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AMAZING PAPERS IN NEUROSCIENCE
Teaching Synaptic Transmission Using Primary Literature: A Skills-Focused Pedagogical Approach

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Neuroscience is a burgeoning and intensive undergraduate major at many institutions of higher education and several areas in neuroscience education need further development. One such needed development is an increased focus on the procurement of career-relevant skills in addition to the traditional acquisition of subject knowledge. Skill development is particularly challenging in neuroscience education as the subject's interdisciplinary nature provides an atypically broad range of potential careers for graduates. Skills common to many careers in neuroscience include the ability to understand and analyze quantitative data and to draw conclusions based on those analyses. Here is presented an active learning pedagogical approach involving the analysis of seminal articles in the primary scientific literature to provide practice in analyzing data and drawing conclusions from those data while at the same time learning the fundamental tenets of synaptic transmission. Articles were selected that highlight principles such as the role of Ca^{2+} in synaptic release, exocytosis, quantal release, and synaptic delay. Figures from these articles that can readily be used to teach these principles were selected, and questions that can help to guide students' analysis of the data are also suggested. Activities like this are needed in greater numbers to facilitate the process of helping students gain skills relevant to a productive career in neuroscience.

Key words: active learning, neuroscience education; primary literature; skills-based learning; backward design

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Lecturing is one of the oldest known forms of education and is still widely used today. In recent years, many have vilified the lecture method of instruction but it remains an effective and popular way of communicating ideas and information, including among those who are antagonistic toward the practice (Friesen, 2011). The modern professional landscape, however, is rapidly changing and has significantly different needs than what existed even a few years ago, including an increasing need for graduates with transferable skills in addition to subject matter knowledge. A recent survey found that 53% of graduates from traditional degree programs had opted to not apply for an entry-level job in their field because they felt underqualified (Cengage Group, 2022). Only 41% of those surveyed felt that a college degree signaled to employers the skills they had to offer. From the other side of the fence, employers also felt that graduates should be better prepared with the skills they need to be successful in the workforce (Hart Research Associates, 2010; Manyika et al., 2011). Historically, there has been a persistent increase in the demand for college degrees (Fuller et al., 2017). Employers, however, are shifting toward skills-based hiring, and there is a trend toward dropping degree requirements for many mid-level and even some high-level positions (Fuller et al., 2022). Amid these recent changes, it is evident that in order to maintain relevance for student populations, institutions of higher learning need to re-center their educational programs on helping students to develop career-ready skills. Failing to compensate for this changing educational climate could impact the nation's ability to stay competitive, which could affect employability and economic prosperity (Oliveri and Markle, 2017).

If a shift toward skill development is needed, then the first question to address is, "What skills should be emphasized?" In general, employers are interested in graduates with significant skills in communication (oral and written), teamwork, collaboration, professionalism, work ethic, critical thinking, problem-solving, and creativity (Casner-Lotto and Berrington, 2006; Markle et al., 2013). Graduates of neuroscience programs, however, are employed in a wide variety of positions, and consequently the institutions of higher learning that educate these students are responsible for equipping them with the skills and tools needed to be successful in a broad range of careers (Akil et al., 2016). For example, coding and data analysis skills are used by many neuroscience professionals in academic and industry research and are predictive of higher salaries (Shah and Juavinett, 2021). Shifting the focus from acquisition of knowledge to developing career skills, however, can be difficult since after identifying the appropriate skills to target, the program must then figure out how to effectively teach and assess those skills, neither of which is trivial (Oliveri and Markle, 2017). It may be beneficial in these circumstances to take observations from career-focused programs that already do this well, such as certain programs in industrial design, computer programming, or trade school programs such as welding.

Many of the skills that graduates, and in particular neuroscience graduates, are going to need are not easily obtained through a traditional pedagogical model. As such, an active learning paradigm would be more appropriate for this type of learning. Active learning in which students are led to construct their own learning framework has also been shown to lead to improved grades and lower rates of failure provided the instructor has sufficient skill to be effective in using the pedagogical technique (Andrews et al., 2011; Freeman et al., 2014). Additionally, significant work has been done demonstrating various benefits of using primary scientific literature in STEM classrooms, including improvements in understanding research questions and hypotheses, interpreting figures and experimental results, and drawing conclusions from data (Goudsouzian and Hsu, 2023). The approach discussed here is designed to help neuroscience students develop skills with broad appeal to a variety of industries through an active learning model utilizing primary scientific literature. The goal is to provide practice in data analysis, teamwork, problem-solving, and communication.

To accomplish these objectives, we present five seminal articles in the topic of synaptic transmission that can be used to teach the fundamentals of this subject while reaping the benefits of active learning and the utilization of primary scientific literature in the classroom. These articles are presented in order of conceptual flow rather than in chronological order. Specific figures from the articles that best lend themselves to classroom analysis are also highlighted, and potential questions for the students to discuss in groups or in a class-level discussion on the subject are suggested. Ideas for how to implement this approach are also addressed. These articles are examples of a larger base of primary literature that could be utilized to teach the fundamental principles of neuroscience in the undergraduate neuroscience classroom with an enhanced focus on the development of skills relevant to a career in neuroscience.

ROLE OF Ca^{2+} IN VESICLE RELEASE

Considering the conceptual flow of synaptic transmission, Ca^{2+} influx into the presynaptic terminal precedes synaptic release. Therefore, prior to addressing the topic of synaptic release, it may be prudent to help students gain an understanding of action potentials and action potential propagation prior to beginning this exercise since these are the processes that immediately precede and lead to Ca^{2+} influx and synaptic release. These points can, therefore, be used as a starting point for this discussion.

The release of neurotransmitters is initiated by membrane depolarization. This depolarization causes an influx of Ca^{2+} ions through voltage-gated Ca^{2+} channels. A landmark article implicating a role for Ca^{2+} in synaptic transmission was published by Katz and Miledi in 1967 (Katz and Miledi, 1967). They used a preparation of a frog neuromuscular junction paralyzed by tetrodotoxin and kept it in a low Ca^{2+} solution. They pulsed $CaCl_2$ by iontophoresis onto the preparation immediately before and after a depolarizing stimulus and then recorded the end plate potential (EPP) that resulted. They found that there was a narrow window of time during which the $CaCl_2$ pulse was able to facilitate an EPP. This window immediately preceded the application of the excitatory stimulus, suggesting that Ca^{2+} was required for the EPP to be realized.

Figures 1 and 2 are effectively identical for our purposes. They both illustrate in the form of sample traces that the $CaCl_2$ pulse must come immediately before the excitatory stimulus in order for an EPP to be observed. Either figure may be used to illustrate this. Questions for the students to consider could include, “What is iontophoresis?”, “What effect does calcium have on the depolarization event?”, and “What significance does the timing of the pulses have?”

Figures 3 and 4 illustrate in histogram form the difference in total number of EPPs recorded between two groups, namely the events where the excitatory stimulus was preceded by a Ca^{2+} pulse (white bars) and the events where the excitatory stimulus was followed by a Ca^{2+} pulse (shaded bars). As a tangential benefit, these figures introduce the concept of synaptic delay, which is addressed more directly in another paper. Questions for the students to consider could include, “What does the Y axis represent in these figures?”, “What does the X axis represent in these figures?”, and “Why are there shaded bars (representing EPPs recorded in the *P + Ca* configuration) in Figure 3 but not in Figure 4?” Figures 5 and 6 are not used here.

Figure 7 may be included or omitted. It illustrates that when a Mg^{2+} pulse is applied before the Ca^{2+} pulse and excitatory stimulus, it inhibits EPPs. This is not essential to the discussion of the role of Ca^{2+} in synaptic transmission but can be included as a more challenging discussion point, if desired. Questions for the students to consider could include, “What effect does Mg^{2+} have on the depolarization?” and “Does timing matter?”

VESICLE DYNAMICS IN EXOCYTOSIS

The nature of synaptic release was a topic of much debate among neuroscientists in the mid to late 20th century. We now understand that following the intake of Ca^{2+} into the presynaptic terminal, Ca^{2+} ions find their way to the SNARE complex and initiate fusion of vesicles with the presynaptic membrane. This results in a release of neurotransmitter into the synaptic cleft.

To introduce the topic of vesicular release, two studies are recommended, both published by Heuser and Reese. In the first, they utilized freeze fracturing, which involves stimulating synaptic transmission immediately prior to freezing and breaking open the affected synapse, to capture synaptic events in a preparation they could then inspect under a microscope (Heuser et al., 1979). They used this technique to show the membrane perturbations that result when synaptic release occurs. Five figures from this study are recommended for highlighting principles of vesicular release for students.

Figure 1 illustrates the experimental apparatus for freeze fracture and is useful mainly to ensure that students understand the other figures from the paper. A question for the students to consider could be, “What is this machine, and how does it operate?”

Figures 3 and 4 illustrate the impact on EPPs of adding the voltage-gated K^+ channel blocker, 4-Aminopyridine (4-

AP). Questions for the students to consider could include, “Why did they use 4-AP?” and “What does this illustrate about synaptic transmission?”

Figures 7 and 8 show the freeze-fractured synapses and illustrate the membrane depressions left by the synaptic fusion events. Questions for the students to consider could include, “What is an active zone?” and “What do we learn about neurotransmitter release from these images?”

The second paper shows similar freeze-fracture images, and some of these images could be used instead of figures from the 1979 manuscript, if desired (Heuser and Reese, 1981). Three sets of figures are recommended for use from this publication.

Figures 1 and 2 illustrate similar principles as the previous paper except that it is possible to view the proceedings from a “side view” perpendicular to the freeze-fracture images. Questions for the students to consider could include, “What do you see that is the same or different from the freeze-fracture images?” and “What are the broad undulations in the membrane in Figure 2?”

Figure 27 summarizes some of the findings from this paper and illustrates the progression of vesicular fusion as captured through freeze-fracture microscopy. A question for the students to consider could be, “What hypothesis of synaptic release do these data support?”

Additional studies could be added to address in further detail the previously prevailing hypothesis of kiss-and-run versus the current understanding of vesicular fusion.

QUANTAL RELEASE

Perhaps to the surprise of some early neuroscientists, synaptic release is, in fact, quantal. The contents of a single vesicle would be considered the smallest unit of neurotransmitter that can be excreted during synaptic transmission. All other levels of neurotransmitter release are a rough multiple of this vesicular quantum. To introduce this topic, a 1954 paper by Del Castillo and Katz can be utilized (Del Castillo and Katz, 1954). Three figures from this paper are recommended for introducing the topic of quantal release.

Figure 1 illustrates clearly the fluctuation in amplitude of the EPPs that occur from stimulating the nerve leading to a particular neuromuscular junction. Questions for the students to consider could include, “What does it mean that the amplitudes are not equal?”, and “What are miniature or spontaneous potentials?”

Figures 4 and 5 include histograms showing the distribution of end plate potential amplitudes at two different end plates. These figures help to identify the end plate potential produced by releasing a single quantum of neurotransmitter into the synapse. Questions for the students to consider could include, “Why would the researchers be interested in calculating ‘m’ as in equation 1?”, and “What does the mean amplitude of the end plate potential represent in this figure?”

Figure 7 includes a histogram of end plate potentials produced by spontaneous vesicular release. The authors highlight in this figure the multimodal nature of the histogram, with the second and third peaks occurring at multiples of the first peak. This suggests that the second and third peaks are release events involving multiple vesicles. Questions for the students to consider could include, “Why might there be multiple peaks in this histogram?”, and “Why is it significant that the responses are clustered around multiples of an average spontaneous potential?”

SYNAPTIC DELAY

One of the early discussions in the science of synaptic transmission was whether synapses communicated electrically or chemically. We now understand that both mechanisms are viable and that they are found in operation at different synapses. Electrical synapses occur commonly in the developing mammalian brain and continue to be present in the adult brain as well (Nagy et al., 2018). One of the discoveries that supported the argument of chemical communication, which is most common in the adult brain, is the fact that there is a delay between the moment the action potential hits the presynaptic terminal and the moment the post synaptic potential is generated. This synaptic delay supports the hypothesis that a chemical communication is occurring during this delay period. To introduce the topic of synaptic delay, a 1965 paper by Katz and Miledi can be studied (Katz and Miledi, 1965). Five figures from this paper are recommended for highlighting principles of synaptic delay.

Figures 1 and 2 show in diagrammatic form and in actual traces the delay that exists between the presynaptic potential and the post synaptic potential. Questions for the students to consider could include, “What is the significance of the delay between the stimulus and the presynaptic potential?”, “What is the significance of the delay between the presynaptic potential and the post synaptic potential?”, and “What are possible causes for these delays?”

The authors propose three potential explanations for this synaptic delay. Figure 5 addresses the possibility that the delay is explained by the time it takes for the neurotransmitter to diffuse across the synaptic cleft. Since this figure contains arbitrary axes, the students will need to rely more heavily on the text to understand this possible explanation. Questions for the students to consider could include, “How long should it take for acetylcholine to diffuse across the synaptic cleft?”, and “How did they figure this out?”

Another of the potential explanations the authors included was the time it takes for the neurotransmitter to activate the postsynaptic receptor. Figures 6 and 7 illustrate that postsynaptic acetylcholine potentials begin to be visible approximately 0.1 ms after the presynaptic potential occurs, suggesting that this explanation could account for approximately 20% of the 0.5 ms delay observed between the presynaptic and postsynaptic potentials. Questions for the students to consider could include, “How long does it take for the acetylcholine to activate the receptor and produce a response once it arrives?”, “How did they figure that out?”, and “To what do the authors attribute the delay?”

IMPLEMENTATION

The manuscripts mentioned here could be used in a number of ways. One suggested implementation is to divide the students into groups and assign each group a topic to prepare in advance. Those groups then prepare to present the figures to the class as a group and answer any questions that the class may have so that they become the main instructors for their given topic. Questions asked may be directed first to the group before the course instructor offers any additional insights. The course instructor may also interject as needed to ensure that the correct conclusions are being drawn and that all of the important principles pertaining to the subject are covered sufficiently. There also may be times when the discussion stagnates, and the instructor can ask questions, including some of the questions listed here, to stimulate additional discussion and discovery learning. Some students accustomed to traditional lecture-style learning will experience discomfort in this approach, making it essential to proactively address their individual needs and capacities before implementation.

The implementation described above was preliminarily validated with an undergraduate 200-level neuroscience course, and the results were favorable. Almost all students reported either a typical or increased level of engagement, and performance on the chapter quiz was improved over other chapters. An assortment of supportive comments from students who participated indicated that they appreciated how the strategy caused them to think more deeply and practice drawing conclusions from data.

CONCLUSION

In a rapidly changing professional landscape, additional efforts are needed to ensure that graduates from institutions of higher learning, and in particular graduates from neuroscience programs, possess the skills that will enable them to be successful in the varied careers in which they are likely to engage. Using active learning pedagogical approaches that implement the analysis of figures and data from primary scientific literature has the potential to improve learning and provide students additional opportunities to develop skills in data analysis and drawing conclusions from experimental findings. The proposed articles and implementation presented here may be used as one such tool in the ongoing effort to render to students a greater skillset relevant to a career as a neuroscience professional.

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