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Title: Senescence Associated with Neurodegeneration: Simultaneous Assessment of β -gal Activity and Nissl Stain in Histological Sections of Rodent Brain

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

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

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **06/05/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 19

Number of Shots: 36

Interviews

INTRODUCTION:

NOTE: The name of the person delivering the interviews is not provided

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

- 1.1. This study defines spatial relationships between senescence burden and neuronal cytoarchitecture in the central nervous system.
 - 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. Conventional approaches lack co-localization; this protocol preserves anatomical context by integrating SA- β -gal detection with Nissl staining in situ.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. This protocol is suitable for rodent models of aging, neurodegeneration, acute brain injury, and senescence-targeting therapeutic interventions.
 - 1.3.1. 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?~~

- 1.4. This protocol enables simultaneous measurement of senescence burden and neuronal integrity within the same histological brain section.

- 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. Future studies can explore cell-type specificity, causal roles of senescence, and therapeutic modulation using senolytic or senomorphic interventions.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

This study was approved by the Facultad de Medicina, Universidad Nacional Autónoma de México

Protocol

2. Detection of Senescence-Associated β -Galactosidase (SA- β -gal) Activity

Demonstrators: Verónica Salas -Venegas and Jair Ramírez-Carreto

- 2.1. To begin, prepare the senescence-associated beta-galactosidase or SA- β -gal (*S-A-beta-Gal*) staining solution fresh on the day of use [1] from the solutions presented here [2].
 - 2.1.1. WIDE: Talent at the working bench with the required components placed in front of him.
 - 2.1.2. LAB MEDIA: Table 1
- 2.2. Remove the prepared brain slides from the cold storage [1]. Place the slides in a Coplin jar and allow them to equilibrate at room temperature for 5 minutes [2].
 - 2.2.1. Talent taking slides out from a cold storage unit.
 - 2.2.2. Talent placing slides into a Coplin jar and leaving them on the bench.
- 2.3. Add enough PBS to the Coplin jar to hydrate the sections [1] and place it on a low-speed orbital shaker for 5 minutes at room temperature [2].
 - 2.3.1. Talent pouring phosphate-buffered saline into the Coplin jar until slides are submerged.
 - 2.3.2. Talent placing the Coplin jar onto an orbital shaker.
- 2.4. After the second wash, remove excess PBS gently from the slide edges using absorbent paper [1].
 - 2.4.1. Talent dabbing the edges of the slides with absorbent paper.
- 2.5. Then, draw a hydrophobic barrier around each section using a water repellent barrier pen, avoiding contact with the tissue [1].
 - 2.5.1. Talent outlining tissue sections on the slides with a barrier pen.
- 2.6. To perform SA- β -gal staining, place the slides in a humidity chamber [1]. Using a micropipette, add approximately 200 microliters of staining solution per section to ensure complete coverage of the tissue [2]. Then, incubate the slides at 37 degrees Celsius for 48 hours [3].
 - 2.6.1. Talent placing slides into a humidity chamber.
 - 2.6.2. Talent pipetting staining solution onto each tissue section on the slides.
 - 2.6.3. Talent placing the slides into an incubator.

- 2.7. After incubation, remove the staining solution using absorbent paper [1].
 - 2.7.1. Talent removing staining solution from the slides using absorbent paper.
- 2.8. Then, place the slides in a Coplin jar and add PBS to fully cover the slides [1]. Place the Coplin jar on a low-speed orbital shaker and agitate gently for 5 minutes at room temperature [2].
 - 2.8.1. Talent placing slides into a Coplin jar and pouring PBS until submerged.
 - 2.8.2. Talent placing the Coplin jar onto a low-speed orbital shaker.

3. Nissl Staining

- 3.1. Prepare the Nissl working solution by mixing 15 milliliters of cresyl violet solution with 85 milliliters of acetate buffer from the stock solutions [1]. Filter the solution using a filter paper to remove precipitates [2].
 - 3.1.1. Talent mixing cresyl violet solution with acetate buffer in a container.
 - 3.1.2. Talent filtering the solution through filter paper into a clean container.
- 3.2. Pre-warm the filtered solution at 37 degrees Celsius for at least 30 minutes [1]. Then, add 10 drops of 0.1 molar acetic acid immediately before use to the Nissl working solution [2].
 - 3.2.1. Talent placing the solution into a heating device for pre-warming.
 - 3.2.2. Talent adding drops of acetic acid into the Nissl working solution.
- 3.3. Next, prepare acidified ethanol by adding 95 milliliters of absolute ethanol to a volumetric flask and adding 15 drops of 0.1 molar acetic acid [1]. Bring the volume to 100 milliliters with distilled water and mix thoroughly [2].
 - 3.3.1. Talent adding absolute ethanol and acetic acid into a volumetric flask.
 - 3.3.2. Talent adding distilled water to the volumetric flask and mixing the solution thoroughly.
- 3.4. Place the slides in a Coplin jar and add PBS to fully cover the slides [1], then incubate at 37 degrees Celsius for 5 minutes [2].
 - 3.4.1. Talent placing slides into a Coplin jar, adding phosphate-buffered saline.
 - 3.4.2. Talent placing the jar into an incubator.
- 3.5. Immerse the slides carefully in the pre-warmed Nissl solution one slide at a time [1] and incubate at 37 degrees Celsius for 5 to 20 minutes [2].
 - 3.5.1. Talent immersing slides individually into a container of pre-warmed Nissl solution.
 - 3.5.2. Talent placing the slides aside for incubation.

3.6. To wash the section, rinse the slides gently in distilled water for 1 minute [1]. Then, transfer the slides to a Coplin jar containing fresh distilled water for an additional 1 minute [2].

3.6.1. Talent rinsing slides under distilled water.

3.6.2. Talent placing slides into a Coplin jar with fresh distilled water.

3.7. Evaluate staining quality by observing the slides under a bright-field microscope [1]. Confirm intense purple punctate staining with minimal background in the bright-field microscope [2].

3.7.1. View of stained tissue sections under a bright-field microscope at 10 times magnification.

3.7.2. View showing intense purple punctate staining with minimal background.

3.8. Immediately transfer the slides to absolute ethanol for 10 seconds to stop differentiation [1]. Then, immerse the slides in xylene for at least 5 minutes [1].

3.8.1. Talent placing slides into a container with absolute ethanol.

3.8.2. Talent placing slides into a container filled with xylene.

NOTE: Add a title scard: Slide Mounting

3.9. To mount the slides, remove the slides from xylene and blot the edges gently [1], then place the slides on a flat surface [1].

3.9.1. Talent removing slides from xylene, dabbing the edges with absorbent paper.

3.9.2. Talent placing the slides on a flat surface.

3.10. Apply approximately 120 microliters of mounting medium over the tissue [1] cover it with a coverslip, and allow the slides to dry for at least 5 minutes at room temperature [1].

3.10.1. Talent pipetting mounting medium onto the tissue sections on the slides.

3.10.2. Talent leaving slides on a flat surface to dry.

3.11. Observe the slides under a bright-field microscope and acquire images of regions of interest [1]. After processing the acquired images using ImageJ, quantify the senescence burden using the presented equation [2].

3.11.1. LAB MEDIA: Figure 2.tif

3.11.2. TEXT ON A PLAIN BACKGROUND

$$\text{Senescence burden (\%)} = (\text{SA-}\beta\text{-gal-positive cells} / \text{Total cells}) \times 100$$

Results

4. Results

- 4.1. Successful SA- β -gal staining was indicated by the presence of a distinct blue precipitate localized within cells [1].
 - 4.1.1. LAB MEDIA: Figure 1.
- 4.2. At low magnification, major brain regions such as the hippocampus and cerebral cortex were identified [1]. At higher magnification, individual cells displaying intracellular blue staining were observed, particularly within regions such as the CA3 subfield [2].
 - 4.2.1. LAB MEDIA: Figure 1A.
 - 4.2.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the annotated points in the 10x image*
- 4.3. Successful Nissl staining is characterized by intense purple labeling of neuronal somata with clear contrast against the surrounding neuropil [1].
 - 4.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the purple-stained cell layer along the curved structure*
- 4.4. At low magnification, anatomical regions such as the hippocampus and cortex were distinguished [1]. At higher magnification, neuronal populations in specific subregions, such as CA3, were identified [2], and cortical layer organization was assessed by differences in cell density and structure [3].
 - 4.4.1. LAB MEDIA: Figure 2A.
 - 4.4.2. LAB MEDIA: Figure 2B. *Video editor: Zoom in on the dense purple band of cells*
 - 4.4.3. LAB MEDIA: Figure 2C.
- 4.5. When both staining methods were applied sequentially, both blue SA- β -gal-positive cells and purple Nissl staining were preserved within the same section [1].
 - 4.5.1. LAB MEDIA: Figure 3B. *Highlight the blue and purple dots*
- 4.6. At low magnification, regional distribution across the hippocampus and cortex was verified [1]. At higher magnification, the spatial relationship between senescent cells and neuronal structures was assessed in CA3 [2] and cortical regions [3].
 - 4.6.1. LAB MEDIA: Figure 3A.
 - 4.6.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the blue dots and nearby purple-stained cell layer*
 - 4.6.3. LAB MEDIA: Figure 3C. *Video editor: Highlight the blue spots indicated by arrows*

within the purple background

1. Senescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/senescence>
IPA: /sɪˈnesəns/
Phonetic Spelling: sih·NESS·uhns
2. Neurodegeneration
Pronunciation link: No confirmed link found
IPA: /ˌnʊroʊdɪˌdʒenəˈreɪʃən/
Phonetic Spelling: noor·oh·dih·jen·uh·RAY·shuhn
3. β -galactosidase
Pronunciation link: <https://www.merriam-webster.com/medical/beta-galactosidase>
IPA: /ˌbɛrtəˌɡəˌlæktˈtɑːsəˌdeɪs/
Phonetic Spelling: BAY·tuh guh·lak·TOS·uh·days
4. Nissl
Pronunciation link: <https://www.merriam-webster.com/medical/Nissl>
IPA: /ˈnɪsəl/
Phonetic Spelling: NISS·uhl
5. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
IPA: /ˌhɪstəˈlɑːdʒɪkəl/
Phonetic Spelling: hiss·tuh·LOJ·ih·kuhl
6. Neuroimmunología
Pronunciation link: No confirmed link found
IPA: /neuroɪnmunoloˈxia/
Phonetic Spelling: neh·oo·roh·een·moo·noh·loh·HEE·ah
7. Cytoarchitecture
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoarchitecture>
IPA: /ˌsaɪtoʊˈɑːrkəˌtektʃər/
Phonetic Spelling: sy·toh·AR·kuh·tek·cher
8. Co-localization
Pronunciation link: No confirmed link found
IPA: /ˌkoʊˌloʊkələˈzeɪʃən/
Phonetic Spelling: koh·loh·kuh·luh·ZAY·shuhn
9. Neurodegeneration
Pronunciation link: No confirmed link found
IPA: /ˌnʊroʊdɪˌdʒenəˈreɪʃən/
Phonetic Spelling: noor·oh·dih·jen·uh·RAY·shuhn
10. Senolytic
Pronunciation link: No confirmed link found

- IPA: /ˌsenəˈlɪtɪk/
Phonetic Spelling: sen·uh·LIT·ik
11. Senomorphic
Pronunciation link: No confirmed link found
IPA: /ˌsenəˈmɔːrfɪk/
Phonetic Spelling: sen·uh·MOR·fik
12. Galactosidase
Pronunciation link: <https://www.merriam-webster.com/dictionary/galactosidase>
IPA: /ɡəˌlæktˈɑːsəˌdeɪs/
Phonetic Spelling: guh·lak·TOS·uh·days
13. Equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ɪˈkwɪləˌbreɪt/
Phonetic Spelling: ih·KWIL·uh·brayt
14. Hydrophobic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrophobic>
IPA: /ˌhaɪdrəˈfoʊbɪk/
Phonetic Spelling: hy·druh·FOH·bik
15. Micropipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/micropipette>
IPA: /ˌmaɪkroʊpaɪˈpet/
Phonetic Spelling: my·kroh·py·PET
16. Cresyl violet
Pronunciation link: No confirmed link found
IPA: /ˈkresəlˈvaɪələt/
Phonetic Spelling: KRESS·uhl VY·uh·luht
17. Acetate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>
IPA: /ˈæsəˌteɪt/
Phonetic Spelling: ASS·uh·tayt
18. Precipitates
Pronunciation link: <https://www.merriam-webster.com/dictionary/precipitate>
IPA: /prɪˈsɪpətəts/
Phonetic Spelling: prih·SIP·ih·tuhts
19. Molar
Pronunciation link: <https://www.merriam-webster.com/dictionary/molar>
IPA: /ˈmoʊlər/
Phonetic Spelling: MOH·ler
20. Acetic acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetic%20acid>
IPA: /əˈsiːtɪkˈæsɪd/
Phonetic Spelling: uh·SEE·tik ASS·id
21. Acidified
Pronunciation link: <https://www.merriam-webster.com/dictionary/acidify>

- IPA: /əˈsɪdəˌfaɪd/
Phonetic Spelling: uh·SID·uh·fyd
22. Volumetric
Pronunciation link: <https://www.merriam-webster.com/dictionary/volumetric>
IPA: /ˌvɒːljəˈmetrɪk/
Phonetic Spelling: vol·yuh·MET·rik
23. Punctate
Pronunciation link: <https://www.merriam-webster.com/dictionary/punctate>
IPA: /ˈpʌŋ(k)-,tāt/
Phonetic Spelling: PUNK·tayt
24. Xylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/xylene>
IPA: /ˈzaɪˌliːn/
Phonetic Spelling: ZY·leen
25. Coverslip
Pronunciation link: <https://www.merriam-webster.com/dictionary/coverslip>
IPA: /ˈkʌvərˌslɪp/
Phonetic Spelling: KUV·er·slip
26. Hippocampus
Pronunciation link: <https://www.merriam-webster.com/dictionary/hippocampus>
IPA: /ˌhɪpəˈkæmpəs/
Phonetic Spelling: hip·uh·KAM·puhs
27. Cerebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>
IPA: /səˈriːbrəl/, /ˈserəbrəl/
Phonetic Spelling: suh·REE·bruhl / SER·uh·bruhl
28. Intracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/intracellular>
IPA: /ˌɪntrəˈseljələr/
Phonetic Spelling: in·truh·SEL·yuh·ler
29. Neuronal
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuronal>
IPA: /nʊˈroʊnəl/
Phonetic Spelling: noo·ROH·nuhl
30. Somata
Pronunciation link: <https://www.merriam-webster.com/dictionary/soma>
IPA: /ˈsoʊmətə/
Phonetic Spelling: SOH·muh·tuh
31. Neuropil
Pronunciation link: <https://www.merriam-webster.com/medical/neuropil>
IPA: /ˈnʊrəˌpɪl/
Phonetic Spelling: NOOR·uh·pil
32. Senescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/senescent>

IPA: /sɪˈnesənt/

Phonetic Spelling: sih·NESS·uhnt