

Teacher Resource for:
**Optical Control of Muscle Function by Transplantation of Stem Cell–
Derived Motor Neurons 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.

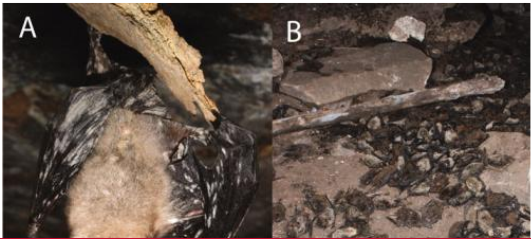
ABSTRACT

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, WNS is 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 naïve 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 naïve 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

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.

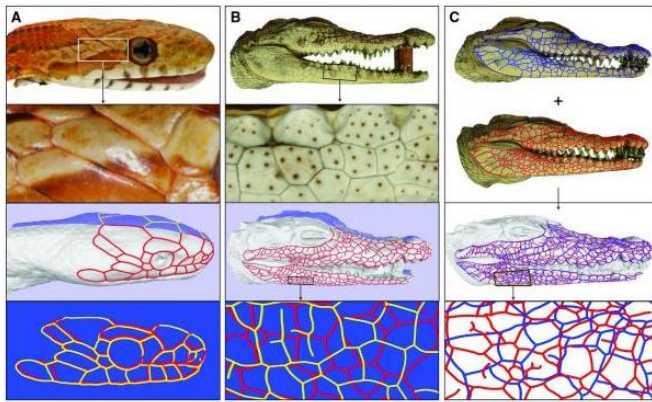


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). **(B)** Polygonal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

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- 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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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.

Science in the Classroom

A collection of annotated research papers and accompanying teaching materials

Audience High School **University** **Edit** TOPIC Biological

Lemmings: They're What's for Dinner



EDITOR'S INTRODUCTION
Cyclic Dynamics in a Simple Vertebrate Predator-Prey Community.
Gilg et al.

Scientific studies often involve more than one discipline. In this case of lemming population dynamics, scientists use both ecology-related methodology to collect data in Greenland, and mathematical equations to construct a predictive model. Similar to the cyclic dynamic described in this study, this interdisciplinary research would not have been complete if one of these two scientific disciplines had been missing.

annotated by Fanny Bernardon
original paper published 10/31/2003
annotations posted on 1/31/14

ABSTRACT
The collared lemming in the high-Arctic tundra in Greenland is preyed upon by four species of predators that show marked differences in the numbers of lemmings each consumes and in the dependence of their dynamics on lemming density. A predator-prey model based on the field-estimated predator responses robustly predicts 4-year periodicity in lemming dynamics, in agreement with long-term empirical data. There is no indication in the field that food or space limits lemming population growth, nor is there need in the model to consider those factors. The cyclic dynamics are driven by a 1-year delay in the numerical response of the stoat and stabilized by strongly density-dependent predation by the arctic fox, the snowy owl, and the long-tailed skua.

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THOUGHT QUESTIONS

1. Why is this study important?
2. What is the objective?
3. What are the conclusions?
4. What is the supporting evidence?
5. Are there any doubts that this conclusion is right?
6. What would you do next?

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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

Student Learning Goals:

Connections to the nature of science from the article

- Developing and using an animal model for human disease.
- Develop hypothesis based on previous works.
- Designing experiments to test hypotheses.
- Make predictions based on preliminary data.
- Validate novel experimental approach by comparison with established and previously described approach.
- Provide explanations to observations and data.
- Recognize challenges and limitations of an experimental model

The importance of this scientific research

- Combined optogenetics and regenerative medicine to validate a novel method to restore muscle function after nerve injury in a live animal.
- Established experimental protocol to generate ESC-derived ChR2 motor neurons for engraftment in the site of peripheral nerve injury.
- This is a proof-of-principle study with implications in clinical use of optogenetics in regenerative medicine.

The actual science involved

- Modeling muscle denervation
- Develop hypothesis based on previous optogenetics studies
- Validate novel method using in vitro experiments
- Design in vivo experiments based on in vitro studies
- Design methods for data collection
- Compare previous methods and established principles
- Test experimental approach using established parameters
- Demonstrate novel methods in vivo

Connect to Learning Standards:

Next Gen. Science Standards 8 practices (page 54)

http://www.nap.edu/openbook.php?record_id=13165&page=54

- Practice 1 - Asking questions and defining problems.
 - The authors asked two questions in this study, which are highlighted here.
- Practice 2: Developing and Using Models
 - The authors developed and used an animal model to study nerve damage in which a surgical procedure is performed to produce muscle denervation that resembles early-stage ALS in humans.
- Practice 3: Planning and carrying out investigations.
 - From this point forward in the report, the authors use a number of parameters to test muscle function to determine whether optical stimulation of engrafted ChR2 motor neurons generates comparably normal muscle contraction. Optical stimulation is compared to electrical stimulation (control) of the sciatic nerve in Figure 3.

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:

After nerve injury or neurodegenerative disease, patients suffer paralysis and loss of muscle mass due to limited neuron regeneration. Major obstacles to restoring muscle function include the ability to effectively replace damaged motor axons, make connections to muscles, and to generate normal muscle activity. In this report, scientists engineer light-sensitive motor neurons derived from embryonic stem cells and transplant these motor neurons into mice with nerve injury. By switching on a light, scientists can control these light-sensing motor neurons and restore muscle activity.

Topics Covered:

- Transgenic/transgene technology
- Embryonic stem cells
- Skeletal muscle physiology
- Neuromuscular physiology
- Motor neuron structure and function
- Modeling nerve damage and neurodegenerative disease
- Quantification of muscle contractile force
- Muscle contraction physiology
- Cell type identity

Why this Research is Important:

- Combined optogenetics and regenerative medicine to validate a novel method to restore muscle function after nerve injury in a live animal.
- Established experimental protocol to generate ESC-derived ChR2 motor neurons for engraftment in the site of peripheral nerve injury.
- This is a proof-of-principle study with implications in clinical use of optogenetics in regenerative medicine.

Methods used in the Research:

1. Transfection of ESCs with electroporation

Transfection is an experimental method to introduce DNA into cells, followed by expression of the gene(s) of the introduced DNA. Various transfection methods, conditions, and reagents can be used depending on the cell type. An example of transfection reagents can be found [here](#), along with an overview of transfection:

<http://www.lifetechnologies.com/us/en/home/life-science/protein-expression-and-analysis/transfection-selection/lipofectamine-3000.html>. For embryonic stem cells in this study, the authors used electroporation for transfection; an overview of transfection methods used in this study can be found here: <http://www.biorad.com/en-us/applications-technologies/transfection-stem-cells>.

2. Generation of stable ESC clones

Hb9::CD14-IRES-GFP transgenic ESCs were transfected, differentiated (directed to become motor neurons), and selected based on expressions of fluorescent proteins and chemical resistance. In other words, cells that stably express the transfected genes should show fluorescence and survive chemicals that would normally kill un-transfected cells.

3. Magnetic-activated cell sorting (MACS)

Magnetic-activated cell sorting (MACS) is used here to isolate population of cells from a larger heterogeneous population. The basic principles of MACS are summarized here: https://www.miltenyibiotec.com/en/products-and-services/macs-cell-separation/macs-technology/microbeads_dp.aspx. Video description of the MACS Microbeads used in this study and tutorials about the method can be found here:

http://www.miltenyi.tv/vsc_2318_1848_1_vid_62670/Hands-on-MACS-Technology.html.

4. Immunohistochemistry and immunocytochemistry

Immunohistochemistry and immunocytochemistry use the same principles to analyze tissue using a microscope. The two methods differ in sample source and processing, which can be found here:

<http://www.piercenet.com/method/immunohistochemistry-vs-immunocytochemistry>. The basic principles of immunohistochemistry are summarized in this video: <https://www.youtube.com/watch?v=HdBqTAAi3rU>.

5. Confocal microscopy

A microscope imaging technique used to examine live or fixed specimen labeled with fluorescence. The principles of confocal microscopy is detailed here:

<http://www.microscopyu.com/articles/confocal/confocalintrobasics.html>.

6. In vitro Electrophysiology

Recordings of motor neuron action potential, or nerve impulse and activity, were performed in cultured neurons. An overview and video of patch-clamp recordings of brain tissue and cultured cells can be found here: <http://www.leica-microsystems.com/science-lab/the-patch-clamp-technique/>.

7. Surgery

Sciatic nerve ligation and engraftment by injection of embryoid bodies
Sciatic nerve ligation is a common surgical procedure used in research to produce chronic constriction of the sciatic nerve in animal models for

investigations of pain mechanisms, nerve damage, and neurodegenerative disease. A video explanation of the procedure can be found here:

<http://www.jove.com/video/3393/chronic-constriction-sciatic-nerve-pain-hypersensitivity-testing>.

8. In vivo isometric muscle tension physiology

Isometric muscle contraction generates tension, or force, without changing the length of the muscle. This video explains the basic principles of isometric vs. isotonic muscle contraction: <https://www.youtube.com/watch?v=pbXML3m2hSE>. The experimental set-up for isometric muscle tension recordings performed in this study is shown in Movie S1, as part of the supplementary material in *Science*: <http://www.sciencemag.org/content/344/6179/94/suppl/DC1>.

Conclusions:

- Established method of generating ESC-derived ChR2 motor neurons suitable for engraftment and survival in peripheral nerve environment.
- ESC-derived ChR2 motor neurons functionally mature in 35 days in vitro and in vivo after engraftment. ChR2 motor neurons morphologically resemble and are functionally comparable, to normal, mature motor neurons.
- ESC-derived ChR2 motor neurons engrafted at the site of peripheral nerve injury can grow, extend axons, and reinnervate muscle fibers.
- Optical stimulation of engrafted ChR2 motor neurons effectively generates muscle contraction in a controlled manner with characteristics of normal muscle function.
- Optical stimulation of engrafted ChR2 motor neurons induced motor-unit recruitment in physiological order, which indicates graded muscle contraction.
- Successful engraftment and optical stimulation of ESC-derived ChR2 motor neurons implicate the restoration of muscle function after peripheral nerve injury.
- An animated summary of the results and proposed future applications of the techniques discussed in this paper, narrated by Barney Bryson, can be found here:
<https://www.dropbox.com/s/1ramx9vyk9f4f9b/Animation%20and%20Audio%20for%20Bryson%20et%20al..mp4>.

Areas of Further Study:

- Stem cell technology: directed differentiation of embryonic stem cells and implications for clinical therapies using stem cell technology.
- Muscle physiology: various types of skeletal muscle contraction and the molecular mechanisms of skeletal muscle function.
- Muscle cell types: compare characteristics of smooth muscle, skeletal muscle, and cardiac muscle.
- Skeletal Muscles: fast-twitch vs. slow-twitch muscle fibers

- Optogenetics with applications in central and peripheral nervous system function. The use of ChR2 in optogenetics research has been applied to the study of memory, neurological disorders, and brain function.

Resources for Interactive Engagement:

1. Discussion Questions

1. Can you give an example of tissue engraftment, implantation, or transplantation used in the clinical setting? What are some major challenges? How do we know when an implantation is successful?

Answer: Patients who receive organ transplants require immune suppression for the rest of their lives. Another major challenge is the failure of engrafted tissue to function normally in the recipient. If engraftment is successful, normal organ or tissue function should be restored.

2. The study demonstrates the principle of stimulating a specific population of neurons that respond to light. Can you think of another instance or context where stimulating a specific population of neurons would be useful?

Answer: Optical stimulation of ChR2 neurons, in transgenic animals or virally introduced, has allowed cell population-specific activation which improves our understanding of specific neuronal processes. Examples would be studies in memory formation, behavioral responses, etc.

3. Give an example of isometric muscle contraction (i.e. muscle contraction that generates force, but does not change the length of the muscle). Can you demonstrate this type of muscle contraction?

Answer: Isometric muscle contraction occurs, for example, when you carry groceries. Your bicep muscles generate force without shortening. In contrast, curling weights would cause shortening of the biceps while generating contractile force.

4. Why is it important to restore motor neuron function and muscle function after nerve injury?

Answer: After nerve injury, denervation of target muscles can cause irreversible loss of muscle function, decrease muscle use and consequently muscular dystrophy or loss of muscle mass. Restoration of motor neuron function after nerve injury may not fully restore muscle function, but importantly preserve and prevent additional loss of muscle function.