Real Science Review: Concussion

Boy: "I play football. Am I going to experience brain injuries like those being seen in some professional football players?"

Girl: I often hit the soccer ball with my head. Is that dangerous?"

Well, some scientists are asking questions like this too, maybe because they also played football or soccer in school. The scientists who study the brain know that concussion can have life-changing effects. That's why they are using research to help them find ways to reduce concussions and treat them when they happen. Cell biology is the place to start this research.

Researchers are trying to figure out what happens in neurons during concussion. If scientists understood the mechanisms, they may be able to develop drug treatments to reduce or compensate for the damage. An obvious place to start to study what happens to neurons in a concussion is with the proteins in cell membranes that link adjacent cells together. We should expect that the force of concussion would disrupt the linking of proteins that form the bridge between cells. Concussion could tear these "sticky" proteins and separate cells from each other.

Below is our revised version of an original research report that was approved by real peer reviewers (fellow scientists) before it was accepted for publication.

Original report: Hemphill MA, Cabiri BE, Gabriele S, Kerscher L, Franck C, Goss JA, et al. (2011) A Possible Role for Integrin Signaling in Diffuse Axonal Injury. PLoS ONE 6(7): e22899. https://doi.org/10.1371/journal.pone.0022899. Adapting author: W. R. Klemm

Vocabulary Used in the A Report

Axons, dendrites: neuron membrane extensions of the neuron cell membrane. Think of it like branches on a tree.

Concussion: temporary or permanent brain damage caused by a strong force that affects the head.

Integrin: a class of proteins anchored in cell membranes that help neurons stick together.

Intracellular: inside the cell

Extracellular: outside the cell

Synapses: junction points between neurons. These are patches of neuron membrane though which currents are most able to flow.

A Possible Role for Integrin Signaling in Axonal Injury

Abstract

Blows to the head can cause the brain damage known as concussion. Common causes of concussion include vicious football tackles or "head hits" in soccer, baseball pitches, boxing. Scientists are discovering what concussion does to nerve cells. The hypothesis of this study was that a key part of the response to concussion could be proteins that help neurons stick to each other. These proteins are called “integrins,” because they promote tissue integrity by helping cells stay attached to each other. Neurons need close contact to communicate with each other. To mimic the effect of concussion on neurons, the researchers used a bath of nutrient fluid to grow rat-brain neurons on an underlying plastic sheet. This sheet could be stretched, which mimicked concussion by stretching the neurons attached to it.

In a second series of tests, the researchers tested the damage caused by the force of magnetic micro-tweezers acting to pull neurons away from their point of attachment in the culture dish. These methods revealed that local damage was caused by breakage of the neuronal membrane “integrins.” In the study, the stretching and pulling damage was reduced by treating the neurons with a drug that helps make intracellular structural proteins more stable.

Why do this kind of study on cultured neurons? Why not just hit an animal in the head and see what damage is produced? The answer is that this is inhumane and not necessary to get the scientific questions. By using this cell culture method, they can study live neurons and get precise measures at the individual cell level.

Introduction

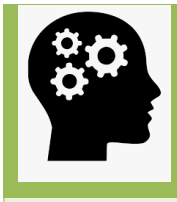
Previous studies had shown that the most frequent wound suffered by U.S. troops in Iraq and Afghanistan is blast-induced trauma. The force alone can damage the brain even when flying metal does not damage tissue. Brain scans (using magnetic resonance imaging or MRI) of blast victims indicate that blast force damages the large fiber tracts of nerve cell processes that connect different parts of the brain and spinal cord. Not much is known about how concussion affects the white matter or neurons at the individual cell level.

Introduction: Questions to Answer

1. If there was a hypothesis, either stated or implied, what was it?
2. How well did the authors justify doing this study?
3. What are some other related ideas that they did not test?

One unique way to study concussion would be to culture nerve cells and subject them to mechanical force, while observing what happens to certain cellular structures. The authors hypothesized that one target of blast injury could be the membrane-bound proteins that act like glue to hold the membranes of adjacent neuron cell bodies and processes together.

These proteins, known as "integrins," also extend through the plasma membrane and link to structural proteins inside the neuron. Concussion damage to integrins could therefore disrupt structures inside the neurons as well as outside. Altering internal structural proteins would disrupt cellular synthesis processes and ionic currents that underlie the electrical activity of neurons.



Think About It!

In your notebook, state:

- **Ideas related the questions on the Introduction. (As you move to other sections of the paper, make notes on the questions that you will want to develop in your final report).**
- **Note what integrins are and why they are important to issues involving concussion.**
- **Summarize the basic method and strategy for testing effects on integrins.**
- **State any ideas on other ways concussions could be studied scientifically.**

Methods

Researchers grew neurons placed on a plastic sheet in fluid containing nutrients and salts that kept the

Methods: Questions to Answer

1. What acts as a control group by receiving no treatment? What is the purpose for having this group and how well does it serve that purpose?
2. What factors (variables) that might affect the results are not considered?
3. What are the advantages and disadvantages of the procedures and equipment used?

neurons alive and well. An adhesive chemical was placed on the plastic sheet to help neurons stick to the sheet (yellow band in Figure 1). They also added a dye that would penetrate any points of the neuronal membranes that became porous because of breakage.

One force was to pull on (stretch) the plastic sheet, which also stretched the attached neurons. The researchers examined the neurons under a microscope to identify how much damage was done by various levels of stretching.

The processes (axons and dendrites) that extend out from neuron cell bodies contain numerous, tubules that can be damaged by physical forces, such as occur in concussion. The tubules can be very long. Think about how long these tubules must be in the neck of a giraffe. Simple diffusion would not be sufficient to transport chemicals along such great distances. Any distortion of the microtubules would disrupt transport.

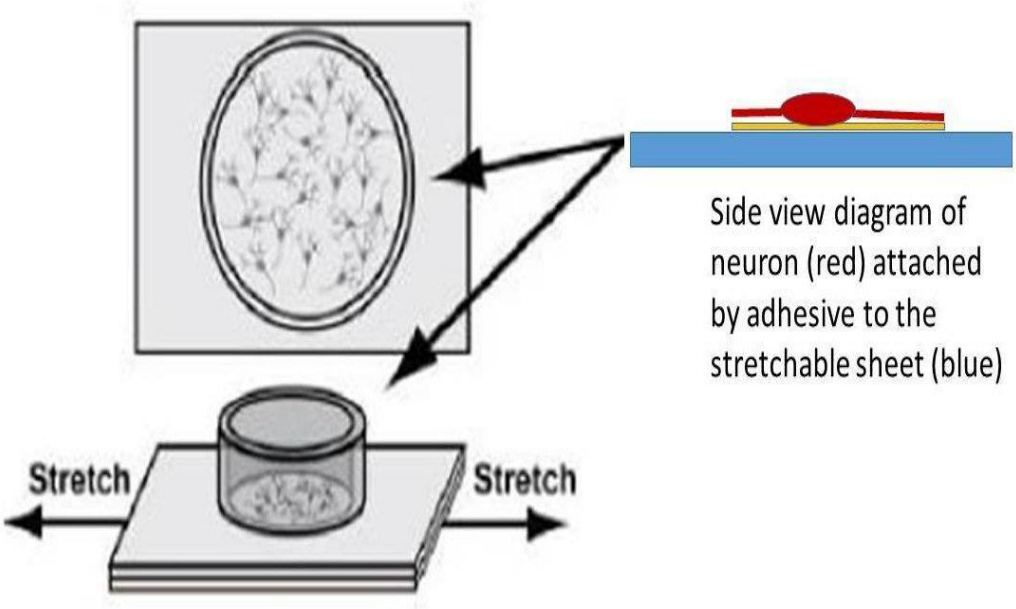


Figure. 1. To measure effects of stretching neurons attached to the plastic sheet, the researchers stretched the sheet in various degrees and then examined the neurons under a microscope to see how they were damaged.

In the second set of experiments, researchers used a different kind of force to evaluate damage in finer detail. To do this, they cultured neurons in a grooved chamber so that the ridges of the grooves would direct growth of the neurites along the lines of attachment. They bathed the neurons in a fluorescent reagent and magnetic beads that specifically bound membrane integrin to intracellular microtubules. Unlike most proteins in the cell membrane, integrins span the entire cell membrane. The reagent they used bound simultaneously to both microtubule protein and the integrin. The attached magnetic beads allowed the magnetic tweezers to pull on different parts of the neurons. The linkage of the fluorescent tag with both integrin and microtubules thus provides a way to see which parts of the neuron are most vulnerable to stretching. (Figure 2).

They used magnetic micro-tweezers to pull with various levels of force on various portions of neuronal membrane. Thus, if pulling on the neuron is damaging, it will break the bridge between tubules on the inside and the film on the outside. This breakage showed up as localized swelling.

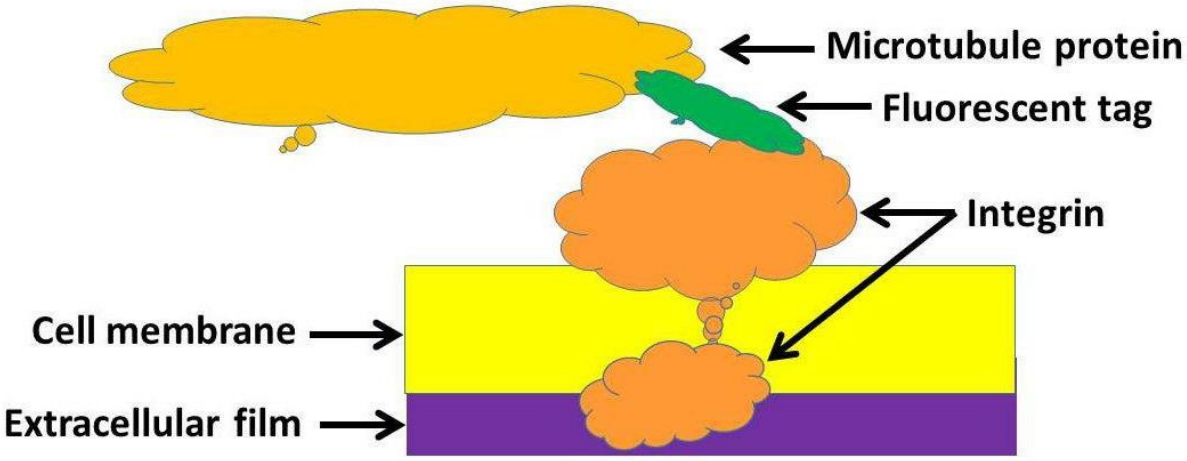


Figure 2. Membrane tagging technique to allow seeing which parts inside a neuron remain attached after being pulled on by micro-tweezers.

Finally, the researchers used this second system to conduct a preliminary study of a couple of compounds that, when added to their system, might reinforce integrins and make them more resistant to disruptive force.

Results

The first set of experiments showed that stretching breaks the contact of neurons with the plastic sheet. The amount of neuron damage was indicated by localized swelling and cell death. Degree of damage increased with increasing stretch. With increasing stretch some neurons actually died, while a large percentage of them became swollen (Figure 3).

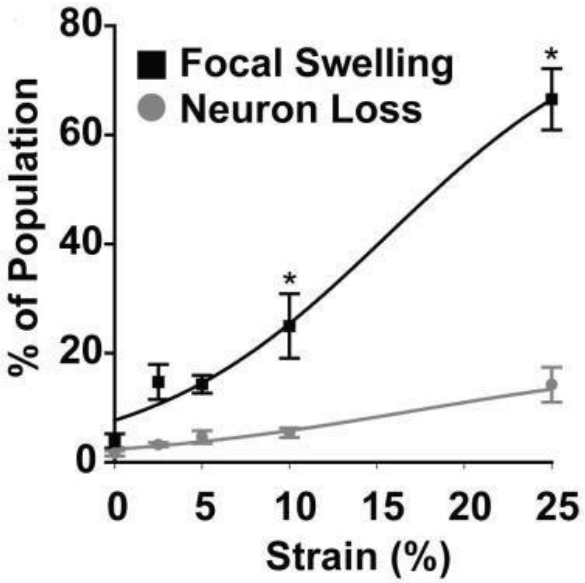


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Figure 3. Increasing the stretch (Strain % on the x axis) increased the percentage of damaged neurons, as indicated by swelling and cell death. (The little “T” above the bars represents the “standard error” variation around the mean of all tests. Bars labeled with * indicate that the odds that this difference could have occurred by chance alone is less than 5%.).

When a dye was added that would enter neurons at points where breakage caused

neurons to be porous, a sudden breakaway point was seen between 25% and 40% stretch (Figure 4).

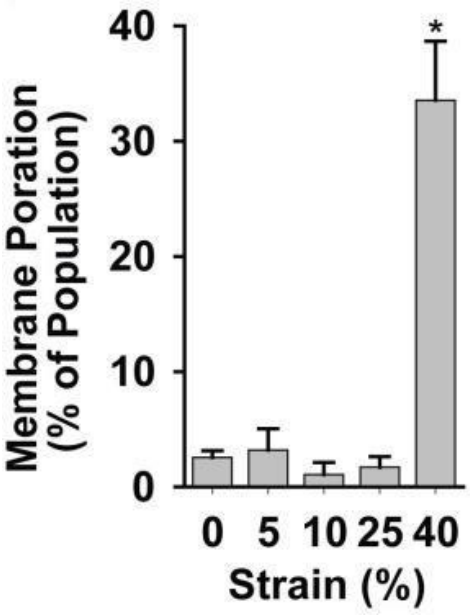


Figure 4. Effects of different degrees of stretch on the percentage of neurons exhibiting signs of membrane breakage as indicated by the extracellular dye entering the neurons (poration). A sudden breakaway response occurs at 40% of maximum strain.

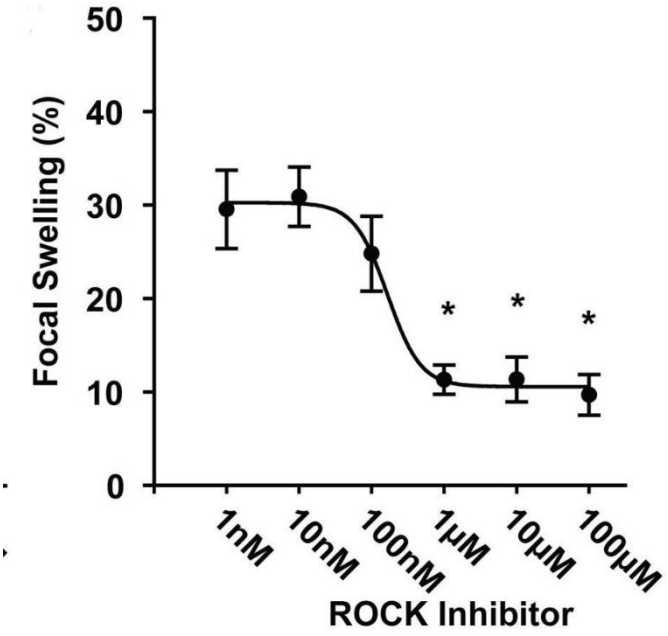
Damage, even if applied in only one spot, spread throughout the cell as a result of the disturbance to the integrin.

Finally, using the first method of longitudinal stretching, the researchers tested the effect of adding to the tissue bath a compound (referred to as a ROCK inhibitor) known to protect neurotubules from disruption. When added to the cell bath in this stretch system immediately after a stretch, this compound caused a dose-dependent decrease in the percentage of neurons that had local swelling after stretching (Figure 5). This apparent protection occurred with both 5% and 10% stretch (Figure 6).

Results: Questions to Answer

1. Do the results support the hypothesis or not? How convincing is that support?
2. Do you notice anything of possible importance in the data that authors failed to mention?
3. Is the variation in data large enough to suggest that some unknown variables interfere with reliable results? What might these be?
4. How big is the ‘treatment’ effect? Is it large enough to be of much practical importance?

Figure 5. Adding an integrin-stabilizing compound (ROCK inhibitor) to the tissue bath provided a dose-dependent protective effect against disruptive force.



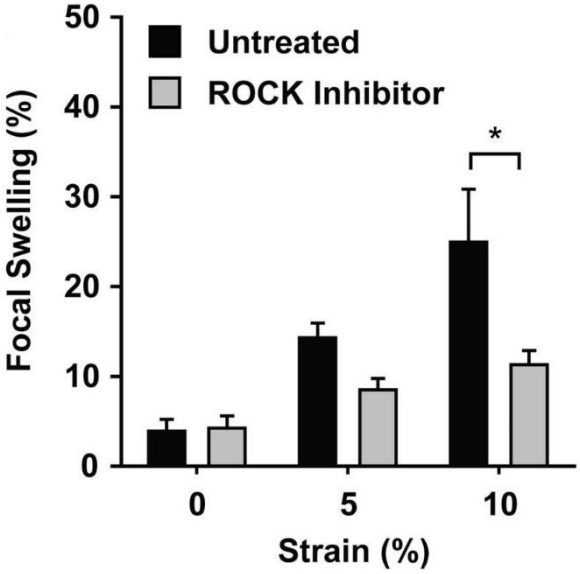


Figure 6. The strain protection occurred against both 5% and 10% strain magnitude. The bracket with the * indicates that the difference at the 10% strain was a statistically reliable indicator of the chemical's protective effect.

Discussion

The authors believe that the experiments tested the effects of mechanical stretching on neuronal membrane integrins and the extracellular binding of the membranes to the intracellular protein structures. Even stretching too weak to cause tears in the neuronal membrane disrupted integrins and their coupling to the proteins of neurotubule proteins.

The authors think these findings are especially significant, because others have shown integrins to be crucial to normal cell function at synapses, in growth of axons and dendrites, and even for memory (which is stored in changes in synapse structure).

The forces that cause concussion are likely to produce stretching and tearing like that in these experiments. Therefore, the authors believe that these results indicate what happens to neuron membranes and neurotubules during concussion. The data indicated that local damage often spreads through wide areas inside the neuron because of the way integrins link to other intracellular structural proteins. The authors cite studies by others who used different methods and still arrived at

similar conclusions.

Discussion: Questions to Answer

1. Summarize how the authors discussed the results in terms of their original hypothesis.
2. Did the authors point out ideas that go beyond the hypothesis?
3. What ideas for future research did the authors generate?
4. What ideas for future research do you generate?
5. How would you state the "so what" or take-home lesson?

The authors noted that they were able to reduce damage to neurites with a ROCK inhibitor. These studies suggest that concussion might cause damage by way of an integrin-mediated signaling cascade involving a ROCK-mediated pathway. Thus, these key chemical systems may point to possible targets for drug development that might prevent or reduce concussion damage.