

Teacher Resource for:
The gut microbiota influences blood-brain barrier permeability in mice.

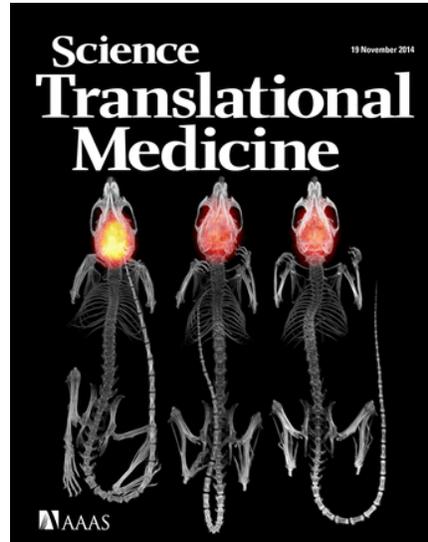


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GENERAL USE OF Science in the Classroom

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

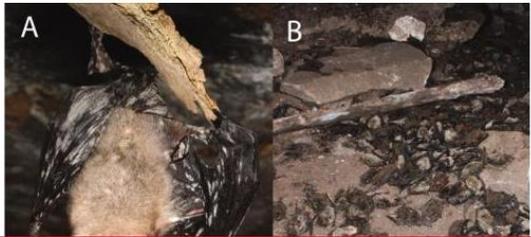
Learning Lens:

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.

ABSTRACT
[White-Nose Syndrome \(WNS\)](#) is an emerging disease affecting hibernating bat mortality and precipitous population declines in winter [hibernacula](#). First discovered spreading rapidly across eastern North America and currently affects seven species, causing a regional population collapse and is predicted to lead to regional extinction of the [little brown myotis \(Myotis lucifugus\)](#), previously one of the most common bat species in North America. Novel diseases can have serious impacts on [naive wildlife populations](#), which in turn can have substantial impacts on ecosystem integrity.

REPORT
[Emerging infectious diseases](#) are increasingly recognized as [direct and indirect agents of extinction](#) of free-ranging wildlife (1–4). [Introductions of disease into naive wildlife populations](#) have led to serious declines or [local extinctions](#) of different species in the past few decades, including amphibians from [chytridiomycosis](#) (5, 6), rabbits from [myxomatosis](#) in the United Kingdom (7), [Tasmanian devils](#) from infectious cancer (3), and birds in North America from [West Nile virus](#) (8). Here we demonstrate that [White-Nose Syndrome \(WNS\)](#), an emerging infectious disease, is causing unprecedented mortality among hibernating bats in eastern North America and has caused a population collapse that is [threatening regional extinction](#) of the little brown myotis (*Myotis lucifugus*), a once widespread and common bat species.

[WNS is associated with a newly described psychrophilic fungus \(Geomyces destructans\)](#) that grows on exposed tissues of hibernating bats, apparently causing premature arousals, aberrant behavior, and [premature loss of critical fat reserves](#) (9, 10) (Fig. 1). [The origin of WNS and its putative pathogen, G. destructans, is uncertain](#) (9). A plausible hypothesis for the origin of this disease in North America is [introduction via human trade or travel from Europe](#), based on recent evidence that *G. destructans* has been observed on at least one [hibernating bat species in Europe](#) (11). [Anthropogenic](#) spread of invasive pathogens in wildlife and domestic animal populations, so-called [pathogen pollution](#), poses substantial [threats to biodiversity and ecosystem integrity and is of major concern in conservation efforts](#) (1, 2).



Learning Lens

A species of bats:
http://www.mnh.si.edu/mna/image_info.cfm?species_id=199

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

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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.

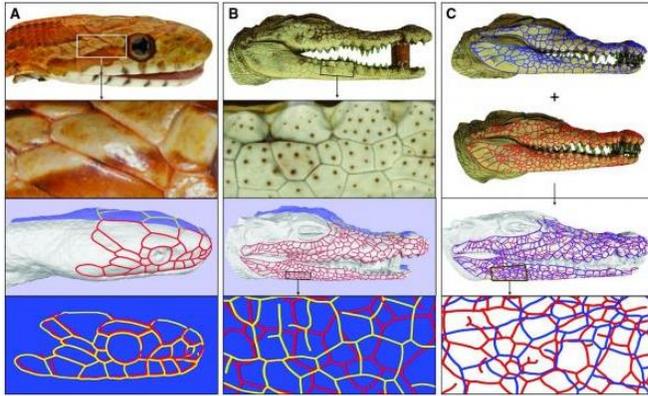


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). **(B)** Polygonal 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
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- 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.

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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

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.

flexible use of spatially precise

brain plasticity. *Front Neurosci* 4,

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.

The screenshot shows the 'Science in the Classroom' website interface. At the top left, the logo 'Science in the Classroom' is displayed with the tagline 'A collection of annotated research papers and accompanying teaching materials'. Below this, the 'Audience' is set to 'University' and the 'TOPIC' is 'Biological'. The main content area features a resource titled 'Lemmings: They're What's for Dinner' with a thumbnail image of a brain scan labeled 'Brain Disease'. The text includes an 'EDITOR'S INTRODUCTION' by Gilg et al. and an 'ABSTRACT' describing the study on lemming population dynamics in Greenland. On the right sidebar, a 'Thought Questions' section is highlighted with a green circle, containing six questions about the study's importance, objectives, conclusions, supporting evidence, and next steps. Below this is a 'Learning Lens' section with a category selector and a list of links: GLOSSARY, PREVIOUS WORK, AUTHOR'S EXPERIMENTS, CONCLUSIONS, and NEWS AND POLICY LINKS. A red navigation bar at the bottom contains links for Home, Download PDF, Related Science News, Paper Details, Questions?, Activities, Teaching Resources, and Contact Us.

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

This article is an excellent illustration of the additive and collaborative nature of science. Independent lines of research had shown that the gut microbiota could shape intestinal barrier permeability by regulating tight junctions, and that similar tight junctions affected BBB permeability. The authors combined these and other findings to produce the startling hypothesis that microorganisms in the digestive tract could shape the developing brain. Their work required expertise in both biological systems, and involved collaboration among many departments on three continents. In turn, the model put forth by this study will be used to form new hypotheses, and will be tested, refined, and expanded by future studies.

The importance of this scientific research

See Thought Question 2: This research impacts everyone, at a basic level: All human embryos develop a BBB, and this study suggests that the maternal gut microbiota may impact it meaningfully. Additionally, many diseases such as Alzheimer's disease, epilepsy, and multiple sclerosis involve increased BBB permeability, and so these results may eventually impact our understanding and treatment of those illnesses.

The actual science involved

The science forming the core of this study was simple: The BBB acts as a filter to prevent harmful molecules from passing from the bloodstream into the brain. The authors employed techniques that are straightforward and intuitive to understand, though they are technically sophisticated and challenging. Many of their assays involved the delivery of detectable molecules (Evans blue dye, raclopride, and the R4A antibody) that produced obvious signals indicating that the respective molecule had leaked through the BBB into the brain. The authors added to their understanding of those results by using Western blotting, which allowed them to determine the expression level of selected proteins. Next, the authors explored interventions like fecal transfer and colonization with single bacterial strains, measuring their success using these previously identified, straightforward, and easily interpretable indicators of impaired BBB function.

Connect to Learning Standards:

[The Next Generation Science Standards](#)

- Science and Engineering Practice 1: Asking questions for science. The authors ask the question: "Through what mechanism could the blood-brain barriers of germ-free mice become more permeable?" They rely on previous studies that have revealed that the permeability of blood vessels in the brain is partially determined by the status of tight junctions, structures that join endothelial cells together to form a barrier. This information forms the basis of their hypothesized explanation for the phenomenon, which is referred to as a "model." Sp.3: The authors plan to test their model that germ-free mice have abnormal tight junctions by examining the expression levels of several proteins that form the tight junctions.
- Science and Engineering Practice 2: Developing and using models. Students can be expected to evaluate and refine models through iterative cycles of comparing predictions with real-world results.
- Science and Engineering Practices 4 and 5: Analyzing and interpreting data and using mathematics and computational thinking.
 - The authors analyze their data to determine whether tight junction proteins are expressed at lower levels in germ-free mice.
 - First, they visually inspect bands representing the total amount of each protein: see for example, in Figure 4A, the four bands in the row marked "Occludin." The two bands in pathogen-free (PF) columns are notably darker than the two bands in germ-free (GF) columns, indicating a reduction in the amount of occludin protein in germ-free mice. They digitally analyze the gels to determine the intensity of the bands, then use a statistical analysis to determine that, indeed, occludin is downregulated in germ-free mice (see Figure 4D).
- Science and Engineering Practices 6, 7, and 8: Constructing explanations, engaging in argument from evidence, and obtaining, evaluating, and communicating information.
 - They synthesize the data collected throughout their study to construct an explanation for the blood-brain barrier (BBB) leakiness in germ-free mice: Their tight barriers are malformed because of reduced expression of key proteins like occludin.
 - They continue to argue that, based on their evidence, the gut microbiota regulates BBB development, likely through mechanisms involving the production of short chain fatty acids that promote tight junction formation.

They communicate their conclusions clearly, and outline a model that other research groups can test in their own future studies.

- Crosscutting Concept 4: Systems and System Models. Students can investigate or analyze a system by defining its boundaries and initial conditions, as well as its inputs and outputs. They can use models to simulate the flow of energy, matter, and interactions within and between systems at different scales.

[The Common Core English and Language Arts Standards](#)

- ELA-Literacy.RST.11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11 and 12 texts and topics
- ELA-Literacy.RST.11-12.8: Evaluate the hypothesis, 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.

Topics Covered:

See Thought Question 1: The authors are arguing that processes carried out by microorganisms living in the digestive tract can have a powerful impact on the formation and maintenance of the BBB. They support this novel idea through careful investigations of the brains of germ-free mice, and by improving the health of germ-free mice through treatment with microbes and molecules that restore, improve, or mimic the functions of healthy gut flora.

Why this research is important:

See: The importance of this research

Methods used in the Research:

See: The actual science involved above

Conclusions:

The authors found that the BBB of embryonic mice whose mothers were germ-free were more permeable than those carried by pathogen-free mice. They showed that these deficits lasted through adulthood in germ-free mice, and that a particular structural component of the BBB, tight junctions, was disrupted and may account for the increased BBB leakiness. They demonstrated that BBB function could be improved by introducing normal gut microbiota into adult germ-free mice, suggesting that BBB health can be restored long after development. Furthermore, they found that a specific class of molecules produced by the gut, SCFAs, were capable of decreasing BBB permeability to levels comparable to controls. Overall, these results suggest that gut flora shape the development and possibly the maintenance of the BBB, and that treatment with healthy gut microbiota or even with SCFAs may be an effective method for treating abnormal or deficient BBBs.

Areas of Further Study:

See Thought Question 4.

1. Future work should investigate the role of diet and gastrointestinal health of pregnant women in embryonic BBB development, and consider whether treatments to improve maternal gut flora health could positively impact the embryo.
2. Additional studies should assess whether existing protocols for administering antibiotics to pregnant women and young children can be improved in light of these findings.
3. Future studies should determine whether less severe manipulations of gut microbiota cause changes in BBB permeability.
4. Additional work should investigate the role of gut microbiota health in the myriad neurological diseases that involve BBB abnormalities.
5. More studies should be conducted to investigate possible treatments that can be employed to improve gut flora health and BBB function in mice and humans.

Resources for Interactive Engagement:

Discussion Questions

1. What is the “big picture” conclusion that the authors are trying to communicate? What do they hope that readers will take away from this article?
 - a. Possible answers:
 - i. The authors are arguing that processes carried out by microorganisms living in the digestive tract can have a powerful impact on the formation and maintenance of the BBB. They support this novel idea through careful investigations of the brains of germ-free mice, and by improving the health of germ-free mice through treatment with microbes and molecules that restore, improve, or mimic the functions of healthy gut flora.
2. Why is it important to understand the connection between gut microbiota and the BBB? For what human populations might research like this matter?
 - a. Possible answers:
 - i. This research impacts everyone, at a basic level: all human embryos develop a BBB, and this study suggests that the maternal gut microbiota may impact it meaningfully. Additionally, many diseases such as Alzheimer’s disease, epilepsy, and multiple sclerosis involve increased BBB permeability, and so these results may eventually impact our understanding and treatment of those illnesses.
3. How could the authors have improved this research? What are the limitations of the work?
 - a. Possible answers:
 - i. The authors used a wide array of methods to convincingly demonstrate the link between gut microbiota and the BBB. However, in their own words, “... the results should be interpreted cautiously until more physiological data are acquired to corroborate these findings.”
 - ii. The authors have shown that germ-free mice display abnormal BBB permeability and tight junction development. However, the entirely germ-free environment of the mice in this study is an extreme situation that is seldom, if ever, experienced by humans. Additional work will need to establish whether the results of this study apply to mice undergoing more typical stresses to their gut microbiota.

- iii. Additionally, the authors have shown that BBB permeability can be impacted by the gut microbiota, but have not demonstrated that these changes directly lead to specific negative health outcomes. It seems likely that the increased permeability of the BBB in germ-free mice to both dye and antibodies would have some negative consequence for brain development or function, but this remains to be shown. Furthermore, this will need to be redemonstrated for less extreme manipulations of gut microbiota, before the full impact of these findings can be understood.
 - iv. Finally, all studies utilizing mice suffer to a degree from uncertain generalizability: it is currently unknown whether the mechanisms uncovered in this research will apply to human development.
4. What are the next experiments that should be done to expand on this study?
- a. Possible answers:
 - i. Future work should investigate the role of diet and gastrointestinal health of pregnant women in embryonic BBB development, and consider whether treatments to improve maternal gut flora health could positively impact the embryo.
 - ii. Additional studies should assess whether existing protocols for administering antibiotics to pregnant women and young children can be improved in light of these findings.
 - iii. Future studies should determine whether less severe manipulations of gut microbiota cause changes in BBB permeability.
 - iv. Additional work should investigate the role of gut microbiota health in the myriad neurological diseases that involve BBB abnormalities.
 - v. More studies should be conducted to investigate possible treatments that can be employed to improve gut flora health and BBB function in mice and humans.