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Signaling by Neuronal Swelling

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Abstract

Several physical phenomena accompany the firing of electrical impulses by axons. Some of these, such as the microscopic swelling of axons, alter the transmission of light through axons. This produces what are called “intrinsic optical signals” because optical methods can be used to see axons fire without adding voltage-sensitive dyes or using electronic amplifiers. These physical changes allow neurons to communicate through nonsynaptic signals to adjacent cells, such as other neurons or glia. Two of the three videos in this Teaching Resource show the optical manifestations of the microscopic swelling of axons that accompanies the firing of action potentials in cultured neurons, and one shows the nonsynaptic release of ATP that occurs through membrane channels that are stimulated by neuronal swelling.

Keywords

single photon; luciferase; neuron; microscope; imaging; movie

Description

Electrical excitation of nerve axons also produces many other effects (1–3), including rapid heating and cooling of the axon (3–6), volume changes in the axon (6–14), and changes in the optical properties of the axon that alter the transmission of light, its polarization, and scattering (15–22). This set of movies shows optical changes, and physical displacement of axons that occur when neurons fire action potentials, as well as a nonsynaptic mechanism of signaling that occurs as a response to the physical changes: the release of the neurotransmitter ATP through membrane channels. The physical changes can be observed with optical methods without requiring the use of dyes and thus are called “intrinsic optical signals” (1, 23–25). One of these changes is axonal swelling, which can trigger the nonsynaptic release of adenosine triphosphate (ATP) (1), a neurotransmitter and cell-cell signaling molecule (26). This released ATP was detected by adding an enzyme to the culture medium that “lights up” in the presence of ATP. These movies are intended as educational tools that may be useful in a neuroscience course or in a course focused on imaging techniques or lectures about cellular communication or neuronal signaling.

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Educational Details

Learning Resource Type: Video

Context: Undergraduate upper division, graduate, professional (degree program)

Intended Users: Teacher, learner

Intended Educational Use: Learn, research, teach

Discipline: Neuroscience

Shown in Fig. 1A is a standard transmitted light microscope image of mouse dorsal root ganglion (DRG) axons grown in cell cultures that were equipped with electrodes for stimulating action potentials in the axons. Changes in light transmission through the axons can be seen by subtracting a reference image (one frame of a time-lapse movie before stimulation) from all the other images in the video sequence. Movie 1 shows an increase in the transmission of light through the axons after they are stimulated to fire impulses (Fig. 1B). The increased transmission of light is caused by microscopic swelling of the axons, which reduces light scattering through them. The increase in light transmission is shown as a pseudocolored image, with warmer colors indicating greater light transmission and thus axonal swelling. Movie 2 shows microscopic displacement, another visual manifestation that is also indicative of microscopic axonal swelling (Fig. 2). Such changes can be seen in many types of nerves and nerve tissue, including retina (27, 28), olfactory bulbs (6, 29), dorsal root ganglia (11), invertebrate axons (8, 14, 22, 30, 31), mammalian neurohypophysis (21, 32), and frog spinal cord (33). The displacement results from small volume changes in the axons that are caused by the transmembrane flux of ions and water, which accompany membrane polarization and depolarization. This movie was captured from a neuron stimulated with trains of impulses, which cause accumulated swelling, but by using more sensitive methods, the rapid swelling and shrinking of axons during a single electrical impulse can be seen (9, 30, 32, 34–36). After cessation of action potential firing, the axon ultimately returns to the original position after axon volume recovers. Channels in the axonal membrane open to release water to restore the normal cell volume. ATP and other neurotransmitters pass out of the axon through these open channels to signal other cells and neurons without synapses (1).

The release of ATP from neurons through the volume-activated chloride channels activated in response to axonal swelling can be seen with single-photon imaging (1). This type of signaling is distinct from the synaptic signaling that occurs at the nerve terminal and does not involve vesicular release that characterizes synaptic neurotransmitter release. In Movie 3, single-photon imaging was used to detect the release of ATP. ATP was detected by adding the firefly proteins (luciferase and Luciferin) that generate light in the tail of a firefly to cultures of mouse sensory neurons (37). The reaction that generates light requires ATP, so that when ATP is released from a neuron when it fires electrical impulses, a flash of photons is seen (Fig. 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Images of mouse DRG axons grown in cell cultures equipped with electrodes for stimulating action potentials in axons. **(A)** Transmitted light microscope image. **(B)** High magnification image of microscopic swelling of axons of neurons firing electrical impulses. (B) is a still image from Movie 1 (<http://stke.sciencemag.org/cgi/content/full/4/155/tr1/DC1>). Increased light transmission, showing axon swelling, is displayed as warmer colors. Changes in light transmission through these axons were revealed by subtracting a reference image, one frame of the video sequence captured before electrical stimulation, from the other frames in the time-lapse video. Scale bar, 10 μm .

Fig. 2.

High-magnification images of an axon of a cultured neuron stimulated to fire electrical impulses. These still images are from Movie 2 (<http://stke.sciencemag.org/cgi/content/full/4/155/tr1/DC1>). The images appear fuzzy because the high magnification is near the limit of resolution of the light microscope and the pixel density of the video camera. In the movie, the axon displaces approximately 0.5 μm . The red dot and arrow in the still images serve as a reference mark to help visualize the displacement. **(A)** A still image from the beginning of the movie before stimulation. **(B)** An image taken after the axon has been stimulated to fire.

Fig. 3. Single-photon image of ATP released from cultured neurons, from Movie 3 (<http://stke.sciencemag.org/cgi/content/full/4/155/tr1/DC1>). The red spots are photons released by luciferase and luciferin, which was added to the culture medium, in response to ATP.