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Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21181078>

Title: A Comparative Approach for Quantitative Cell Counting Studies in Widely Different Mammalian Brains

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, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? No, you can record everything in the same building - only the floor changes (yes, there is an elevator to move around if you need to transport your equipment).
- 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. **No.**

Current Protocol Length

Number of Steps: 14

Number of Shots: 36 (25 SC)

Introduction

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

INTRODUCTION:

Authors: The questions will not appear in the video and hence the following statements need to be answered in full sentences with context

~~What are the most recent developments in your field of research?~~

- 1.1. **Luca Bonfanti:** Recently, it was discovered that brain structural plasticity is markedly different across mammals, thus requiring systematic quantitative analyses to be revealed.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.2. **Luca Bonfanti:** Our systematic analysis of widely different brains gives comparable results to be put in a phylogenetic context.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How will your findings advance research in your field?~~

- 1.3. **Luca Bonfanti:** Our findings can reveal evolutionary trade-offs between different types of plasticity also involving different brain regions.
 - 1.3.1. 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.

Ethics Title Card

This research has been approved by the Italian Ministry of Health

Protocol

NOTE: The screen recordings are slightly blur at the top, but authors have an old system and this is the best they can provide

NOTE from Utkarsh: Please make the timeline 16:9 () 1920x1080) as the screen captures are in 4k and wont be visible in 1280 X 720, I tried downgrading 1 screenshot and it looks much better with 16:9 ration.

2. Processing Brain Sections and Brain Level Selection

Demonstrator: Alessandro Zanone

- 2.1. To begin, take a 12-well plate from the minus 20 degrees Celsius refrigerator containing the free-floating brain sections [1] and remove some slices into a petri dish filled with PBS solution [2]. Select the sections and place them on a slide [3].
 - 2.1.1. WIDE: Talent taking a 12-well plate from the refrigerator and placing it on the bench.
 - 2.1.2. Talent places the sections in a dish containing PBS.
 - 2.1.3. Talent selecting some samples and placing them on a slide.
- 2.2. After histologically staining the sections [1], identify the brain levels based on specific neuroanatomical features. Define it into Levels 1 through 4 using the same number of 40-micrometer-thick serial sections [2-TXT] and pick 3 equally spaced coronal sections from each brain level [3] and perform immunohistochemical staining [4].
 - 2.2.1. Talent arranging the histologically stained reference sections on the workspace.
TXT: Use histologically stained reference sections for identification
 - 2.2.2. LAB MEDIA: Figure 3 *Video editor: Mark Level L1, L2, L3 and L4 regions in both the mouse and chimpanzee brain section.*
 - 2.2.3. Talent selecting and lifting 3 equally spaced coronal sections and placing them in new well.
 - 2.2.4. Talent adding PBS to the sections. **Authors:** This will just be a representative shot of adding some solution as we are not explaining immunohistochemical staining procedure in the video.

3. Cell Counting Using Neurolucida Software

- 3.1. Open the Neurolucida (*neuro-lucida*) software on the workstation [1] and place the slide under the microscope using the selected objective for cell counting [2].
 - 3.1.1. Talent turning on the workstation and launching the Neurolucida software.
 - 3.1.2. Talent positioning the microscope slide on the stage and selecting the appropriate objective.
- 3.2. At low magnification, outline cortical layer two using the Freehand contour line tool in the **Trace** menu of the software [1]. Trace the perimeter to obtain the length of cortical layer two, including both paleocortex and neocortex [2].
 - 3.2.1. SCREEN: 69446_3.2.1_SCREEN.
 - 3.2.2. SCREEN: 69446_3.2.1_SCREEN.
- 3.3. Select the preferred symbols for cell counting from the **Markers** toolbar [1]. Count type 1 cells, identified by a small soma with a bipolar shape and a soma diameter ranging from 3 to 9 micrometers, in each region of interest [2]. Count type 2 cells, identified by a larger soma with complex dendritic arborization and a soma diameter ranging from 9 to 18 micrometers [3].
 - 3.3.1. SCREEN: 69446_3.3.1-3.3.2-3.3.3_SCREEN. 00:00-00:05
 - 3.3.2. SCREEN: 69446_3.3.1-3.3.2-3.3.3_SCREEN. 00:06-00:17
 - 3.3.3. SCREEN: 69446_3.3.1-3.3.2-3.3.3_SCREEN. 00:18-00:26
- 3.4. Now, collect the data for the phylogenetic analysis from the paleocortex, the neocortex, and the cerebral cortex obtained by combining data from the paleocortex and neocortex [1].
 - 3.4.1. SCREEN: 69446_3.4.1-3.4.2-3.4.3_SCREEN.
- 3.5. Collect the cortical layer two perimeter, measured in millimeters and visible in the **Contour Measurements** toolbar, into a spreadsheet file [1].
 - 3.5.1. SCREEN: 69446_3.5.1-_SCREEN.
- 3.6. On the same spreadsheet, collect the number of type 1 cells and type 2 cells visible in

the **Markers** toolbar [1]. Calculate and record the total number of cells by adding type 1 and type 2 cells for each brain region and brain level, using three sections per level for a total of 12 sections per specimen [2].

3.6.1. SCREEN: 69446_3.6.1_SCREEN.

3.6.2. SCREEN: 69446_3.6.2_SCREEN 00:05-00:19.

- 3.7. Use the collected values to calculate the linear cell density for each section in each region by dividing the number of cells by the perimeter in millimeters [1]. Calculate the percentage of type 1 cells and type 2 cells by dividing the type 1 or type 2 cell number by the total number of cells and multiplying by 100 [2].

3.7.1. SCREEN: 69446_3.7.1_SCREEN 00:05-00:19.

3.7.2. SCREEN: 69446_3.7.2_SCREEN 00:15-00:33.

- 3.8. Next, calculate the percentage of type 1 and type 2 cells in each specimen using the formula [1]. Obtain the mean value of these percentages among the 4 specimens considered per species using the **Average** function on the spreadsheet [2]. Use the linear cell density to perform phylogenetic analyses, and use the total cells counted in each coronal section to estimate the total number of immature neurons per hemisphere [3].

3.8.1. SCREEN: 69446_3.8.1_SCREEN.

3.8.2. SCREEN: 69446_3.8.2_SCREEN.

3.8.3. SCREEN: 69446_3.8.3_SCREEN.

- 3.9. Calculate the density median value of the 12 sections considered per specimen using the **Median** function on the spreadsheet [1]. Use the median as the central measure because the sample per group is relatively small and some groups exhibit asymmetrical distributions [2].

3.9.1. SCREEN: 69446_3.9.1_SCREEN 00:05-00:14

3.9.2. SCREEN: 69446_3.9.2_SCREEN 00:05-00:09.

- 3.10. Use the **Freehand** contour tool on the Neurolucida software to draw a line across the soma diameter perpendicular to the main axis [1]. Measure at least 100 randomly selected DCX-positive cells of both types in each species, using 25 cells per specimen, in each brain region to obtain the soma diameter range for type 1 and type 2 cells [2-TXT].

3.10.1. SCREEN: 69446_3.10.1_SCREEN.

3.10.2. SCREEN: 69446_3.10.2_SCREEN. **TXT: DCX: Doublecortin**

4. Phylogenetic Analyses

Demonstrator: Alessia Pattaro

- 4.1. Now, create a list of the analyzed species in Notepad using their scientific names [1]. Upload the list to the website timetree.org (*time tree dot O-R-G*) and export as a newick file [2]. On Mesquite Project software upload the newick file, integrate the data and save the resulting file as a PDF file [3].

4.1.1. SCREEN: 2025-03-31 115402-Phylogenetic Tree" 00:11 – 00:18

4.1.2. SCREEN: 2025-03-31 115402-Phylogenetic Tree" 00:27 – 00:33 and 00:40 – 00:48

4.1.3. SCREEN: 2025-03-31 115402-Phylogenetic Tree" 01:28 – 01:38 and and 04:41 – 04:55 *Video editor: Please speed up*

- 4.2. Finally, in the previously created data sheet, add a column containing the brain size for each species [1]. Calculate the natural logarithm of the DCX-positive cell densities and the natural logarithm of the brain size [2]. Perform statistical analyses on the chosen statistical software and download the obtained files [3]. Enter the residuals into a new column in the data sheet so a residual graph can be made [4].

4.2.1. SCREEN: 2025-03-31 124938-Correlation brain size or neocortical extension and DCX density 2.mp4" 00:00-00:13

4.2.2. SCREEN: 2025-03-31 124938-Correlation brain size or neocortical extension and DCX density 2.mp4" 00:13-00:20

4.2.3. SCREEN: 2025-03-31 124938-Correlation brain size or neocortical extension and DCX density 2.mp4" 00:22 - 00:28 and 00:50 - 01:00

4.2.4. SCREEN: 2025-03-31 124938-Correlation brain size or neocortical extension and DCX density 2.mp4" 01:33 – 01:38 and 01:55-02:00

Results

5. Results

- 5.1. DCX-positive cells in the cerebral cortex of all species studied consistently showed a topographical arrangement forming a monolayer along cortical layer II (2) [1], included predominantly small, unipolar or bipolar cells and a minor fraction of large, complex cells [2]. The cells co-expressed PSA-NCAM (P-S-A-N-cam) and DCX [3-TXT] while lacking Ki67 expression [4] and contained a subpopulation of complex cells that expressed NeuN (New-N) [5-TXT].
 - 5.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the strip of cells under the label “topographical distribution” label*
 - 5.1.2. LAB MEDIA: Figure 4A. *Video editor: Zoom in on the pie charts and the image to the left of the charts showing type 1 and type 2 cell drawings under the label “cell morphology.”*
 - 5.1.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the images marked PSA-NCAM and DCX.* **TXT: PSA-NCAM: Polysialylated Neural Cell Adhesion Molecule**
 - 5.1.4. LAB MEDIA: Figure 4A. *Video editor: Highlight the image marked Ki67 on the right*
 - 5.1.5. LAB MEDIA: Figure 4A. *Video editor: Highlight the image marked NeuN DCX* **TXT: NeuN: Neuronal Nuclei**
- 5.2. The linear density of cortical immature neurons varied greatly across species [1].
 - 5.2.1. LAB MEDIA: Figure 4B.
- 5.3. Phylogenetic comparative analyses showed that the Laurasiatheria group had a higher density of cortical immature neurons [1] than the Euarchontoglires [2].
 - 5.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the blue lines in all 3 trees that cover “Felix catus, Vulpes vulpes, Canis lupus, Equus caballus, Ovis aries”*
 - 5.3.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the white lines in all 3 trees that cover “Oryctolagus cuniculus, Mus musculus, Heterocephalus glaber, Pan troglodytes”*
- 5.4. A strong covariance was observed between cortical immature neuron density and brain volume [1], and between cortical immature neuron density and neocortical layer II

perimeter [2].

5.4.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the left scatter plot showing the correlation between cIN density and brain volume*

5.4.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the right scatter plot showing the correlation between cIN density and neocortical layer II perimeter*

1. Histologically
Pronunciation link: <https://www.merriam-webster.com/dictionary/histologically>
IPA: /ˌhɪs.təˈlɑː.dʒɪ.kli/
Phonetic Spelling: his·tuh·laa·ji·klee
2. Neuroanatomical
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuroanatomical>
IPA: /ˌnɒr.oʊ.əˈnɑː.mɪ.kəl/
Phonetic Spelling: nyur·oh·a·nuh·taa·mi·kuhl
3. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkraː.mɪ.tər/
Phonetic Spelling: my·kraa·muh·ter
4. Immunohistochemical
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunohistochemical>
IPA: /ɪˌmjuː.noʊ.ˌhɪs.təˈkɛm.ɪ.kəl/
Phonetic Spelling: ih·myoo·noh·his·tuh·ke·mi·kuhl
5. Neurolucida
Pronunciation link: No confirmed link found
IPA: /ˌnɒr.oʊˈluː.sɪ.də/
Phonetic Spelling: nyur·oh·loo·sih·duh
6. Paleocortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/paleocortex>
IPA: /ˌpeɪ.li.ʊˈkɔːr.tɛks/
Phonetic Spelling: pay·lee·oh·kor·teks
7. Neocortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/neocortex>
IPA: /ˌniː.ʊˈkɔːr.tɛks/
Phonetic Spelling: nee·oh·kor·teks
8. Phylogenetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/phylogenetic>
IPA: /ˌfaɪ.loʊ.dʒəˈnɛ.tɪk/
Phonetic Spelling: fy·loh·juh·ne·tik
9. Coronal
Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>

- IPA: /kə'roʊ.nəl/
Phonetic Spelling: kuh·roh·nuhl
10. Dendritic
Pronunciation link: <https://www.merriam-webster.com/dictionary/dendritic>
IPA: /den'dri.tɪk/
Phonetic Spelling: den·dri·tik
11. Arborization
Pronunciation link: <https://www.merriam-webster.com/dictionary/arborization>
IPA: /ˌɑːr.bə.ɪ'zeɪ.ʃən/
Phonetic Spelling: ar·buh·rih·zay·shuhn
12. Cerebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>
IPA: /sə'riː.brəl/
Phonetic Spelling: suh·ree·bruhl
13. Median
Pronunciation link: <https://www.merriam-webster.com/dictionary/median>
IPA: /'miː.di.ən/
Phonetic Spelling: mee·dee·uhn
14. Doublecortin
Pronunciation link: No confirmed link found
IPA: /ˌdʌb.əl'kɔːr.tɪn/
Phonetic Spelling: duh·buhl·kor·tin
15. Laurasiatheria
Pronunciation link: <https://www.howtopronounce.com/laurasiatheria>
IPA: /ˌlɔːr.eɪ.ʒi.ə'θɪr.i.ə/
Phonetic Spelling: lor·ay·zhee·uh·thair·ee·uh
16. Euarchontoglires
Pronunciation link: <https://www.howtopronounce.com/euarchontoglires>
IPA: /ˌjuː.ɑːr.kən'toʊ.glɑɪr.iːz/
Phonetic Spelling: yoo·ar·kon·toh·glyrz
17. Covariance
Pronunciation link: <https://www.merriam-webster.com/dictionary/covariance>
IPA: /koʊ'veɪ.i.əns/
Phonetic Spelling: koh·vair·ee·uhns
18. Polysialylated
Pronunciation link: <https://www.merriam-webster.com/dictionary/polysialylated>
IPA: /ˌpɑː.li.sai'æl.i.leɪ.tɪd/
Phonetic Spelling: pah·lee·sy·a·lih·lay·tid
19. Ki67
Pronunciation link: No confirmed link found
IPA: /ˌkaɪ'sɪk.stiːn/
Phonetic Spelling: kye·six·teen
20. NeuN
Pronunciation link: No confirmed link found

IPA: /'nu:.ɛn/

Phonetic Spelling: noo·en