Teacher Resource for:

Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages

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GENERAL USE OF *Science* in the Classroom

Student Learning Goals:

"One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge."[1]

The U.S. National Academy of Sciences defines science as: "Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim."

An important student learning goal, central to any understanding of "the nature of scientific knowledge," is to give each student an appreciation of how science is done.

This includes knowing why:

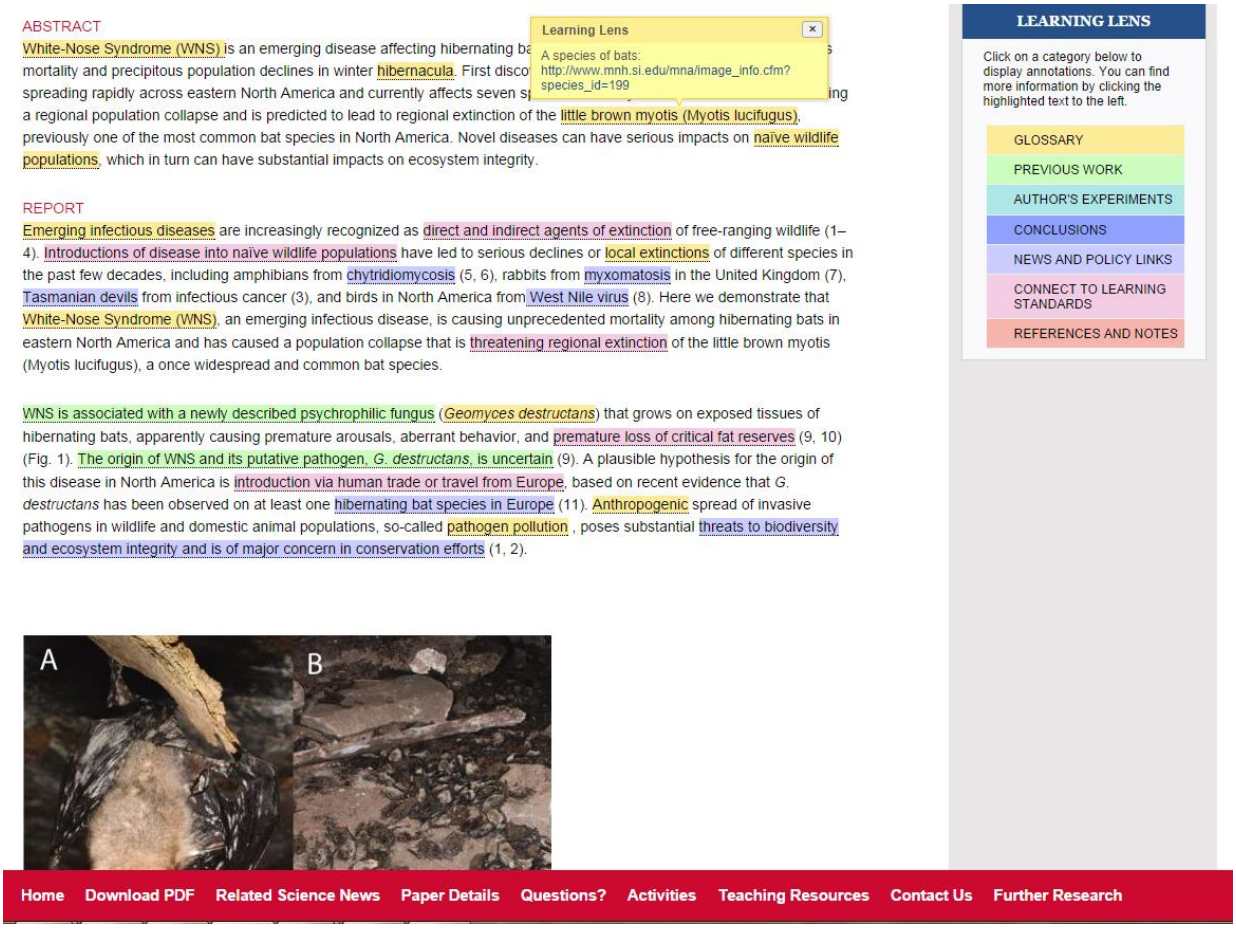
- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist's conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

[1] *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

Learning Lens:

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.



An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

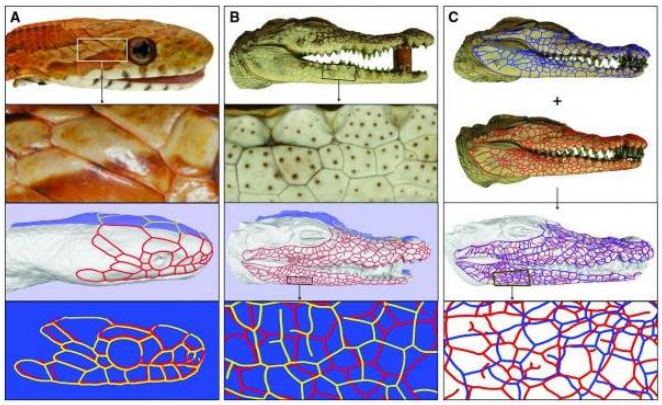


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). (B) Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. (C) Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).

Method: 3D geometry and color-texture reconstruction | Panel A | Panel B | Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

- GLOSSARY
- PREVIOUS WORK
- AUTHOR'S EXPERIMENTS
- CONCLUSIONS
- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

6. L. Lewejohann, B. Zipser, N. Sachser, "Personality" in laboratory mice used for biomedical research: A way of understanding variability? Dev. Psychobiol. 53, 624 (2011).
7. G. Kempermann, The neurogenic reserve hypothesis: What is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163 (2008).
8. Lewejohann et al., Behavioral phenotyping of a murine model of Alzheimer's disease in a seminaturalistic environment using RFID tracking. Behav. Res. Methods 41, 850 (2009).
9. B. Steineret al., Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46, 41 (2004).
10. Garthe, J. Behr, G. Kempermann, Ad… flexible use of spatially precise learning strategies. PLoS ONE 4, e54…
11. G. Kempermann, New neurons for 'su… 27 (2012).
12. G. Kempermannet al., Why and how … brain plasticity. Front Neurosci 4, 189 (2010).
13. K. Fabelet al., Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Front. Neurosci. 3, 50 (2009).
14. Amrein, H. P. Lipp, Adult hippocampal neurogenesis of mammals: Evolution and life history. Biol. Lett. 5, 141 (2009).
15. G. Kempermann, Why new neurons? Possible functions for adult hippocampal neurogenesis. J. Neurosci. 22, 635 (2002).
16. C. Crabbe, D. Wahlsten, B. C. Dudek, Genetics of mouse behavior: Interactions with laboratory environment. Science 284, 1670 (1999).
17. Lewejohann et al., Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. Genes Brain Behav. 5, 64 (2006).
18. D. W. Bailey, How pure are inbred strains of mice? Immunol. Today 3, 210 (1982).
19. R. Lathe, The individuality of mice. Genes Brain Behav. 3, 317 (2004).
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21. R. P. Talenset al., Epigenetic variation during the adult lifespan: Cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11, 694 (2012).
22. P. B. Baltes, J. R. Nesselroade, S. W. Cornelius, Multivariate antecedents of structural change in development: A simulation of cumulative environmental patterns. Multivariate Behav. Res. 13, 127 (1978).
23. M.E. Raijmakers, P. C. Molenaar, Modeling developmental transitions in adaptive resonance theory. Dev. Sci. 7, 149 (2004).
24. K. Friston, M. Breakspear, G. Deco, Perception and self-organized instability. Front. Comput. Neurosci. 6, 44 (2012).
25. Van de Weerd et al., Effects of environmental enrichment for mice: Variation in experimental results. J. Appl. Anim. Welf. Sci. 5, 87 (2002).
26. D. P. Wolferet al., Laboratory animal welfare: Cage enrichment and mouse behaviour. Nature 432, 821 (2004).
27. K. Lewin, Dynamic Theory of Personality (McGraw-Hill, New York, 1935).
28. K. L. Jang, R. R. McCrae, A. Angleitner, R. Riemann, W. J. Livesley, Heritability of facet-level traits in a cross-cultural twin

Learning Lens

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

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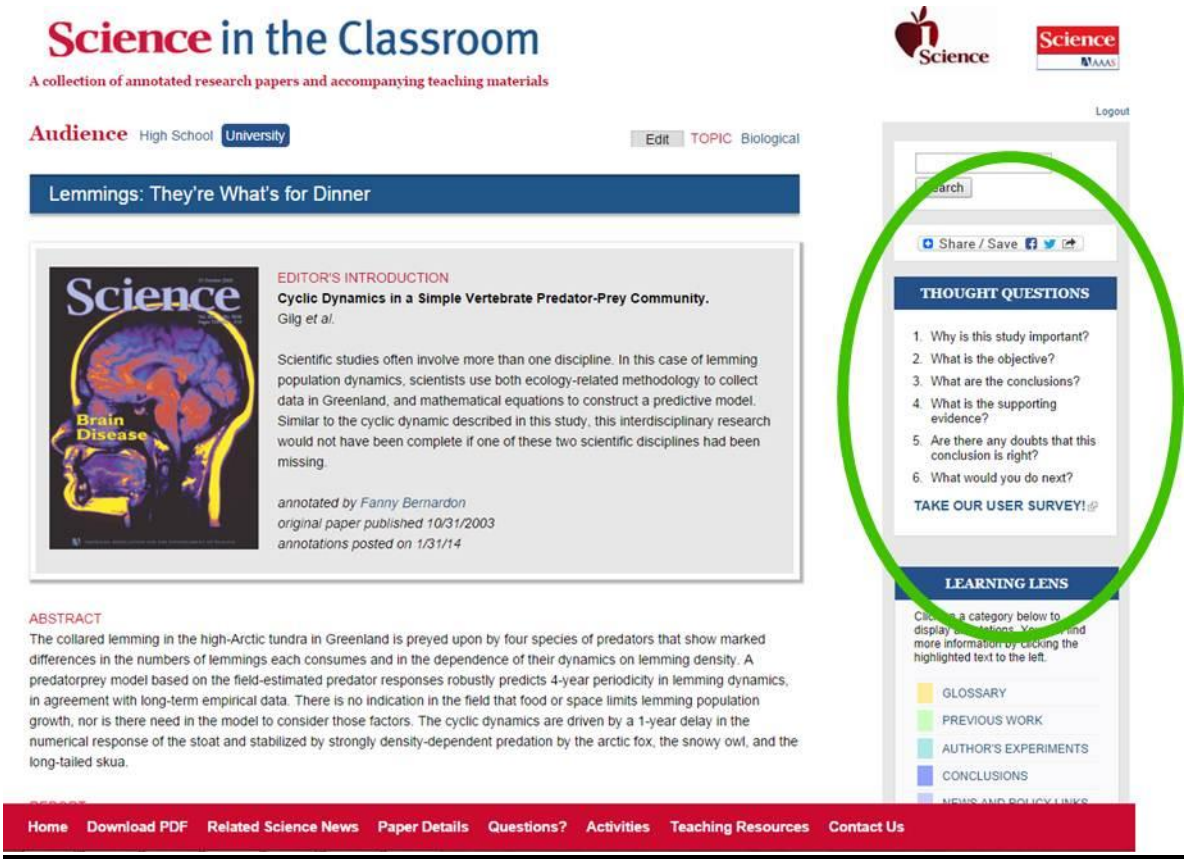
Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.



Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.

2. Activities tied to the articles.

Some ways to use the *Science* in the Classroom articles:

1. Assign to student groups to read and discuss during class.

2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.

3. Assign to individual students to complete during class or as homework.

4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).

2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.

3. Have students edit the article, or parts of the article, to a simpler reading level.

4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier

publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.

6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- Why is it important to determine whether blood monocytes are microglial precursors?
- How did the progress of research methods advance our understanding of this relationship?

The importance of this scientific research

- Understanding brain-immune interactions is paramount for the development of new treatment options for the diseases of the central nervous system (CNS).

The actual science involved

- Genetic techniques
- Fluorescent cell sorting
- Immunocytochemistry
- In vivo imaging

Connect to Learning Standards:

The Next Generation Science Standards Science and Engineering Practices

- Practice 1: Asking questions (for science) and defining problems (for engineering)
- Practice 2: Developing and using models
- Practice 3: Planning and carrying out investigations
- Practice 4: Analyzing and interpreting data
- Practice 5: Using mathematics and computational thinking
- Practice 6: Constructing explanations (for science) and designing solutions (for engineering)
- Practice 7: Engaging in argument from evidence
- Practice 8: Obtaining, evaluating, and communicating information

The AP Biology Standards

- Essential knowledge 2.D.2: Homeostatic mechanisms reflect both common ancestry and divergence due to adaptation in different environments.
- Essential knowledge 2.E.1: Timing and coordination of specific events are necessary for the normal development of an organism, and these events are regulated by a variety of mechanisms.
- Essential knowledge 3.B.1: Gene regulation results in differential gene expression, leading to cell specialization.

Common Core English Language Arts

- 11-12.2: Determine the central ideas or conclusions of a text; summarize complex concepts, processes, or information presented in a text by paraphrasing them in simpler but still accurate terms.
- 11-12.6: Analyze the author's purpose in providing an explanation, describing a procedure, or discussing an experiment in a text, identifying important issues that remain unresolved.
- 11-12.8: Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

The main defense system in a live organism is the immune system. Your peripheral and central immune systems are not identical. One of the main differences is the type of cells recruited to mitigate the effects of an immunological challenge. Although your periphery is teeming with a plethora of immune cells, your brain only relies on one cell type. They are called microglia. Microglia are very similar to macrophages that live outside the brain. As a matter of fact, microglia can become macrophages if CNS homeostasis is disturbed. They can phagocytize pathogens and alert other cells of their presence. Because microglia and macrophages are so similar, scientists hypothesized that, like peripheral macrophages, microglia originate from peripheral monocytes that infiltrate the brain around the time of birth. Early studies supported this hypothesis. Scientists speculated that this transformation of peripheral monocytes into brain microglia continues even in an adult animal. That meant that peripheral macrophages could be genetically transformed into “Trojan horses” to be sent into the brain to stop progression of various CNS disorders. However, as our methods advanced and became more nuanced, it became clear that peripheral monocytes do not enter the brain in an adult animal under normal conditions. Moreover, microglia could be related to these cells only distantly, originating from an early population of macrophages in the yolk sac. To determine whether that new hypothesis is correct, researchers used advanced genetic methods to label various populations of hematopoietic cells in mice during different time points of prenatal and postnatal development.

Topics Covered:

- Prenatal and postnatal development of hematopoietic cells
- Ontogenetic differences between immune cells in the CNS (microglia) and peripheral monocytes
- Development of genetic models to study cellular differentiation
- Time- and tissue-specific gene expression
- Cell trafficking
- Cell imaging and quantification techniques

Why this research is important:

The brain is a very well-protected organ. The barrier between the brain and the rest of the body is a highly controlled environment. Only some molecules and cells are allowed to go in and leave the brain under normal conditions. This protective environment can be disturbed by physical and nonphysical stressors, leading to an influx of compounds that can either help the brain heal or further exacerbate the insult. In many diseases of the CNS, scientists observed peripheral immune cells migrating into the brain. The results of their trespassing have varied: Sometimes they were “agents of recovery” and sometimes “agents of destruction” (Bertnic et al., 2000). Thus, it is important to determine whether these migrating monocytes are developmentally related to microglial cells that reside in the brain and have evolved to respond to disruptions in brain homeostasis. Knowing the origin of these cell types would allow us to harness their potential for treatment of brain-related diseases.

Methods used in the Research:

- Gene recombination technology
- Fluorescent cell sorting
- Fluorescent immunostaining
- In vivo imaging

Conclusions:

- Yolk sac macrophages and microglia have similar phenotypes .
- Both yolk sac macrophages and microglia depend on the presence of the CSF-1R receptor on their surface for differentiation. Absence of this receptor does not affect development of circulating monocytes, further supporting the prediction these cells are not precursors of brain microglia.
- Runx1 yolk sac progenitors give rise to microglia, but not to blood and tissue macrophages, granulocytes, or lymphocytes.
- Blood circulation needs to be developed to allow the influx of yolk sac precursors into the brain.
- The influx of yolk sac progenitors into the brain happens between embryonic days 8.5 and 9.5.

Areas of Further Study:

- How severe should brain insult be in order for peripheral monocytes to cross the blood-brain barrier (BBB)?
- Can these cells enter the brain in response to nonphysical stressors?
- How do peripheral monocytes and microglia communicate at the level of the BBB?
- Can peripheral monocytes acquire microglial phenotype upon entering brain parenchyma in response to injury?

Discussion Questions:

Discussion questions associated with the learning standards

- *Essential knowledge 2.D.2:* Homeostatic mechanisms reflect both common ancestry and divergence due to adaptation in different environments.
    - The authors of this experiment found that microglia and peripheral monocytes have evolved from different progenitors. Microglia evolved from yolk sac macrophages, whereas peripheral blood cells arise from fetal liver, spleen, and bone marrow. How can the differential environments in which these cells develop affect their morphology and function?
- *Essential knowledge 2.E.1:* Timing and coordination of specific events are necessary for the normal development of an organism, and these events are regulated by a variety of mechanisms.
    - The authors showed that differentiation of microglial cells depends on the presence of specific molecules. Differential expression of which molecules distinguished developmental trajectories of microglial cells and macrophages?
- *Essential knowledge 3.B.1:* Gene regulation results in differential gene expression, leading to cell specialization.
    - Despite having identical genomes, cells acquire distinct phenotypes as a result of tissue- and/or time-specific differences in gene expression profiles in distinct cell types. How did the authors use the restricted time window of *Runx1* expression to determine the origin of microglial cells?
- *Practice 2: Developing and using models:*
    - How did the authors determine that microglial seeding in the brain depends on the developed vasculature?
- *Practice 7:* Engaging in argument from evidence
    - With an example of one graph show how the authors used obtained data to support their hypothesis.