AMAZING PAPERS IN NEUROSCIENCE
Getting Graded: Teaching Principles of Chemical Synaptic Transmission Without Action Potentials

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The study of synaptic transmission is foundational for undergraduate neuroscience students. Chemical synaptic transmitter release is usually presented as evoked by action potentials, but it can also be caused by subthreshold, 'graded' changes in presynaptic membrane potential. In a 1980 publication in the *Proceedings of the National Academy of Sciences*, Graubard and colleagues measured synaptic activity in the crustacean stomatogastric ganglion; they found that postsynaptic voltage changes occur in response to both action potentials and to graded depolarizations in presynaptic neurons. This was one of the first papers to clearly demonstrate that both graded and spike-mediated chemical synaptic transmission could occur concurrently at an identified synapse. Discussion of this work in undergraduate classrooms can encourage students to think in greater depth about the diversity of transmission mechanisms available to neurons.

Key words: action potential; graded chemical synaptic transmission; stomatogastric ganglion; electrophysiology

Chemical synaptic transmission between neurons requires the release of neurotransmitter molecules from a presynaptic neuron into a synaptic cleft. Once released, neurotransmitters bind to receptors on the membranes of postsynaptic neurons. Action-potential-evoked release is common within the nervous system. In this mode, an action potential is generated and travels along the axon to the presynaptic terminal. At the presynaptic terminal, it causes a brief depolarization, which results in the opening of voltage-gated calcium channels. The resulting influx of calcium into the presynaptic terminal triggers fusion of neurotransmitter vesicles to the presynaptic membrane so that neurotransmitter can be released (Siegel and Sapru, 2015).

Graded chemical synaptic transmission is an alternative mode in which transmitter release is dependent on presynaptic membrane fluctuations, even when an action potential does not occur in the presynaptic neuron. In 1980, Graubard, Raper and Hartline published a paper in the *Proceedings of The National Academy of Sciences* that was part of the early work using electrophysiology techniques to explore graded chemical synaptic transmission in the stomatogastric ganglion of the lobster, *Panulirus interruptus*. The paper offers a unique opportunity for educators, as it demonstrates graded chemical synaptic transmission in direct comparison to the action-potential-evoked transmission that occurs between the same neurons, so students gain an understanding of how to measure both types of chemical synaptic transmission.

In comparison to action-potential-evoked transmission, which is taught in detail to undergraduate neuroscience students, graded chemical synaptic transmission is often not thoroughly discussed as a pervasive mechanism. Graded synaptic transmission is common in a variety of neural networks, including the retina (Werblin and Dowling, 1969), the crustacean stomatogastric ganglion (Graubard et al., 1980), and the olfactory bulb (Castro and Urban, 2009). Therefore, teaching this type of synaptic transmission to undergraduate neuroscience students can expand understanding of the diversity of synaptic transmission mechanisms.

RESEARCH SUMMARY

Graubard et al. (1980) used intracellular recordings of the motor neurons of the pyloric network, a central pattern generator network that is part of the crustacean stomatogastric ganglion. In this network, a triphasic motor pattern is generated by synaptic connections (Marder and Bucher, 2007) between a single pacemaker interneuron (anterior burster, AB) and three motor neurons (pyloric, PY; lateral pyloric, LP; and pyloric dilator, PD).

Graubard et al. (1980) demonstrated that at chemical inhibitory synapses in the pyloric network of the lobster stomatogastric ganglion, the same neurons that release neurotransmitter in response to action potentials also do so in response to subthreshold, graded voltages. They showed that both mechanisms of release could happen together and occurred as part of the standard communication between the network neurons. They compared two recordings of depolarization in a presynaptic neuron and resulting inhibitory postsynaptic potentials (IPSPs) in a postsynaptic neuron. For both recordings, the experimenters used the same depolarizing presynaptic current, which hovered near the threshold of action potential generation, sometimes eliciting an action potential and other times not. Comparison of the presynaptic recordings show the same initial depolarization, resulting in identical IPSPs. After a maintained depolarization, one presynaptic recording showed a small action potential occurring, while the other did not. The corresponding postsynaptic recording showed a small increase in the IPSP amplitude occurring at the time of the presynaptic spike, not seen in the other recording where there was no presynaptic spike. The IPSP amplitude increase was clearly seen in response to the action

potential, while the initial IPSP was in response to the graded neurotransmitter release that occurred before the action potential. These recordings showed that although a presynaptic action potential evoked a response in the postsynaptic neuron, the graded depolarizations of the presynaptic cell actually resulted in a larger hyperpolarization in the postsynaptic cell than the action potential did.

Results from further recordings also showed that neurotransmitter was being released from the presynaptic neuron even at relatively hyperpolarized 'resting' membrane potential (-55mV). This observation has important implications; it means that information is being transmitted at these types of synapses over a wide range of presynaptic voltages, which in turn enables fine tuning of postsynaptic responses.

VALUE

Graubard et al. (1980) provides an introduction for undergraduate neuroscience students to a more detailed understanding of graded chemical synaptic transmission. It is not the first paper to demonstrate graded chemical synaptic transmission; however, it is short, easily accessible, and has figures that provide a clear illustration of key findings. The paper also highlights the advantages of using small, well understood invertebrate neural networks. Because the stomatogastric ganglion is a system of only roughly 30 neurons, with a defined connectome, it is an ideal place to study principles of basic synaptic physiology and neuromodulation. Understanding these principles is critical as they fundamentally govern how neurons communicate with one another.

At the conclusion of their paper, Graubard et al. (1980) discuss the neuronal qualities that support graded transmission at chemical synapses. They argue that key features are: 1) closely situated sites for input and output; and 2) a transmitter release threshold that is suitably different from the threshold for action-potential-evoked transmission. In their study, each neurite was both receiving and releasing neurotransmitter, as opposed to classic neuronal polarization, as seen in the vertebrate motor neuron for example, with release sites at axon terminals, and input sites at dendrites and the soma. Input and output sites must be close together as graded signals will diminish with distance, unlike action potentials which are regenerative. While these neurons may not have typical polarity of neurite function, Graubard et al. (1980) point out that the stomatogastric neurons are representative of other neuron types. For example, they share a structural similarity with vertebrate neurons found in close packed systems that have dendrodendritic synapses in addition to standard axon-dendrite synapses, such as olfactory bulb cells (Castro and Urban, 2009). Therefore, if these structurally similar cells also have the appropriate release threshold, they conclude that their findings could represent a "common and important" neuronal mechanism.

This conclusion to the paper lends readily to discussion exploring whether graded transmission can be said to be a common and/or important mode of neuronal computation. Neurons in the stomatogastric ganglion produce both spike-evoked and graded transmission, but graded synaptic transmission is the only means of transmission at chemical synapses for rod and cone photoreceptors in the retina (Siegel and Sapru, 2015). These photoreceptors are primary sensory neurons that signal onto bipolar cells. Neuroscience textbooks such as *Essential Neuroscience* (Siegel and Sapru, 2015) can be used to facilitate discussion on how the cells of the retina compare to those of the stomatogastric ganglion. This textbook describes the response of rods and cones to light as graded membrane responses, and that their release of glutamate is constant in the absence of light. Reading Graubard et al. (1980) gives more depth to this explanation. Stomatogastric ganglion cells, like the cells in the retina, are continuously releasing neurotransmitter due to voltage fluctuations, except when hyperpolarized. However, while neurons of the stomatogastric ganglion have neurites that are both presynaptic and postsynaptic, the photoreceptors of the retina absorb light at their outer segments and release neurotransmitter from their axon terminals. Comparison of these two systems provides a platform for discussing multiple aspects of synaptic transmission.

Graubard et al. (1980) may also help students engage with even earlier papers on graded synaptic transmission. Some of the first studies to discover graded synaptic transmission in neurons include Werblin & Dowling (1969), who studied the retina of the vertebrate mudpuppy. Although their paper is much longer and more extensive than Graubard et al. (1980), highly motivated students could gain further insight from Werblin and Dowling (1969) into how graded synaptic transmission was first discovered in the vertebrate retina.

Graded transmission is sometimes thought of as less common than action-potential-evoked transmission, but this may not be the case. Roberts and Bush, in their 1981 book *Neurones Without Impulses* provide a compilation of early work showing how common this type of signaling is in nervous systems. Examples of graded synaptic transmission continue to be documented in more recent work. For example, Castro and Urban (2009) discovered that mitral cells in the olfactory bulb of the mouse release glutamate at dendrodendritic synapses in response to both graded signals and action potentials. They suggest this provides neurons with options for qualitatively different outputs. Additionally, neurons in other sensory systems, such as in the vertebrate hair cells in the inner ear, also use graded chemical synaptic transmission (reviewed in Juusola et al., 1996). Graubard et al. (1980) is an excellent introduction to this modern work and can be used for comparison and discussion of the prevalence of graded chemical synaptic transmission.

Pinel and Barnes (2018) in their textbook *Biopsychology* remark that an understanding of some of the exceptional abilities of some cerebral neurons, with graded chemical synaptic transmission being only one example, can help students put their essential learning of the foundations of neuronal signal transmission in a wider perspective, and appreciate the remarkable diversity of cerebral neurons. Graubard et al. (1980) can provide an ideal platform to discuss the importance of graded and action-potential-

evoked transmission acting separately or in concert at synapses.

AUDIENCE

After students have been taught principles of action-potential-evoked synaptic transmission, Graubard et al. (1980) can be taught to 3rd and 4th year undergraduates to solidify the difference between action-potential-evoked transmission and graded transmission. The methodology will already be familiar to students who have learned about chemical synaptic transmission and the use of electrophysiology to study action potentials. This paper could also be taught in a final year neuroscience course on synaptic transmission, where students who have long understood the standard functioning of action-potential-evoked chemical synaptic transmission will have the opportunity to explore an interesting alternative. To provide students with background information on the mechanisms of the stomatogastric ganglion in the lobster there is a useful video on YouTube (iBiology, 2013) that teaches these mechanisms including the neuronal circuits described in Graubard et al. (1980) and compliments the learning from their paper.

Multiple student activities could be planned around Graubard et al. (1980). For example, students could discuss in groups which of the figures they think are most useful for clearly depicting the findings of the paper and explain their reasoning to the class. Some of the relevant findings of the more complex Werblin and Dowling (1969) and Castro and Urban (2009) papers could be explained in lecture or tutorial by the instructor and used to build discussion points on synaptic transmission and where current research investigating graded transmission could lead. The relevant sections of textbook chapters, for example Siegel and Sapru (2015) Chapter 15 on the visual system can be taught in class or given as reading for class preparation. Finally, Graubard et al.'s (1980) paper and the resulting discussions can supplement understanding of the importance and significance of action potentials, and help students understand when graded transmission could actually be more advantageous than action-potential-evoked transmission.

CONCLUSION

Graubard et al. (1980) discovered that spiking neurons in the pyloric network of the lobster stomatogastric ganglion also inhibit each other through increases and decreases in presynaptic voltage that occur at subthreshold levels. The constant release of neurotransmitter from these neurons is increased or decreased as a function of these presynaptic voltage changes and results in corresponding graded changes in voltage in the postsynaptic cell. This graded synaptic transmission had a consequential impact on the circuit and was important for establishing the rhythmic pattern of this motor system. Graubard et al. (1980) not only presents important research but also provides an excellent teaching tool for undergraduate neuroscience students to expand their understanding of a prevalent but often overlooked mechanism of chemical neurotransmission.

REFERENCES

Castro JB, Urban NN (2009) subthreshold glutamate release from mitral cell dendrites. J Neurosci 29:7023-7030.

Graubard K, Raper J, Hartline D (1980) Graded synaptic transmission between spiking neurons. Proc Natl Acad Sci U S A 77(6):3733-3735.

iBiology (2013) Eve Marder (Brandeis University) Part 1: introduction to central pattern generators. YouTube, January 17, Available at https://www.youtube.com/watch?v=dGRQmndrUzA.

Juusola M, French AS, Uusitalo RO, Weckström M (1996) Information processing by graded-potential transmission through tonically active synapses. Trends Neurosci 19(7):292-297.

Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu Rev Physiol 69:291-316.

Pinel J, Barnes S (2018) Biopsychology. 10th edition. London, UK: Pearson Education.

Roberts A, Bush BMH, eds (1981) Neurones without impulses. Cambridge, MA: Cambridge University Press.

Siegel A, Sapru H (2015) Essential Neuroscience 3rd edition. Philadelphia, PA: Wolters Kluwer.

Werblin F, Dowling J (1969) Organization of the retina of the mudpuppy, *Necturus maculosus.* II. intracellular recording. J Neurophysiology 32(3):339-355.

Received July 15, 2020; accepted August 18, 2020.

The author thanks Dr. Stefan Pulver, Dr. Bruce Johnson, and the University of St Andrews' MRes Neuroscience class of '20 for sharing ideas and providing feedback on the manuscript.

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