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Scriptwriter Name: Poornima G

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Title: Lentiviral-Induced Striatal Pathology as a Preclinical Model of Polyglutamine Spinocerebellar Ataxias

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES**

We recommend using the screen capture program [OBS](https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, please upload all screen-captured video files to your project page as soon as possible.

3. Filming location: Will the filming need to take place in multiple locations? **YES**

If **Yes**, how far apart are the locations? **290 meters (corresponding to 4 minutes)**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **YES**

Current Protocol Length

Number of Steps: 25

Number of Shots: 57 (18 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Clévio Nóbrega:** The research focuses on creating a lentiviral-based mouse model of neurodegeneration to uncover disease mechanisms and therapies for neurodegenerative disorders, namely spinocerebellar ataxias.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Clévio Nóbrega:** This protocol has some advantages compared to conventional transgenic models by providing a fast, controllable, and region-specific *in vivo* model for mechanistic and therapeutic studies.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Tiago Moreira-Gomes:** This protocol allows the assessment of aggregation and neuronal loss within a specific brain region, namely the striatum, in response to therapeutic interventions.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Tiago Moreira-Gomes:** The main challenge is to achieve precise lentiviral delivery via accurate stereotaxic injection, controlled viral dosing, and minimizing tissue damage for consistent results.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Tiago Moreira-Gomes:** Future work can use this model to identify mechanisms underlying disease pathogenesis and to accelerate the development of targeted therapies and biomarkers.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JOVE article address?

- 1.6. **Clévio David Rodrigues Nóbrega, Full Professor, President of ABC:** (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Clévio David Rodrigues Nóbrega, Full Professor, President of ABC:** (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Authors: Could you please also deliver the above statements in Portuguese?

Videographer: Please film the testimonials in both English and Portuguese

Ethics Title Card

This research has been approved by the Direção-Geral da Alimentação e Veterinária (DGAV)

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of the Mouse and Syringe for Lentiviral Injection

Demonstrator: David Brito

- 2.1. To begin, position the anesthetized C57BL-6J (*C-Fifty-Seven-B-L-Six-J*) mouse on a clean desk [1-TXT] and confirm the depth of anesthesia by assessing the toe pinch reflex [2]
 - 2.1.1. WIDE: Talent placing the anesthetized mouse on the clean work desk. **TXT: Anesthesia: IP injection of Medetomidine + Midazolam + Fentanyl**
 - 2.1.2. Talent performing a toe pinch reflex test on the animal.
- 2.2. After shaving the animal's scalp, place it in the stereotaxic frame on a heating pad [1] and position the head using the ear bars and bite bar [2].
 - 2.2.1. Talent placing the animal onto the heating pad within the stereotaxic frame.
 - 2.2.2. Talent securing the animal's head with ear bars and a bite bar.
- 2.3. Mount the syringe containing 2 microliters of lentiviral vector solution into the automated microinjection pump [1-TXT]. ~~and prime the syringe in infusion mode until a drop of liquid appears at the needle tip while the bevel is facing forward [2].~~ **NOTE: The VO is edited for the deleted shot**
 - 2.3.1. Talent securing the syringe into the microinjection pump. **TXT: 400 ng of p24 antigen/mL/injection**
 - 2.3.2. ~~Talent priming the syringe until a droplet forms at the needle tip with the needle tip showing the bevel oriented forward.~~ **NOTE: Not filmed**
- 2.4. Set the pump parameters, including a speed of 0.25 microliters per minute, a volume per hemisphere of 2 microliters, and the appropriate syringe type [1].
 - 2.4.1. Display the microinjection pump interface and input settings for Speed, Volume, and Syringe Type.

3. Lentiviral Delivery into the Mouse Brain Striatum

- 3.1. Disinfect the animal's scalp with 70 percent ethanol [1]. Using a sterile scalpel blade, make a midline incision of approximately 1 to 1.5 centimeters from between the ears to the eyes [2].
 - 3.1.1. Talent applying ethanol to disinfect the scalp.
 - 3.1.2. Talent making a midline incision along the scalp with a scalpel.
- 3.2. Expose the skull and clean the surface by removing the periosteum with cotton swabs [1]. Locate Bregma [2] and record the stereotaxic zero point manually or digitally [3].
 - 3.2.1. Talent exposing the skull and cleaning it with cotton swabs.
 - 3.2.2. Close-up of the skull and talent pointing to the Bregma.
 - 3.2.3. Display recording of the stereotaxic zero point on the device interface.
- 3.3. Determine the injection coordinates relative to Bregma with antero-posterior at plus 0.6 millimeters and medio-lateral at plus or minus 1.8 millimeters [1].
 - 3.3.1. Display coordinate input or reference values for Antero-posterior, Medio-lateral.
- 3.4. Drill carefully at the marked antero-posterior and medio-lateral coordinates slowly to avoid penetrating the brain [1].
 - 3.4.1. Talent drilling at the marked coordinates on the skull.
- 3.5. Lower the injection needle slowly to the dorso-ventral coordinate [1] and then test for needle clogging by injecting a portion of the solution until a drop is clearly visible [2].
NOTE: The VO is added for the moved shot
 - 3.5.1. ~~Talent lowering the injection needle into the drilled hole.~~ NOTE: This shot is moved before 3.6.2)
 - 3.6.1. Talent lowering the injection needle to the target depth. NOTE: This shot is requested to move here
 - 3.5.2. Close-up of the needle tip expelling a visible droplet.
- 3.6. Now, test that the hole is in the correct position by lowering the injection needle [1]. Inject 2 microliters of Lentivirus encoding for the wild-type ataxin into one hemisphere at a rate of 0.25 microliters per minute [2]. After 5 minutes, slowly retract the needle to avoid reflux [3]. Inject the Mutant or expanded protein into the contralateral side

similarly [4]. NOTE: The VO is added for the moved shot

3.5.1 Talent lowering the injection needle into the drilled hole. NOTE: This shot was requested to move here

3.6.1. ~~Talent lowering the injection needle to the target depth.~~ NOTE: This shot is Moved before step 3.5.2]

3.6.2. Talent injecting the virus. *Videographer: Please obtain multiple reusable shots for this step*

3.6.3. Talent retracting the needle.

3.6.4. *Reuse 3.6.2*

3.7. Close the incision with 3 to 5 interrupted 5-0 (5-Oh) nylon sutures [1]. Administer a mixture of antagonistic drugs subcutaneously to reverse the effects of the initial anesthetics [2] and return the animal to its recovery cage [3]. After 12 weeks, remove the animal from its cage for euthanasia and brain dissection [4].

3.7.1. Talent suturing the incision with interrupted stitches.

3.7.2. Talent administering the antagonistic drug mixture via subcutaneous injection.

3.7.3. Talent placing the animal in a recovery cage.

3.7.4. Talent removing the animal from the cage and placing it on a dissection desk.

4. Cryosectioning Isolated Mouse Brain and Collecting Free-Floating Tissue

Demonstrator: Rafael Costa

4.1. Fill the wells of a 48-well plate with PBS containing 0.05 percent sodium azide [1]. Then, transport the brain on dry ice [1] and allow it to equilibrate inside the pre-cooled cryostat for 5 to 10 minutes to prevent tissue damage [2]. NOTE: The VO is added for the moved shot

4.6.1 Talent adding phosphate-buffered saline with sodium azide into the wells of the plate NOTE: This shot is requested to move here

4.1.1. Talent moving towards a cryostat with the brain kept in a container on dry ice in hand.

4.1.2. Talent placing the brain inside the cryostat for equilibration.

4.2. Using a scalpel, remove the olfactory bulbs as they are soft and easy to cut [1]. Apply a

pea-sized drop of fresh O.C.T. compound onto the specimen disk [2-TXT] and place the cerebellum in contact [3]. ~~Position the brain for coronal sectioning [4].~~ Once frozen, add additional O.C.T. to embed the entire brain [4]. **NOTE: The VO is edited for the deleted shot**

4.2.1. Talent removing the olfactory bulbs with a scalpel.

4.2.2. Talent applying a drop of O.C.T. onto the specimen disk. **TXT: O.C.T.: Optimal Cutting Temperature**

4.2.3. Talent placing the cerebellum onto the specimen disk.

~~4.2.4. Talent orienting the brain for coronal sectioning.~~ **NOTE: Not filmed**

4.2.5. Talent adding more O.C.T. to fully embed the brain.

4.3. Mount the specimen disk in the holder [1] and trim until the striatum, visible as two white circles in each hemisphere, is observed [2].

4.3.1. Talent mounting the specimen disk into the holder.

4.3.2. View of the brain showing the striatum as two white circles.

4.4. If hemispheres received different treatments, pierce one hemisphere with a needle to maintain orientation and record this for later analysis [3].

4.4.1. Talent piercing one hemisphere with a needle.

4.5. Now, switch to sectioning mode [1] and collect free-floating sections in a 48-well plate using a fine brush or a fire-polished glass Pasteur pipette [2].

4.5.1. Display switching to Sectioning Mode on the cryostat interface.

4.5.2. Talent transferring sections into a 48-well plate using a brush or Pasteur pipette.

~~4.6. Fill the wells with PBS containing 0.05 percent sodium azide [1].~~ **NOTE: This step is moved before 4.1.1**

~~4.6.1. Talent adding phosphate-buffered saline with sodium azide into the wells of the plate.~~

4.7. Place the first section in well A1 and the second in A2, continuing sequentially [1]. When the plate is full, either continue in a new plate or proceed until the entire striatum is sectioned [2]. Seal the plates with transparent film [3] and store at 4 degrees Celsius until use [4].

- 4.7.1. Talent placing sections into labeled wells A1 and A2.
- 4.7.2. Close-up of placing the section in a new plate.
- 4.7.3. Talent sealing the plate with transparent film.
- 4.7.4. Talent placing the plate in refrigerator.

- 4.8. Perform immunohistochemistry on the sections using appropriate antibodies and develop the staining with Diaminobenzidine [1]. Monitor the development of the brown color and allow the reaction to reach the expected intensity level [2]. Capture images of coronal brain sections spanning the entire striatum [3].
 - 4.8.1. Talent placing the slices into the well with the antibody mix, initiating the reaction.
 - 4.8.2. Shot of the sample showing brown coloration.
 - 4.8.3. Talent placing the sample under the microscope or working at the microscope-attached computer screen capturing images.

5. Quantification of Protein Aggregates in the Mouse Striatum

Demonstrator: Pedro Alexandre

- 5.1. For manual counting, open the image in FIJI to load it and calibrate the pixel size if required when metadata is not available [2].
 - 5.1.1. SCREEN: 69550_screenshot_1.mp4:

- 5.2. Select **Analyze** and choose **Set Scale** to enter the known distance such as 1 pixel equals 0.5 micrometers [1]. Navigate to **Plugins**, then **Analyze, Cell Counter**, and select **Cell Counter** [2]. Click **Initialize** and select the image [3].
 - 5.2.1. SCREEN: 69550_screenshot_2.mp4 : 00:00-00:11
 - 5.2.2. SCREEN: 69550_screenshot_2.mp4 : 00:13-00:19
 - 5.2.3. SCREEN: 69550_screenshot_2.mp4 : 00:19- end *video editor: Highlight the initialize tab*

- 5.3. Select a marker type such as Type 1 for aggregates [1]. Manually click on each visible aggregate to label it [2]. Record the count displayed in the Cell Counter panel for each image or section [3] and export the counts manually or compile results using a macro [4].

5.3.1. SCREEN:69550_screenshot_3.mp4: : 00:00-00:05

5.3.2. SCREEN:69550_screenshot_3.mp4: : 00:05-00:15

5.3.3. SCREEN: 69550_screenshot_3.mp4: : 00:05-00:15 *Video editor: Highlight the increasing number in box next to type 1 tab*

5.3.4. SCREEN: 69550_screenshot_3.mp4: : 00:18-00:23

5.4. For Semi-automated quantification, open IHC-Aggre-Count and click **Upload Image** to load the image [1].

5.4.1. SCREEN: 69550_screenshot_4.mp4: : 00:03-end

5.5. View the image in the Original Image Display and navigate to the **Processing** Tab [1]. Apply HiLo to enhance aggregate and background contrast [2]. Apply a brightness threshold to filter aggregates from the background and use the Min Size Filter to remove small particles [3]. ~~Optionally apply a roundness threshold to exclude non-aggregate shapes [5].~~ **NOTE: The VO is edited for the deleted shots**

5.5.1. SCREEN: 69550_screenshot_5.mp4:: 00:00-00:04

5.5.2. SCREEN: 69550_screenshot_5.mp4: 00:04-00:15

5.5.3. SCREEN: 69550_screenshot_5.mp4: 00:16-end

~~5.5.4. SCREEN: Adjust **Roundness Threshold** settings. 69550_screenshot_5.mp4~~
NOTE: Not filmed

5.6. Finally, navigate to the **General** Tab for immunohistochemistry aggregate detection [1]. Click **Calculate Total Aggregates** and **Calculate Aggregate Area** [2]. Observe detected aggregates highlighted and listed in the Matrix Table with identification, area, and mean size [3].

5.6.1. SCREEN: 69550_screenshot_6.mp4: : 00:00-00:04

5.6.2. SCREEN: 69550_screenshot_6.mp4: : 00:06-00:10

5.6.3. SCREEN: 69550_screenshot_6.mp4: : 00:13-00:20

Results

6. Results

6.1. Robust expression of both wild-type and mutant ataxin-2 was detected twelve weeks after the stereotaxic injection of lentiviral vectors into the striatum of C57BL-6J mice [1].

6.1.1. LAB MEDIA: Figure 3 AB

6.2. Immunostaining for ataxin-2 revealed a diffuse cytoplasmic distribution of wild-type ataxin-2 without forming discrete inclusions [1]. In contrast, the mutant ataxin-2 exhibited cytoplasmic aggregates with a distinct spherical morphology [2].

6.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the yellow-arrowed areas showing the spread-out staining inside the cells*

6.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the red-arrowed dark round spots inside the cells*

6.3. Immunolabeling with DARPP-32 (*Darp-Thirty-Two*), a marker for medium spiny neurons, demonstrated that both wild-type and mutant ataxin-2 were expressed within the striatal neuronal population [1]. While wild-type ataxin-2 expression did not alter the gross morphology of DARPP-32-positive neurons [2], the mutant protein produced neuronal marker loss [3].

6.3.1. LAB MEDIA: Figure 3CD.

6.3.2. LAB MEDIA: Figure 3C.

6.3.3. LAB MEDIA: Figure 3D. *Video editor: Highlight the red-outlined area where the brown neuronal staining is lost*

1. Anesthetized

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>

IPA: /əˈnɛsθəˈtaɪzɪd/

Phonetic Spelling: uh·nes·thuh·tyzd

2. Medetomidine
Pronunciation link: <https://www.howtopronounce.com/medetomidine>
IPA: /mɛˈdetoʊmɪdɪn/
Phonetic Spelling: meh·deh·toh·mih·deen
3. Midazolam
Pronunciation link: <https://www.howtopronounce.com/midazolam>
IPA: /mɪˈdæzəˌlɑːm/
Phonetic Spelling: mih·daz·uh·lahm
4. Fentanyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/fentanyl>
IPA: /ˈfɛn.tə.nɪl/
Phonetic Spelling: fen·tuh·nil
5. Stereotaxic
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereotaxic>
IPA: /ˌstɛr.i.ooˈtæk.sɪk/
Phonetic Spelling: steh·ree·oh·tak·sik
6. Lentiviral
Pronunciation link: <https://www.howtopronounce.com/lentiviral>
IPA: /ˌlɛntiˈvaɪrəl/
Phonetic Spelling: len·tee·vy·ruhl
7. Microinjection
Pronunciation link: <https://www.merriam-webster.com/dictionary/microinjection>
IPA: /ˌmaɪkroʊmˈdʒɛkʃən/
Phonetic Spelling: my·kroh·in·jek·shuhn
8. Hemisphere
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemisphere>
IPA: /ˈhɛmɪˌsfɪr/
Phonetic Spelling: hem·uh·sfeer
9. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /ˈɛθəˌnɔːl/
Phonetic Spelling: eh·thuh·nawl
10. Periosteum
Pronunciation link: <https://www.merriam-webster.com/dictionary/periosteum>
IPA: /ˌpɛrɪˈɑːstiəm/
Phonetic Spelling: peh·ree·os·tee·uhm
11. Bregma
Pronunciation link: <https://www.merriam-webster.com/dictionary/bregma>
IPA: /ˈbrɛgmə/
Phonetic Spelling: breg·muh
12. Antero-posterior
Pronunciation link: <https://www.merriam-webster.com/dictionary/anteroposterior>
IPA: /ˌæn.tə.roʊ.pɑːˈstɪr.i.ər/
Phonetic Spelling: an·tuh·roh·pos·teer·ee·er

13. Dorso-ventral
 Pronunciation link: <https://www.howtopronounce.com/dorso-ventral>
 IPA: /ˌdɔːrsoʊˈvɛntrəl/
 Phonetic Spelling: dor·soh·ven·truhl
14. Ataxin
 Pronunciation link: <https://www.howtopronounce.com/ataxin>
 IPA: /əˈtæksɪn/
 Phonetic Spelling: uh·tak·sin
15. Contralateral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/contralateral>
 IPA: /ˌkɒːntrəˈlætərəl/
 Phonetic Spelling: kon·truh·lat·uh·ruhl
16. Subcutaneously
 Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
 IPA: /ˌsʌbkjuːˈteɪniəsli/
 Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
17. Cryosectioning
 Pronunciation link: <https://www.howtopronounce.com/cryosectioning>
 IPA: /ˌkraɪoʊˈseɪʃənɪŋ/
 Phonetic Spelling: kry·oh·sek·shuh·ning
18. Phosphate-buffered
 Pronunciation link: <https://www.howtopronounce.com/phosphate-buffered>
 IPA: /ˈfɑːsfertˈbʌfərd/
 Phonetic Spelling: fos·fayt buf·erd
19. Cryostat
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cryostat>
 IPA: /ˈkraɪoʊˌstæt/
 Phonetic Spelling: kry·oh·stat
20. Cerebellum
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebellum>
 IPA: /ˌsɛrəˈbɛləm/
 Phonetic Spelling: seh·ruh·bel·uhm
21. Coronal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>
 IPA: /kəˈroʊnəl/
 Phonetic Spelling: kuh·roh·nuhl
22. Immunohistochemistry
 Pronunciation link: <https://www.merriam-webster.com/dictionary/immunohistochemistry>
 IPA: /ɪˌmjuːnoʊˌhɪstoʊˈkɛmɪstri/
 Phonetic Spelling: ih·myoo·noh·his·toh·kem·uh·stree
23. Diaminobenzidine
 Pronunciation link: <https://www.howtopronounce.com/diaminobenzidine>
 IPA: /daɪˌæmɪnoʊˈbenziːdiːn/
 Phonetic Spelling: dye·am·ih·noh·ben·zee·deen

24. Immunostaining

Pronunciation link: <https://www.merriam-webster.com/dictionary/immunostaining>

IPA: /ɪˌmjuːnoʊˈsteɪnɪŋ/

Phonetic Spelling: ih·myoo·noh·stay·ning

25. Cytoplasmic

Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasmic>

IPA: /ˌsaɪtoʊˈplæzmɪk/

Phonetic Spelling: sy·toh·plaz·mik

26. DARPP-32

Pronunciation link: No confirmed link found

IPA: /dɑːrp ˈθɜːrti tuː/

Phonetic Spelling: darp thur·tee too