the system used in animal facilities, the device might be preventing access to the food and water. Ensure the animal is at ease while eating and drinking or adapt the dispensing system accordingly.

NOTE: The range of head movements produced by the animals after a few days with the device on is not modified by the device (see Figure 2¹¹) (i.e., the range of head movements produced remains similar to natural head movements).

4.4. To further ensure mice's well-being, ensure daily surveillance and apply the qualitative scale (**Table 1**) of well-being throughout the duration of the protocol.

4.5. Remove a mouse from the ongoing protocol if one or more of the following criteria apply:

4.5.1. Mice that have a total score higher than 4 points on the aforementioned qualitative scale must immediately be excluded from the experiment (see **Table 1**). Regardless of the score, if the mouse does not regain its initial weight after 6 days, the procedure must be stopped.

4.5.2. The device is not correctly fixed to the headpost if, for instance, the headpost shakes when touched or a part starts to come off. This causes the headpost to come off the mouse's head and consequently interrupts the learning, which explains why daily surveillances are necessary.

4.5.3. When a mouse has its headpost ripped off during any part of the protocol. Due to the skull bleeding associated to this detachment, the reimplantation surgery has a low success rate and is not worth attempting.

5. Removal of the device

5.1 After the learning period (in this protocol 14 days), remove the device following the same instructions as for its fixation (section 3). As soon as the device is removed, test the mice with experiments such as video-oculography tests, or, for instance, with *in vitro* electrophysiology as described previously¹¹.

NOTE: As soon as the device is taken off, mice are exposed back to the standard, visually unobstructed environment. Therefore, perform experiments that aim to test the learning effects of this device directly after its removal.

6. Video-oculography sessions

NOTE: Video-oculography experiments are performed to record the generated eye movements while the animal is being rotated in the dark (vestibulo-ocular reflex, VOR) or by rotating the animal's surroundings while the animal is still (optokinetic reflex, OKR). Each mouse was tested for both these reflexes before and after the adaptation protocol. For more details about the video-oculography set-up, see previously published reports^{12,13}.

6.1. Secure the mouse on the turntable by head-fixing it with the help of screws inserted into the headpost. Place a screen dome surrounding the animal and turn off all the lights in the room except for the optokinetic projector.

6.2. Start the OKR full-field stimulation (white dot pattern projection) and record at several different velocities in both clockwise and counter clockwise directions. As soon as the recordings are over, remove the dome.

6.3. To be able to record the VOR in pitch dark, apply a drop of 2% pilocarpine to the eyes¹⁴. Wait at least 5 min for it to act and gently remove it with a cotton swab. The pilocarpine will keep the pupil constricted with a constant size throughout the measurements, allowing proper quantification of movements in the dark.

6.4. Turn off all the lights in the room and add a box on top of the turntable to keep the animal in pitch dark. Start the horizontal VOR using sinusoidal angular rotations around a vertical axis with different frequencies and/or different velocities.

6.5. Once the recording session is finished, return the mouse to a cage properly illuminated with an infrared lamp. The heat will prevent hypothermia caused by the secondary vasodilator effects of pilocarpine on the body of the mouse.

REPRESENTATIVE RESULTS:

The following figures illustrate the results obtained with mice that underwent the 2 week adaptation protocol wearing either a striped or sham device. **Figure 3** shows an example of raw traces seen during recording sessions. As shown by comparing the traces, the VOR response decreases after the VVM protocol (**Figure 3A**, before vs. after striped). The VOR of sham mice remained unaltered after the adaptation (**Figure 3A**, before vs. after sham). The OKR of mice wearing the striped device (**Figure 3B**) is comparable to the period prior to the VVM protocol and to sham mice. **Figure 4** shows a quantification example of the mean VOR gains at a fixed frequency of 0.5 Hz and at 40 degrees per second, before and after the VVM protocol, for both striped and sham devices. There is a strong gain decrease after mice wore the striped device, while the sham mice did not have significant gain changes. Effects of VOR decrease tested at different velocities/frequencies have been reported by Carcaud et al.¹¹ and Idoux et al.¹⁵.

FIGURE AND TABLE LEGENDS:

Figure 1: Head device depicted with dimensions, in millimeters. Views: (A) back, (B) side, (C) bottom, and (D) aerial.

Figure 2: Headpost depicted with dimensions, in millimeters. Fixed in the implantation surgery, this light (0.2 g) poly (lactic acid) plastic headpost allows the locking of the adaptation device to the mouse and head-fixing of the animal on the turntable during the video-oculography sessions.

Figure 3: Example raw traces of eye movements during VOR and OKR stimulations. (A, left) Left: VOR performed at 0.5 Hz at 40 °/s and (B, right) optokinetic stimulation at a constant velocity of 10 °/s (black line), in a clockwise direction, before (green lines) and after (yellow) wearing the striped or sham (purple) device.

Figure 4: Example mean VOR and OKR gain values after adaptation to either striped or sham device. Gains were plotted according to time (days) for the striped (n = 10) and sham (n = 6) devices at stimulations of 40 °/s and 0.5 Hz for the VOR (left), and 10 °/s clockwise direction for the OKR (right). On the timescale, “before” day represents the day immediately prior to the adaptation and “day 0” represents the day when the device is removed. Error bars represent the standard deviation, ***p < 0.001, not significant.

Table 1: Qualitative scale for the well-being assessment. Listed are the qualitative parameters that must be assessed during the duration of the protocol. The sum weight alterations, physical appearance, and behavior scores should not be greater than four points.

DISCUSSION:

The long-term sensory perturbation described here consists of a visuo-vestibular mismatch produced in freely-behaving mice. To implant the device that mice wear for 14 days, a simple and short surgery using a commercially available surgical kit is performed. Mice recover in less than 1 h from this headpost implantation procedure and show no associated signs of distress from it. Subsequently, in the given example of application of this protocol, VOR and OKR are measured using the video-oculography technique. Nonetheless, this device-induced long-term learning protocol could be used in a variety of experiments such as *in vitro* electrophysiology¹, neuronal imaging, and various behavioral assays. The rationale behind the development of this technique was inspired by the prism-based methodology used in humans and monkeys. This technique, however, differs because it impairs rather than modifies vision. Thus, it constitutes (in its current form) an extreme case of visuo-vestibular mismatch. The authors believe that the provided technical information may be useful for designing a prism-like version of the device or further developing specific feature-restricting devices¹⁶.

Made of a light (0.9 g) poly (lactic acid) plastic, the head device was designed to fit the head of a young adult mouse, allowing protection of the snout and leaving enough space laterally to let the animal groom. The front part of this device exposes the end of the snout to permit feeding and grooming behaviors. The device is slightly opaque, so that the animal is

deprived of precise vision of the surrounding but still receives luminance stimulation. The striped and sham implantations are tested to ensure that the measured effects are due primarily to the visuo-vestibular mismatch caused by the high-contrast visual signal during self-generated movements of the striped device and not by proprioceptive modification (i.e, the weight of the device applied in the mouse's head and neck).

Experimentally, mice that wore the striped device showed a significant VOR gain decrease of ~50% after the learning period; still, there can be an inter-individual variability for absolute gain values. Sham mice showed no significant VOR gain alterations, thus demonstrating that the VOR reduction is caused by the sensory conflict and not by motor impairment. Furthermore, young mice (<P26) showed VOR and OKR gain values lower than older animals¹⁷. For that reason, animal age has to be taken into account while planning the experiment. Finally, the aforementioned mice exclusion criteria (section 4.5) are a crucial step that should be followed to ensure well-being as well as establish reliable results.

One of the advantages of this protocol is the time that it saves experimenters during the learning period, compared to other types of VOR/OKR adaptation protocols. So far, VOR adaptation in mice has been studied by head-fixing and training the animal on a rotating turntable^{6,8,18,19}, which is time-consuming, especially when a lot of animals must be trained. The presented protocol allows the training of several animals at once and saves time. In addition, in these classical experiments the trainings are typically limited to 1 h per day, leaving long periods of putative unlearning that cause adaptation to be an iterated alternation of learning/unlearning with different dynamics²⁰. Here, the head-fixation of the device allows for uninterrupted learning. Another advantage is that since the learning period is generated in a freely behaving head-free situation, mice are able to learn through a range of natural head movements that are actively generated. In the classical protocols, the animal is head-fixed while being passively rotated on the turntable so that the learning occurs at a determined stimulation (one frequency, one velocity)²¹ that does not reflect the natural range of head movements. It is important to note that the vestibular system encodes movements differently when they are actively generated by the subject or when externally applied¹⁰; thus, the cellular mechanisms triggered in both situations may also differ.

Overall, the described methodology is suitable for combined *in vivo/in vitro* studies on long-term sensory adaptations occurring after a visual conflict and/or visuo-vestibular mismatch in freely behaving mice. Sensory conflicts are a recognized cause of motion sickness, which is a field that has recently attracted use of mice^{22,23}. It was recently demonstrated that the gain adaptation caused by the use of this device offers protection against motion sickness when mice are exposed to a provocative stimulus¹⁵. Hence, this protocol could be used to identify the cellular mechanisms underlying adaptation to a sensory conflict as well as to develop anti-motion sickness treatments.

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DISCLOSURES:

The authors declare no conflicts of interest.

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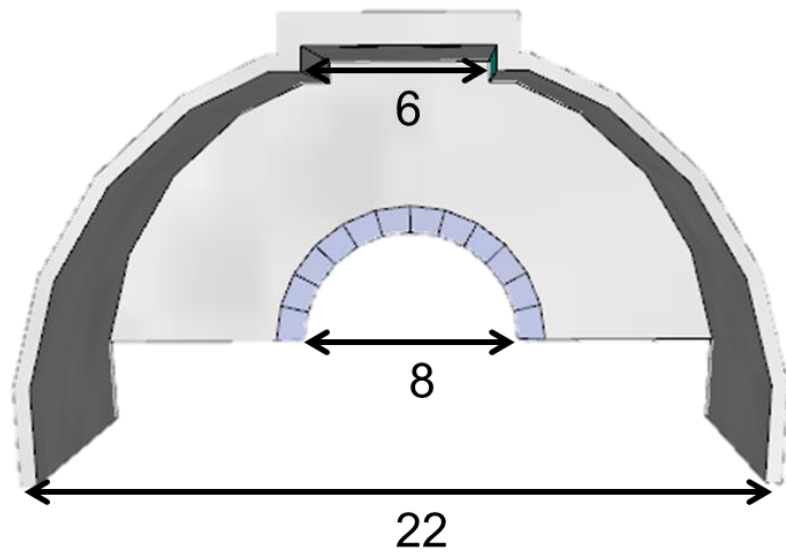
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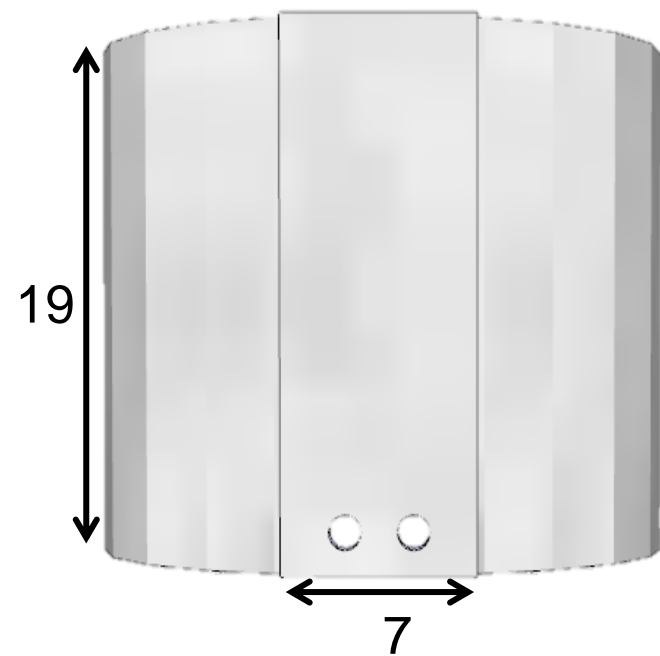
Figure1

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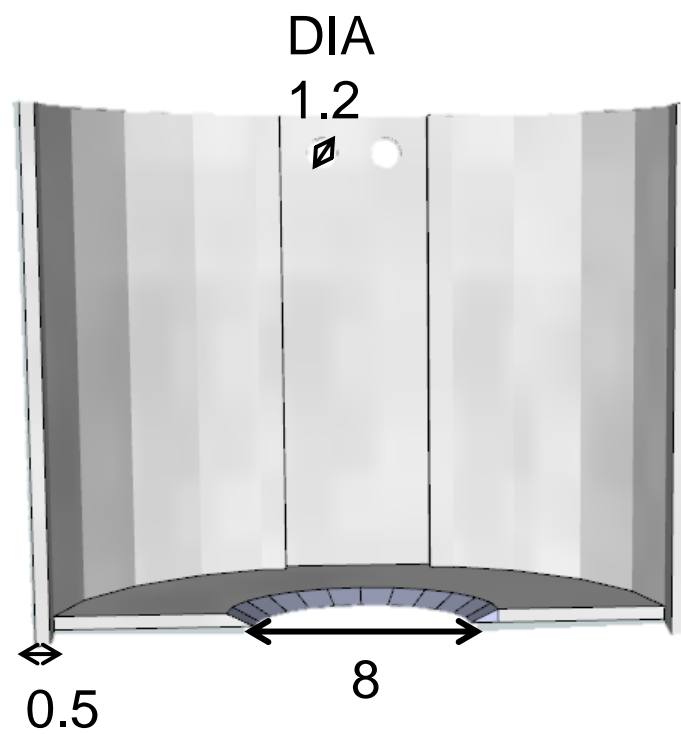
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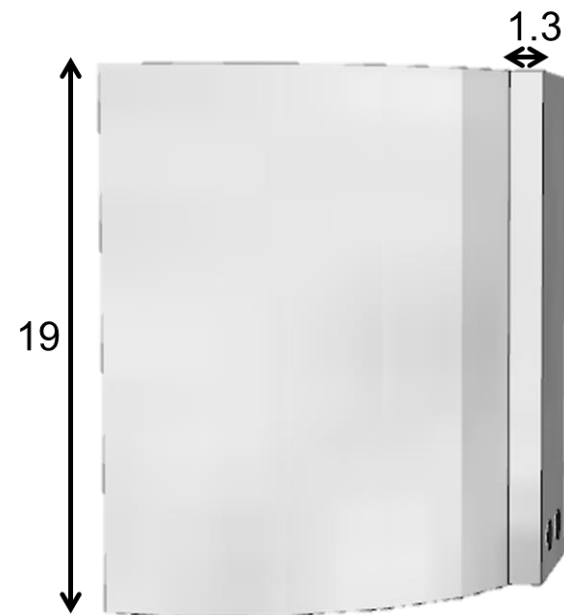
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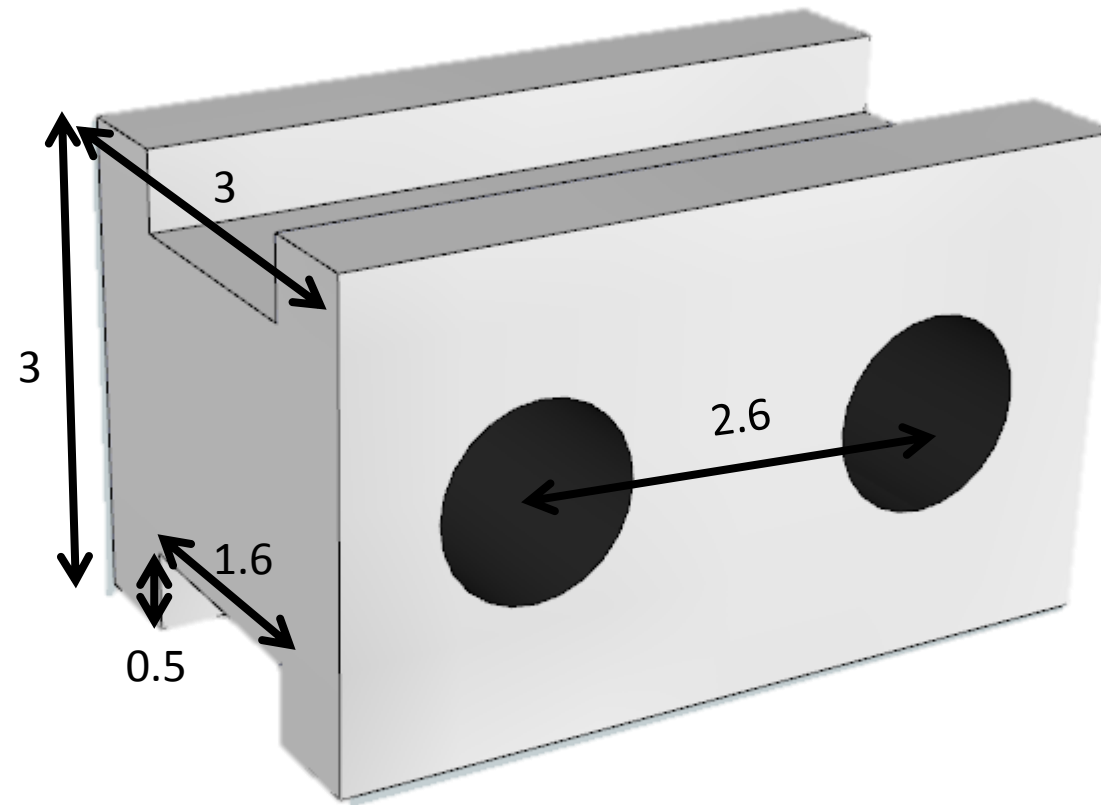


Figure 3

0.5Hz-40°/s

Table/head



Before



After *Striped*



After *Sham*



2s

10°/s

0°/s

Before

After
Striped

After
Sham

5s

10°

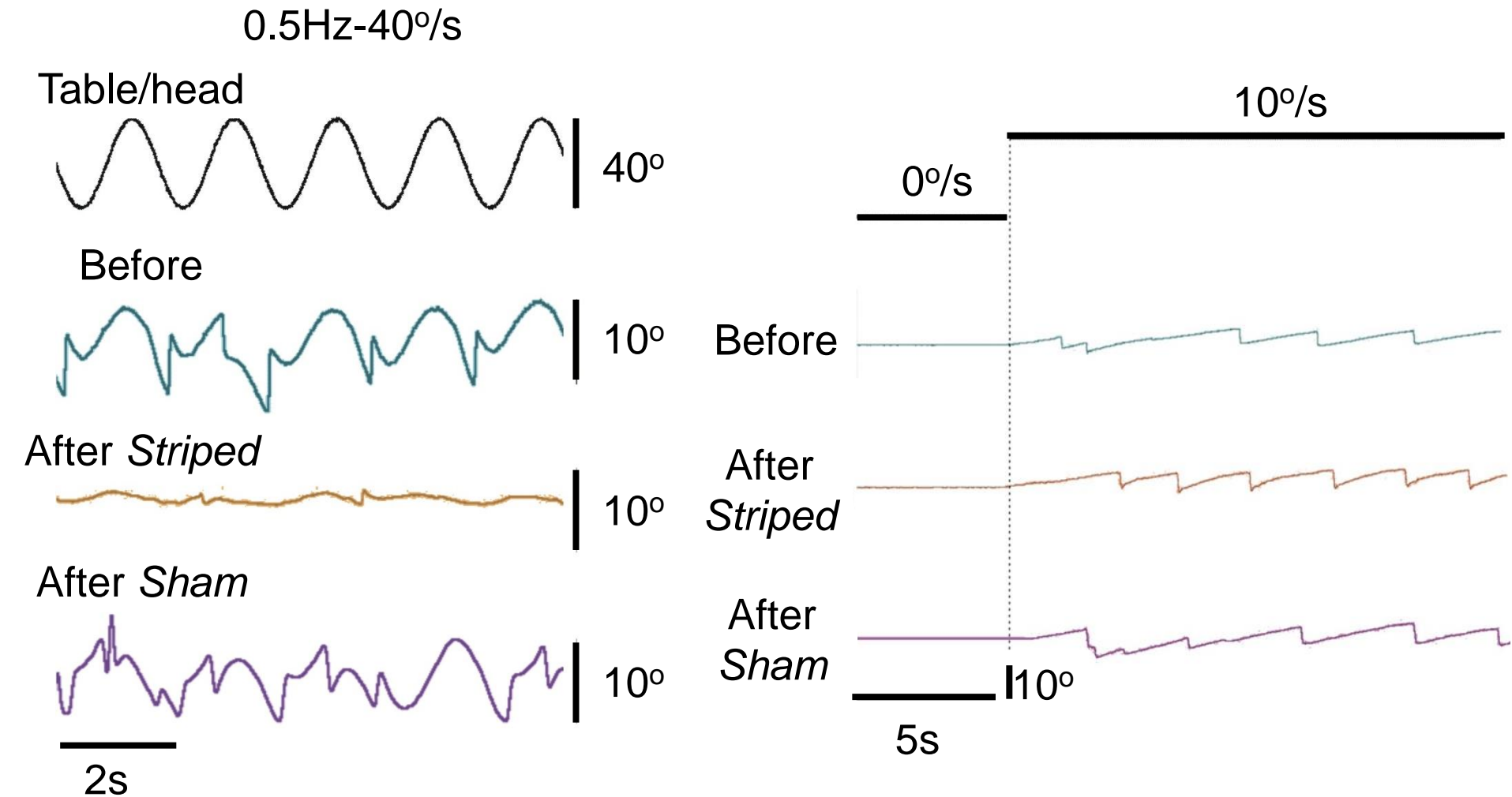
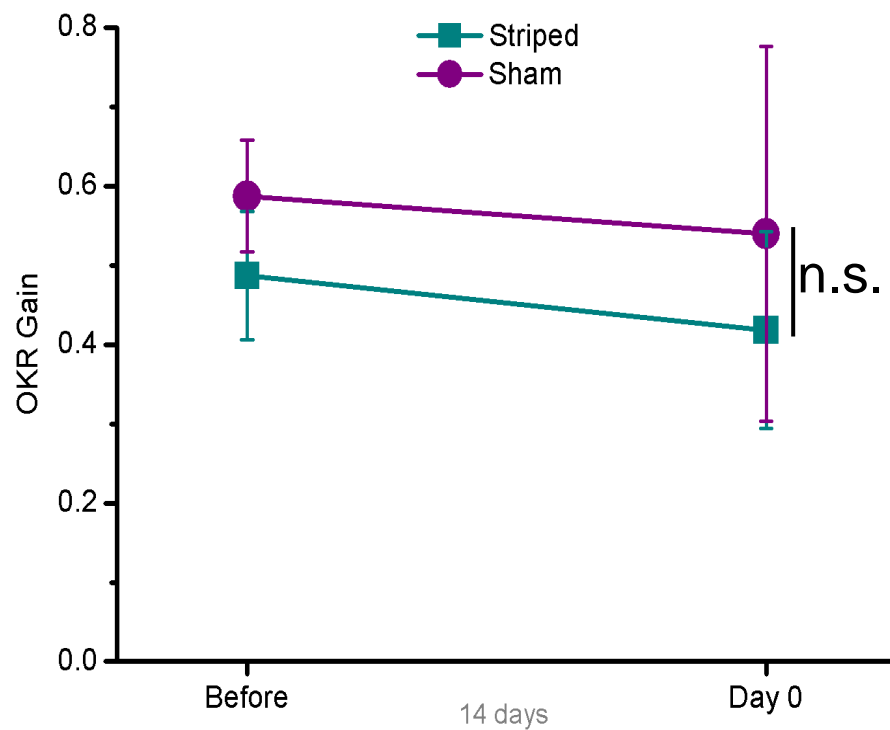
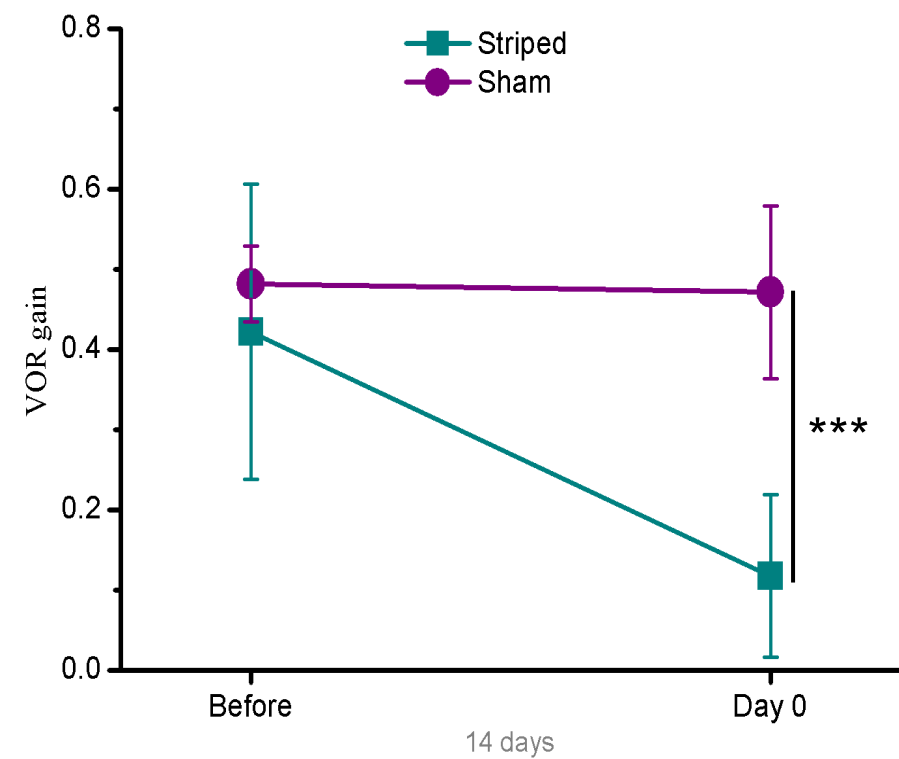


Figure 4



Points	Body weight	Physical appearance	Behavior
0	none or weight gain	standard	no signs of distress and normal
1	weight loss <10%	no body grooming	impaired locomotion or cage orientation
2	weight loss between 10%-	dehydration	--
3	weight loss >20%	wounds	nervous ticks (e.g. scratching, biting)



Name of Material/ Equipment	Company	Catalog Number
3D printer	Ulimaker, USA	S5
Blunt scissors	FST	14079-10
Catalyst V	Sun Medical, Japan	LX22
Dentalon Plus	Heraeus	37041
Eyetracking system and software	Iscan	ETN200
Green activator	Sun Medical, Japan	VE-1
Monomer	Sun Medical, Japan	MF-1
Ocrygel	TvmLab	10779
Polymer L-type clear (cement)	Sun Medical, Japan	TT12F
Sketchup	Trimble	3D modeling software
Turntable		

Comments/Description

Parkell bio-materials, Kit n°S380

Parkell bio-materials, Kit n°S380

Parkell bio-materials, Kit n°S380

Ophtalmic vet ointment

Parkell bio-materials, Kit n°S380

oftware used for the device's ready-to-print design file

Not commercially available



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Author(s):

PROTOCOL FOR LONG-TERM SENSORY CONFlict in FREELY BEHAVING mice
DE BARROS, CARLAUD, BORANZEC

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Editorial comments

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. **AR: done**
2. Please provide an email address for each author. **AR: provided**
3. Abstract (line 25): Please do not include references here. **AR: done**
4. Please revise the Introduction to include all of the following:
 - a) A clear statement of the overall goal of this method **AR: Added, Line 39-41**
 - b) The rationale behind the development and/or use of this technique **AR: the rationale is now stated lines 41-43**
 - c) The advantages over alternative techniques with applicable references to previous studies **AR: Added, Lines 44-54**
 - d) A description of the context of the technique in the wider body of literature **AR: we now discuss the advantages and the context of the technique on lines 44-54. These points are further presented in the discussion in lines 271-284**
 - e) Information to help readers to determine whether the method is appropriate for their application **AR: potential application are now described on lines 60-62**
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6. 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, dashes, or indentations. **AR: indentations removed and dashes replaced by subpoints (1.1.1, 1.1.2, 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. **AR: modification done, the action items are now all in the imperative tense**

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. **Please move the discussion about the protocol to the Discussion.** AR: Done

9. Please add more details 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. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below. AR: Done

10. Line 85: Please specify the concentration of isoflurane gas and mention how proper anesthetization is confirmed. AR: Done, line 83

11. Line 99: How large is the spoon? AR: The spoon is provided in the implantation kit referred in the table of materials. Added in Line 100 ‘(provided in the implantation kit)’ for specification.

12. Please include single-line spaces between all paragraphs, headings, steps, etc. AR: Done

13. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less 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. AR: Done

14. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia. AR: Done

15. Please include all relevant details that are required to perform the step in the highlighting. 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 highlighted. AR: Done

16. Figure 3: Please include a space between numbers and their corresponding units (i.e., 0.5 Hz, 2 s, 5 s). AR: Done

17. Figure 4: Please describe what “****” represent and define n.s. in the figure legend. AR: Done

18. Please include all the Figure Legends together at the end of the Representative Results in the manuscript text. AR: Done

19. Please revise the table of the essential supplies, reagents, and equipment to include the

name, company, and catalog number of all relevant materials. Please sort the items in alphabetical order according to the Name of Material/ Equipment. AR: Done

20. References: Please do not abbreviate journal titles. AR: Done

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The described protocol produces a persistent sensory conflict for experiments aimed at studying long-term consequences of sensory conflict. By permanently wearing a fixed device on their heads, mice are continuously exposed to a sensory mismatch between their visual and vestibular inputs. The proposed protocol provides significant advantages over existing method of inducing long-term vestibulo-visual conflict.

Major Concerns:

None

Minor Concerns:

-My only minor suggestion is to add some details regarding potential aims/relevance of future studies employing this protocol. Sensory conflict is a recognized cause of motion sickness - how proposed technique could help here? Another aspect: it's true that adaptation to visual-vestibular conflict is a form of learning, but does this type of very artificial condition has any significance for real life learning?

AR: The authors thank reviewer #1 for positive evaluation of the work and suggestions for modifications. The interest of the protocol for real life learning and motion sickness-related research is now stated one paragraph of discussion on lines 276-284

-L 141: Replace ground by cage floor AR: Done

-What is the incidence of fixation failure of the headpost with the cement? AR: the incidence is <10%. Added as a note on line 108.

-L 187-205: Description of recording system is missing AR: The description of the recording system is provided in reference number 13 (de Jeu, M. & De Zeeuw, C. I, 2012)

Reviewer #2:

Manuscript Summary:

This study aimed at establishing a new protocol for testing the effects of sensory conflict on long term learning between the visual and vestibular systems. This was performed by applying a persistent visual sensory conflict and then measuring vestibular modalities (vestibulo-ocular reflex) and visual modalities (optokinetic reflex). Therefore enabling the study of multisensory interactions over long period of time and learning.

Major Concerns:

No Major concerns. the manuscript is well prepared and of general interest.

AR: Author's Response

AR: the authors thank reviewer #2 for positive evaluation of the work and for carefully reading the manuscript

Minor Concerns:

*Line 51-52: "allowing protection of the snout and leaving enough space laterally to let the animal groom"- grammar AR: Done

*Line 62: Are the stripes creating the contrast or are they changed by some other means?

*Line 67: Change to "comfortably fit"? AR: Done

*Line 108: change to "where"? AR: Done

*Line 110: change "close back the skin..." to "close the skin..." AR: Done

*Line 138: change "difficulties to properly orientate inside the cage and to reach..." to "difficulties orienting itself inside the cage and when reaching..." AR: Done

*Line 143: change "weight" to "weigh" AR: Done

*Line 155" remove "however" AR: Done

*Line 204: change "avoid" to "prevent" AR: Done

*Line 230: change "here described" to "described here" AR: Done

*Line 259: change "differentially movements" to "movements differentially" AR: Done

*Line 261: change "as well" to "also" AR: Done

Reviewer #3:

Manuscript Summary:

The paper described a device that blocks mice visual inputs during active and passive head movements. It is designed to introduce vestibular-visual conflicts during natural movements to induce motor learning in the vestibulo-ocular reflex. It is an excellent design and will be an important contribution to the field.

Minor Concerns:

The authors need to be aware of the fact that vestibular signals are generated during both active and passive head movements during natural environments. It is also important to note the differences between the current device and the ones used in the monkey literature.

VOR learning is frequency dependent. It is helpful to test more frequencies.

AR: the authors thank reviewer #3 for the positive evaluation of the work. We refer to active vs passive differences on lines 37-42; 245-247. Differences between the current device and the ones used in the monkey literature are stated in discussion lines 259-263. Finally, the authors completely acknowledge the frequency-dependency of the adaptation; this point has been extensively presented in our research papers based on the helmet methodology (Carcaud et al. 2017; Idoux et al. 2018) and we have therefore decided to present a simple case for the Jove, methodology centered, paper. We however indicate this point to interested readers on lines 209-201.

Editorial comments response:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. **AR: Done**
2. Please use standard SI unit symbols and prefixes such as μL , mL, L, g, m, etc., and h, min, s for time units. **AR: Done**
3. Please highlight 2.75 pages or less of the Protocol steps (including headings and spacing) in yellow that identifies the essential steps of the protocol for the video. **AR: Done**
4. Please do not highlight notes for filming. **AR: Removed**
5. Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia. **AR: Already on the text, line: 117. Added as well to the list of materials.**
6. For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain. **AR: the post-surgical treatment is already discussed in Protocol 4; recovery discussion added in line 307-309; post-surgical pain treatment is explained in 107-109.**
7. Discuss maintenance of sterile conditions during survival surgery. **AR: Done, line 100-101**
8. Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency. **AR: Added in line 149-150.**
9. Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered. **AR: Stated in line 150-151.**
10. Please do not highlight any steps describing euthanasia or anesthesia. **AR: Noted**
11. Please split some long steps into two or more sub-steps so that each step contains only 2-3 actions and is less than 4 lines. **AR: Done**
12. Step 4.1: Please ensure that all text is written in imperative tense. **AR: changed, line 186**
13. 4.3: Please ensure that all text is written in imperative tense. **AR: changed, line 197**
14. 4.5.1-4.5.3: Please ensure that all text is written in imperative tense. **AR: changed, line 217, 221, 225.**
15. 5.1: Please ensure that all text is written in imperative tense. **AR: changed, line 232 and 237**
16. Figure 2: Please provide a short description of the figure in addition the title in Figure Legend. **AR: Done, line 285-288**