OPEN ACCESS Freely available online

CourseSource

Lesson

# The Leaky Neuron: Understanding synaptic integration using an analogy involving leaky cups

**Edwin Griff**

Department of Biological Sciences, University of Cincinnati, ML 0006, Cincinnati, OH 45221-0006

## Abstract

Students have difficulty understanding synaptic integration in neural circuits, and how spatial and temporal summation combine in a target neuron to reach threshold. This Lesson uses small cups of water to represent excitatory postsynaptic potentials (EPSPs). Cups of water/EPSPs are added to a large cup, using either many cups at the same time to represent spatial summation, or one cup refilled repeatedly to represent temporal summation; students try to fill the cup to a level (line) that represents threshold for an action potential at the axon hillock. This large cup has a hole in the bottom representing the passive leakage of potential as the EPSPs spread from synapses on the dendrites and cell body to the axon hillock of the target neuron. To get the most out of this Lesson, students need to understand the parts and functions of a typical neuron, the basic sequence of events that occur at a chemical synapse, and the concept and importance of action potentials and their frequency. Students also need to understand the difference between a local potential and an action potential. Students work in groups of four to complete a series of activities in which the size of the cup (amplitude of the EPSPs), and the distance to the axon hillock were varied. We analyzed student responses to questions about the activity and found that the Lesson helped them understand spatial and temporal summation and raised some insightful questions about the process of synaptic integration.

**Citation:** Griff, E. 2018. The Leaky Neuron: Understanding synaptic integration using an analyogy involving leaky cups. *CourseSource*. https://doi.org/10.24918/cs.2018.11

**Editor:** Michelle Smith, Cornell University

**Received:** 12/22/2017; **Accepted:** 04/05/2018; **Published:** 09/28/2018



**Conflict of Interest and Funding Statement:** Travel to the Northstar Summer Institute was funded in part by the McMicken College of Arts and Sciences and the Department of Biological Sciences at the University of Cincinnati. The author has no financial, personal, or professional conflict of interest related to this work.

**Supporting Materials:** S1: Leaky Neuron, Blank data sheet, S2: Leaky Neuron, Blank worksheet, S3: Leaky Neuron, Representative data sheet, S4: Leaky Neuron, Worksheet with key, S5: Leaky Neuron, Homework questions, S6: Leaky Neuron, Sample test questions, S7: Leaky Neuron, Required materials, and S8: Leaky Neuron, Introductory slides.

***Correspondence to:** Department of Biological Sciences, University of Cincinnati, ML 0006, Cincinnati, OH 45221-0006 Email: edwin.griff@uc.edu

## Learning Goal(s)

Students will:

- understand the differences between spatial and temporal summation of synaptic potentials
- understand that local potentials such as synaptic potentials decay with time and distance
- understand how spatial and temporal summation combine to reach threshold for an action potential

## Learning Objective(s)

Students will be able to:

- compare and contrast spatial and temporal summation in terms of the number of presynaptic events and the timing of these events
- predict the relative contribution to reaching threshold and firing an action potential as a function of distance from the axon hillock
- predict how the frequency of incoming presynaptic action potentials effects the success of temporal summation of resultant postsynaptic potentials

## INTRODUCTION

An understanding of how neurons communicate via chemical synaptic transmission is essential to fully appreciate the physiology of the nervous system. The Human Anatomy & Physiology Society (HAPS) has published a comprehensive guideline for Anatomy and Physiology courses (https://www.hapsweb.org/). In Module H - Nervous, HAPS lists eighteen learning outcomes under the topic synapses and an understanding of synaptic potentials and their temporal and spatial summation is included. Over 75% of undergraduate neuroscience faculty who responded to a recent survey of core competencies in an undergraduate neuroscience curriculum ranked "Understanding the cellular and molecular function of neurons, including how neurons communicate" as essential (1). Various aspects of neurophysiology are taught in many high school and college courses including General Biology, Anatomy and Physiology, Psychology, Animal Physiology and Neurobiology.

However, undergraduate students have trouble understanding the physiology of neurons in part because: 1) students do not have

a basic understanding of the physical (electrical) and chemical (ionic) principles needed to comprehend neurophysiological concepts (2); and 2) students generally have less knowledge about the nervous system than the other organ systems (3). The lack of understanding about neurons may be due to an emphasis on the physiology of the digestive, respiratory, and/or cardiovascular systems starting in grade school (4). For example, a website aimed at K-12 students sponsored by the American Physiology Society for Physiology Understanding Week had nothing on the nervous system (http://www.the-aps.org/mm/Education/K-12/EducationProjects/PhUn-Week/PhUnforStudents/PhUn-Experiments/Elementary-School). Furthermore, studies of undergraduate students have revealed significant misconceptions in students' understanding of the resting membrane potential and synaptic transmission (5,6,7). Thus, there is a need for activities that will enhance student understanding of how neurons function.

There are relatively few published hands-on activities that can be done outside of a neurophysiology laboratory setting. One type of activity that has been published involves dramatic demonstrations enacting propagation of an action potential or a simple reflex with students playing the various parts of the cell or circuit (8,9). Another activity simulates neural coding by having students act as inputs that send signals to another student who acts as the output or target neuron. For example, Reardon et al. (10) describe an exercise where "input" students hold up cards for a short, assigned time at a given rate to represent excitatory (E) or inhibitory (I) inputs. The student representing an output neuron writes something relatively short such as "neuron fires" on the board whenever he or she sees a threshold combination (i.e. 3 E's at the same time). While the student is writing, his or her back is turned so that the input cards cannot be seen; this represents a refractory period. By varying the number and type of inputs and the timing and rate of each, both spatial and temporal summation can be simulated.

The Lesson described here provides hands-on experiences that can help students understand a basic neurophysiological concept, synaptic integration. Synaptic integration is a fundamental concept that underlies most animal behaviors, from producing a reflex to reading the words on this page (http://www.mind.ilstu.edu/curriculum/neurons_intro/neurons_intro.php). Almost every neuron in the central nervous system receives many inputs via chemical synapses. At each synapse, chemical neurotransmitters are released when an action potential is conducted/propagated down the axon to the axon terminal. Neurotransmitter diffuses across a synaptic gap where it can bind to receptor proteins on the target neuron. This binding triggers the opening (or closing) of ion channels resulting in a change in the local trans-membrane voltage (membrane potential) of a small patch of membrane of the target neuron. These channels are referred to as ligand-gated or chemically-gated channels; this area of membrane is called the post-synaptic membrane; and the change in voltage is called a post-synaptic potential (PSP). In general, the PSP at one synapse caused by the propagation of one action potential to the presynaptic axon terminal is quite small. In order for the target cell to get excited enough to generate its own action potential and send information along a neural circuit, many synapses must be activated so that their individual PSP's can sum together. This process is referred to as synaptic integration.

Synaptic integration includes both spatial and temporal summation. Spatial summation occurs because different synapses (at different spaces on the dendrites and cell body) can be active at about the same time. The PSPs generated at individual synapses can sum together as they spread toward the region of a neuron where an action potential is initially generated, the axon hillock. An analogy that helps explain spatial summation uses the amount of heat from a candle or small torch to represent a PSP and a frying pan to represent the axon hillock of the target neuron. If more candles are added at different places on the frying pan, the heat sums and the frying pan can get very hot. Imagine that the handle of the frying pan is covered with an explosive material. With a sufficient number of candles at the same time, the handle will get hot enough to trigger an explosion, the action potential (11).

For temporal summation, imagine that the torch is lit repeatedly, again causing the frying pan and its handle to get hot. Temporal summation occurs because neurons typically generate bursts of action potentials one after the other at a frequency as high as 100-300 hertz rather than a single action potential (https://www.khanacademy.org/science/health-and-medicine/nervous-system-and-sensory-infor/neuron-membrane-potentials-topic/v/action-potential-patterns). Each presynaptic action potential in a burst triggers the release of additional neurotransmitter which can produce repeated PSPs. Because a PSP is 20 or more times slower than the action potential, the additional neurotransmitter produces additional PSP's that can sum together in time. Synaptic integration involves both temporal and spatial summation occurring together to determine whether the target neuron is excited and generates its own action potential(s).

While both temporal and spatial summation of PSPs occur at the dendrites and cell bodies of a target neuron, the action potential typically is generated at a region referred to as the axon hillock, initial segment, or spike initiation zone where the axon exits the cell body. This region has a high density of voltage-gated sodium channels, the channels that are responsible for an action potential. An action potential will be generated if the sum of the individual PSPs that spread to the axon hillock reach a level of depolarization called threshold. At threshold, a sufficient number of voltage-gated sodium channels have opened to cause an action potential. If the depolarization exceeds threshold, a higher frequency of action potentials will be generated.

The PSP generated at a given synapse has to spread passively along the membrane from the patch of post-synaptic membrane where it was generated to the axon hillock (http://www.physiologyweb.com/lecture_notes/neuronal_action_potential/neuronal_action_potential_graded_potentials_versus_action_potentials.html). As it spreads, the amplitude of the PSP gets smaller and it takes time for the spreading to occur. This Lesson focuses on the passive spread of Excitatory Post-Synaptic Potentials (EPSPs) where positive charge enters through ligand-gated channels in the postsynaptic membrane. In passive spread some of the positive charge that enters is attracted to the adjacent membrane which is more negative. Some of the positive charge also can leak out of the membrane causing the amplitude of each EPSP to get smaller (and slower) as it spreads. Nonetheless, with sufficient spatial and temporal summation, the axon hillock can reach or exceed threshold

and generate action potentials at a frequency dependent on the level of depolarization.

The activities in this Lesson are concerned only with reaching threshold. The Lesson provides a physical analogy using water and leaky cups to help students understand how PSP's sum by spatial and temporal summation. The activities use a small cup of water to represent an EPSP and a large cup to represent the axon hillock. Pouring several cups into the large cup at the same time represents spatial summation; pouring cups one after the other represents temporal summation. Holes in the bottom of the large cup represent the leak that causes the EPSP to decrease in amplitude as the potential spreads from the synapse to the axon hillock. The water that remains at any moment represents the amplitude of the summed EPSPs that has spread to the axon hillock. The object of each activity is to accumulate enough water in the large cup to reach a line that represents threshold. The Lesson provides a physical analogy that students can see - adding water to a large cup to reach a line - to help them understand the more abstract concept of synaptic integration where positive charge (depolarization) from many synapses spatially sum and charge from individual synapses activated in succession sum temporally to reach threshold for an action potential.

### *Intended Audience*

The initial activity was developed by a faculty team at a National Academies of Science, Engineering, and Medicine Scientific Teaching Alliance's Northstar Summer Institute as a teachable unit. The activity was subsequently used in a neurobiology course at the University of Cincinnati in fall 2015. In spring 2017, parts of the activity were modified as a pseudo-demonstration involving a few students in the neurobiology course.

The Lesson was designed for a one-semester upper-level Neurobiology course in the Department of Biological Sciences at the University of Cincinnati. Neurobiology is a lecture course that requires General Biology, General Biology Lab (2 semesters of each), Genetics, and Cell Biology as pre-requisites. Most students have also had general and organic chemistry. The Lesson was done in the 8$^{th}$ week of the 14-week semester and students had done reading, taken quizzes and one exam, and been involved in group and face-to face classroom activities on the following topics: organization of the nervous system, neural circuits and signaling, including resting membrane potential, action potential, and synaptic potentials, ion channels and transporters, synaptic integration, and a survey of neurotransmitters and their receptors. The 62 students were a mix of junior and senior biological sciences majors. At the beginning of the semester, groups of four or five students were formed such that each group contained some confident and some less confident students based on a survey given before the first class. Students generally sat with their group in lecture and did some group activities each week. The Leaky Neuron activity was done in these same groups in an undergraduate lab setting with running water and sinks that were available at the class time.

### *Required Learning Time*

The Lesson can be completed in a 55-minute class period, if everything is prepared beforehand. Students completed data worksheets during this period and submitted homework questions about the activity separately via Blackboard.

### *Pre-requisite Student Knowledge*

Students should have familiarity with the different functional areas of a neuron: a receiving/input region consisting of the dendrites and cell body, a conducting region consisting of an axon and its branches, and an output region where neurotransmitter is released from the axon terminal. They should be familiar with the concept of a membrane potential and the concept of threshold for generating an action potential. For simplicity, only synapses that are excitatory such that the PSPs (EPSPs) try to bring the membrane at the axon hillock to threshold or beyond are considered in this Lesson.

Specifically, students should be able to describe the following before starting this activity: the structure and function of the dendrites, axon, axon hillock, and axon terminal of a typical neuron; the resting membrane potential, depolarization, action potential, threshold and local potential; the sequence of events starting with an action potential propagating down the axon that results in the release of neurotransmitter from the axon terminal; and the changes in membrane potential at the post-synaptic membrane that generate a local potential called the excitatory post synaptic potential (EPSP).

Students can obtain this knowledge via a lecture, a standard biology (12), physiology (13), or neurobiology (14) textbook, or through pre-class homework assignments. There are many excellent resources to help students understand various aspects of neurophysiology (e.g. http://www.physiologyweb.com/physiology.html, http://neurons.med.utoronto.ca/index.swf, 15, 16).

## SCIENTIFIC TEACHING THEMES

### Active learning

Students will actively engage in learning the concepts by: working in groups of four or five students; following the detailed directions, filling out the data sheet and worksheets; discussing the experiments and results; and by reflecting on the experience by answering several homework questions.

### Assessment

Students will complete one data sheet and one worksheet per group in which they record the number of small cups of water representing the amplitude of the EPSP at the postsynaptic membrane needed to fill a large cup with a hole in the bottom to a line that represents threshold. Students will also record the time this takes and will then measure the amount of water that leaked out of the large cup. These measurements will be made under several conditions such as the distance the small cups must be carried to the large cup, representing synapses farther away from the axon hillock. Course points were awarded for completion of all worksheets; everyone in a group received the same score as long as they were present and participated. A blank sample data sheet (Supporting Files S1: Leaky Neuron, Blank data sheet) and blank worksheet (Supporting Files S2: Leaky Neuron, Blank worksheet) are included. A data sheets with representative data (Supporting Files S3: Leaky Neuron, Representative data sheet) and a worksheet with a key (Supporting Files S4: Leaky Neuron, Worksheet with key) also are included.

Students will articulate how the activity helped them understand spatial and temporal summation by answering several questions assessing the learning goals and objectives

(Supporting Files S5: Leaky Neuron, Homework questions). Student understanding of spatial and temporal summation was also assessed on a lecture exam with both multiple choice and short answer questions (Supporting Files S6: Leaky Neuron, Sample test questions).

Inclusive teaching

Students work in small groups requiring cooperation, distribution of tasks, group discussion as well as individual assessments. Students in a group each had different tasks and could select a task that they could accomplish.

## LESSON PLAN

The Lesson plan contains four parts: timetable, materials required, instructor preparation, and the Lesson description. (See Table 1 for Teaching Timeline)

### *Materials Required*

Required materials are described in Fig. 1, Table 2, and Supporting Files S7: Leaky Neuron, Required materials.

**Figure 1. General set-up of the Lesson.** This figure shows the equipment used for the experiments. A large tray contains a 1000 ml beaker and a student is holding a 500 ml clear cups with holes over it. There is also a 500 ml clear cup without holes, large bowl with water and a 50 ml plastic beaker, four 120 ml (4 ounce) paper cups, and one 200 ml (7 ounce) paper cup. Another student is filling out a data sheet.

See Table 2 for a list of materials needed.

### *Instructor Preparation*

The instructor will need to assemble the materials listed in Supporting File S7: Leaky Neuron, Required materials. The instructor will also need to experiment with the size and/or number of the holes in the large plastic cup so that water will reach the black line in a reasonable amount of time (10-30s). Since students are being timed, they will try to work as fast as they can to fill the large cup. The instructor can vary the number of the holes so that some groups have a larger leak. We used three or four holes either 1 mm (small holes) or 2 mm (large holes) in diameter. The location of the holes is not important since their only function is to let water leak out. Holes are made in the plastic cup by heating a metal dissection needle and pushing the heated needle through the bottom of the cup. The larger holes were made by moving the hot needle in a very small circle. With three large holes, it took about 15 seconds to fill the cup to the black line (threshold) by repeatedly filling and emptying the 50 ml beaker; with four small holes, it took about 10 s (see Activity 2, below). Students will use cups with the hole at the bottom of the cup and the red line that indicates the resting membrane potential also at the bottom. This arrangement of the holes allows the water that leaks out to be collected more easily and therefore makes less of a mess. However, the instructor can demonstrate using a large cup with an alternative arrangement of holes that more clearly shows that a resting membrane potential is maintained at all times. In the alternative arrangement, the red line is drawn a few centimeters from the bottom and the holes are on the side of the cup just above the red line. With holes on the side of the cup, the leaking water covers a wider area and creates more of a mess.

A brief presentation is included that introduces the basic neurophysiological concepts related to this Lesson (Supporting Files S8: Leaky Neuron, Introductory slides).

### *Pre-lesson Information*

Prior to starting the Lesson, the following instructions were read to the students.

You will notice that one of the 16-ounce clear plastic cups has a red line and a black line; the red line represents the resting membrane potential. Your goal is to fill this cup with water up to the black line. This is going to be harder than you think because the cup is extremely leaky!

The level of the water in the large plastic cup represents the membrane potential at the axon hillock. You will start with the water level at the red line. I will demonstrate this with a cup that has holes on the sides just above the red line; when water (positive charge) is added, some water (charge) leaks out. Your cup may have the holes at the bottom of large plastic cup so that it is easier to collect the water that leaks out. If the holes are at the bottom, the red line representing the resting membrane potential also will be at the bottom of the cup (see modifications, below).

Your task is to add water (positive charge) to reach threshold. Each 50 ml plastic beaker represents the depolarization or amount of positive charge produced by an EPSP at an excitatory synapse. You will measure how many cups (active synapses) are needed and how long it takes to reach threshold under different circumstances. These activities will simulate spatial and temporal summation.

The amount of water in a small cup represents the amplitude of the depolarization from an EPSP. Adding several small cups of water at about the same time represents spatial summation of EPSP from different synapses. Adding cups of water one after another represents temporal summation of EPSPs. The holes in the bottom of the large cup represent the leak of depolarization as EPSPs spread from the synapse on the dendrites to the axon hillock. The leak decreases the amount of water at the axon hillock and represents the decrease in the amplitude of an EPSP as it spreads to the axon hillock.

**Figure 2. Trying to reach threshold.** This figure illustrates how threshold is reached in a trial demonstrating temporal summation. One student holds the clear cup with holes over the 1000 ml beaker while another student repeatedly fills and empties a small beaker into the large plastic cup with holes in the bottom.

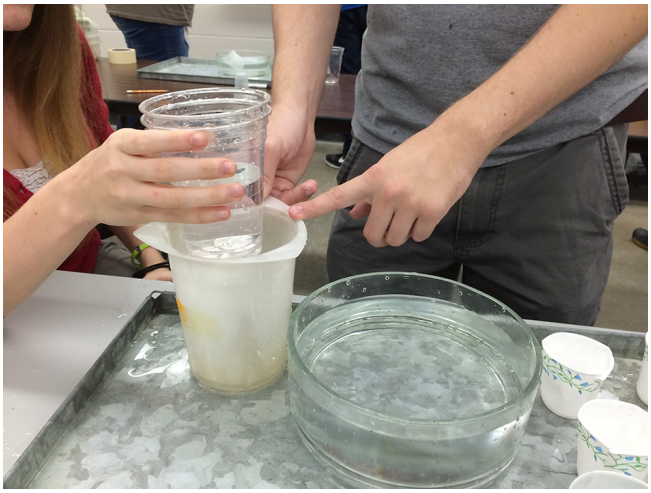


**Figure 3. Stopping the trial.** This figure illustrates how a trial is stopped. To stop a trial and stop the leakage, the clear cup without holes is quickly placed under the cup with holes.

*Lesson Description*

General Instructions

One person will hold the leaky neuron (large cup with holes and threshold marked) over the 1000 ml beaker; he/she will stop the trial by quickly nesting the two cups (quickly putting the cup without holes under the one with the water) when threshold is reached (see Fig. 3). Threshold is reached when the water gets to the black line near the top of the 16-ounce clear cup with holes. This was demonstrated to the class.

- One person will be the timer and record the time elapsed to reach threshold.
- One person will count cups, watch to see when threshold is reached, and immediately tell the timer and the person who stops the trial.
- One or two students will add water to the leaky neuron, dipping the cups or 50 ml beakers into the reservoir bowl to refill them as needed.

Each team will record the:
- number of cups (EPSPs) needed to reach threshold at the axon hillock;
- time to reach threshold
- total amount of water (positive charge) needed to reach threshold
- amount of charge (water) that leaked out
- percentage of charge (water) that leaked out

There is a tray and a large bowl with water at your place. The tray is to contain spilled water; the reservoir bowl may need to be refilled after a few trials.

[Note to instructors: You may want to ask if there are any questions before the students start the first activity.]

Activity 1: Spatial summation of EPSPs from multiple inputs

**1.** Fill the four 120 ml paper cups with water from the reservoir bowl.

Hold the leaky neuron (500 ml cup with holes and threshold marked) over the 1000 ml beaker.

Start the timer and then have two people each dump two cups into the leaky neuron at the same time; stop the experiment (by quickly nesting the clear 500 ml cup without holes under the leaky neuron cup) when the water reaches the threshold line. Record your results (trial 1) on the data sheet (Supporting file S1: Leaky Neuron, Blank data sheet.

Instructor note: the size of the small cups, the size of the hole(s) and the position of the black line must be adjusted so that the four cups can bring the water level above threshold (black line). Four 120 ml cups will easily reach threshold with 500 ml (16 ounce) plastic cup marked with a black line 3-4 cm from the top and three or four large (2 mm) holes in the bottom. Adding many small cups of water each representing a PSP from a separate synapse represents spatial summation.

Repeat the experiment a few times to practice calling out threshold, stopping the experiment, and recording the time. Record your two fastest times (trials 2 & 3) on the data sheet (Supporting Files S1: Leaky Neuron, Blank data sheet).

**2.** Complete the worksheet for Activity 1 (questions 1a, 1b, & 1c; Supporting Files S2: Leaky Neuron, Blank worksheet)). The amount of water in a small cup represents the amplitude of the depolarization from an EPSP. The holes in the bottom of the large cup represent the leak of depolarization as the EPSP spreads from the synapse on the dendrites to the axon

hillock. The black line near the top of the large cup represents a threshold depolarization for an action potential.

Activity 2: Temporal summation of EPSPs from a single input

**3.** Practice:

- Hold the leaky neuron cup over the large beaker.
- Fill the small 50 ml beaker with water.
- Have one person repeatedly empty the water from the small beaker into the leaky neuron (large clear plastic cup with lines and holes), re-fill and re-empty the small beaker until threshold is reached (see Figure 2).
- Stop the trial when threshold is reached. Repeatedly emptying water from a small beaker represents the responses in the target neuron to a burst of action potentials that produces temporal summation.

Repeat the experiment three times and record the data after each trial on the data sheet (Supporting Files S1: Leaky Neuron, Blank data sheet).

**4.** Complete the worksheet for Activity 2 (questions 2a & 2b; Supporting Files 21: Leaky Neuron, Blank worksheet).

Activity 3: Graded local potentials; larger EPSPs

**5.** Repeat Activity 2 using a 200 ml (7 ounce) paper or plastic cup; if using paper, a new cup will be needed for each trial if it becomes soggy. Record the data after each trial on the data sheet (Supporting Files S1: Leaky Neuron, Blank data sheet).

**6.** Complete the worksheet for Activity 3 (questions 3a & 3b; Supporting Files S2: Leaky Neuron, Blank worksheet)).

Activity 4: The effect of distance of the synapse from the spike initiation zone

**7.** Position the bowl with the supply of water at one corner of the tray and hold the leaky neuron cup over the large beaker at the corner diagonally opposite. Repeat Activity 2 under these conditions three times and record your data on the data sheet (Supporting Files S1: Leaky Neuron, Blank data sheet).

**8.** Complete the worksheet for Activity 4 (Supporting Files S2: Leaky Neuron, Blank worksheet).

Activity 5: Homework questions

Students answer questions describing how the activity helped them understand spatial and temporal summation and offer suggestions for improving the activities (Supporting file S5: Leaky Neuron, Homework questions) using a course management system. Supporting file S5: Leaky Neuron, Homework questions also provides sample answers to these questions.

## TEACHING DISCUSSION

The main goal of this Lesson was to provide a concrete analogy so that students can better conceptualize spatial and temporal summation of synaptic potentials and the implications of passive leak currents across the neuronal membrane. Based on my questioning of students about synaptic integration and getting the wrong answers when teaching undergraduate neurobiology courses for over 30 years, reinforced by published studies (5,6), it is known that students have difficulty with concepts such as the decay of synaptic potentials and their integration at the axon hillock. Talking with students while they were carrying out the activities suggested that most students quickly understood the addition of water to represent depolarization of the membrane from rest and the process of spatial and temporal summation. Written responses from the homework questions also showed that the Lesson enhanced student understanding of synaptic integration (Supporting File S5: Leaky Neuron, Homework Questions). Qualitative analysis of these student responses demonstrated that the Lesson helped most of the students understand these concepts (Fig. 4 and Fig. 5). The Lesson produced an unexpected result described by some students in answering the homework questions; the activity helped students get to know the other members of the

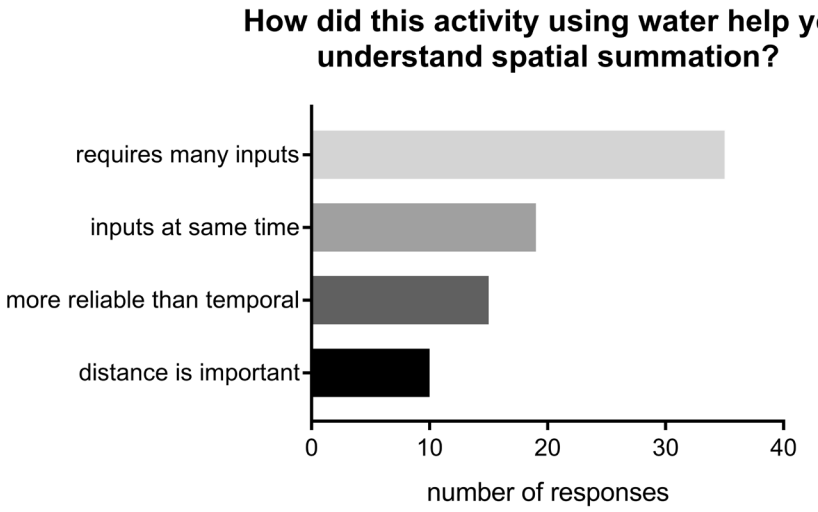



**Figure 4. Qualitative analysis of metacognition question 1.** This figure shows the most common themes found in the responses of students answering the question: "How did this activity using water help you understand spatial summation?" The x axis shows the number of students who replied which each of the most common themes.

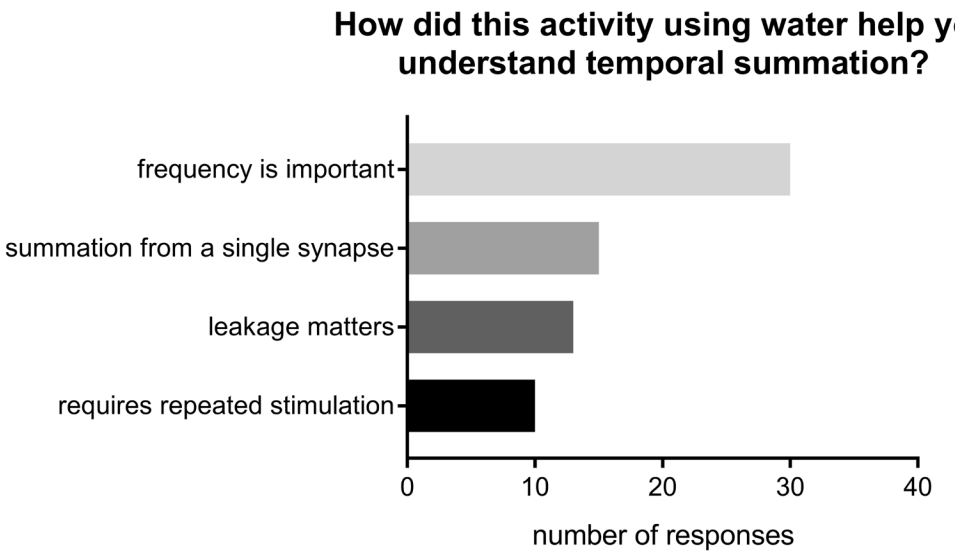



**Figure 5. Qualitative analysis of metacognition question 2.** This figure shows the most common themes found in the responses of students answering the question: "How did this activity using water help you understand temporal summation?" The x axis shows the number of students who replied which each of the most common themes.

group better, increased group cohesiveness, and helped them work together as a team.

The results obtained from the activities (Supporting File S3: Leaky Neuron, Representative Datasheet) were discussed in the next class period. Groups whose large cups were leakier (larger holes) required more time and more water (number of small cups) to reach threshold than groups whose large cups had a smaller leak. Bringing small cups from farther away from the large cup (axon hillock) also took more time and more water, since water continues to leak out of the large cup. The variability in the times and number of cups required to reach threshold between trials and between groups using large cups with approximately the same leak was also discussed; this variability was due primarily to how quickly a particular student could refill the cup and the level to which each small cup was filled. In general, speed in refilling the small cups was more important than whether the small cups were filled completely. The frequency of filling cups is analogous to the frequency of action potentials invading the axon terminal and releasing neurotransmitter. In general, a higher frequency of action potentials is a measure of signal strength.

The volume of water in a small cup (or beaker) represented the amplitude of a single EPSP. In neurons the amplitude of an EPSP at different synapses, and even the amplitude at a given synapse, will vary depending on the amount of current (called the excitatory postsynaptic current, EPSC) that enters the postsynaptic cell. The amount of current reflects the properties of the channel opened by the neurotransmitter and/or the number of channels that open. In the activities above, the amount of water varied between 50 and 200 ml and the voltage needed to reach threshold was about 500 ml of water. In real neurons, threshold will also vary. In the Lesson above the amplitude of the EPSP was 10-40% of the threshold voltage. These values were selected so that threshold could be reached in a reasonable amount of time for each activity. The amplitudes of the EPSPs that spread to the axon hillock in most neurons are typically a much smaller percentage of threshold so that much more spatial and temporal summation is required. For example, the amplitude of neurons in the guinea pig hippocampus was less than 20 uV (17) and threshold was about 9 mV (18). In the hippocampus the amplitude of the EPSP was only 2% of the voltage needed to reach threshold (17,18). At different synapses this percentage will vary with the value of threshold (which itself is not constant), the location of the synapses on the dendritic arborization and distance from the axon hillock, the amount of neurotransmitter released by the presynaptic neuron, the reversal potential of the PSP, and the decay of the PSP amplitude as the potential spreads to the axon hillock. The dynamics of all these parameters allows the nervous system to function under constantly changing conditions.

In more advanced classes, the results of Activity 4 on the effect of distance of the synapse from the spike initiation zone (axon hillock) should be connected with the concept of a space constant, the distance from the synapse at which the amplitude decreases to about 37% (1/e). The decrease in amplitude with distance that occurs for synaptic potentials can be illustrated with another analogy, heat, and a simple demonstration. A metal rod is heated at one end with a torch or candle and students are asked to predict where the rod would be hottest (where it was heated) and be shown that many inches away, the rod is only slightly warm. The distance where the temperature drops to 37% of the hottest value is the space constant. The concept of a space concept then can be applied to the decrease in the amplitude of an EPSP from the postsynaptic membrane where it is generated as it spreads to the axon hillock. In more advanced courses the equations for the space constant and its dependence on internal and transmembrane resistance can be discussed. In the activity, the decrease in the amplitude of the EPSP was represented by the leak of water via holes in the large cup.

### *Student Evaluations*

The last activity of the Leaky Neuron Lesson was a set of homework questions to help students understand the analogy and suggest improvements (Supporting file S5: Leaky Neuron, Homework questions).

Eighty-nine percent of the students (41 out of 46) indicated that the leaky neuron activity helped them understand both spatial and temporal summation, was worthwhile, and was fun. One student did not think the activity was worthwhile or fun, but indicated that it helped with understanding the concepts. Four students did not think the activity was worthwhile because they already understood the concepts and/or thought that the activity would be better as a demonstration or video.

The reasons why the activity helped with understanding spatial and temporal summation were ascertained by descriptive coding of these open-ended questions and the frequency distributions of the most common themes in the student responses are shown in Figures 4 and 5. The questions students reported about synaptic integration after completing the leaky neuron activity provided additional insight concerning the effectiveness of the activity in promoting critical thinking. The most common questions focused on the conditions in the nervous system that would favor spatial versus temporal summation and requests for specific examples. For example, students asked if one type of summation was specific to certain postsynaptic neurons and whether there were any pathologies caused by failure of one type. Many students wanted to know if spatial summation was indeed faster than temporal summation in reaching threshold, as seen in the water activity. Others went deeper, asking about whether a particular input could participate in both spatial and temporal summation, and whether there was a correlation between one type of summation and the proximity of the synapses to the axon hillock. Some students also wanted to know more about the interactions of excitatory and inhibitory synapses in spatial summation, and one student asked how inhibitory synapses can produce temporal summation. A few students were interested in the ionic basis of summation as well as the ionic basis of leakage. About 50% of the students indicated that they did not have any questions.

The students asked excellent questions; the answers will depend on the instructor's background and knowledge of the model systems used to study the nervous system. For example, spatial summation has been well studied in the visual system because the geometry of light stimulation, such as the diameter of a spot, is easily controlled while the response of a single neuron is recorded (19). The larger the spot, the greater the number of photoreceptors that converge onto the next neurons in the circuit and produce spatial summation. One of the models for temporal summation is the gill-withdrawal reflex in the sea slug, *Aplysia* where the frequency of action potentials modifies synaptic strength that occurs in learning via temporal summation. However, in general, spatial and temporal summation combine to determine the responses of target neurons. Thus, the responses of retinal neurons also depend on temporal summation and the responses of neurons in Aplysia also depend on spatial summation. In terms of speed, spatial summation was faster in the Lesson because the water from the four small cups was dumped into the large cup as if the 4 synapses were very close to the axon hillock (large cup). The effectiveness of both spatial and temporal summation depends strongly on how far the synapses is from the axon hillock since both decay (get smaller) with distance. As discussed briefly below (Modifications to the Lesson), excitatory and inhibitory PSP can sum together by both spatial and temporal summation.

Most students thought the activity was worthwhile in term of increasing their understanding of spatial and temporal summation. Students appreciated the relative simplicity of the water analogy. One student said" I liked how simple it was. Sometimes demonstrations can try too hard and end up confusing me more than I was before." Several students commented that they learn better with hands-on activities and that they could visualize the processes better than they could with only words and diagrams. Another said, "Activities help me visualize concepts, something fun you can do with a group, where every activity has an analogy related to what we learn in class." Several students commented that the activity helped bring their group closer together, and that such activities should occur earlier in the semester.

Students also offered several suggestions to improve the activity. The most frequent suggestion was to use a larger room, followed by comments that the paper cups did not hold up well to repeated use. Students also suggested more combinations of cup size, distance, and the size of the leak, but fewer repetitions of a single condition. Some students wanted a smaller leak so that they did not have to work so hard to get the neurons to threshold. One student suggested having the holes on the side of the large plastic cup so that the level of water representing the resting membrane potential was maintained. Other students suggested using specific distances and specific rates of adding water in the temporal summation activities so that the results of the different groups could be compared more easily. Overall, the student responses suggested that the activity was an effective and enjoyable method to demonstrate spatial and temporal summation in synaptic integration.

### *Modifications to the Lesson*

The Lesson could be improved by providing students with the goals before the day of the activity and by restating them at the top of the instructions. The main goals of the Lesson were communicated to students on the day of the activity by reading the pre-lesson information, and the homework questions (Supporting File S5, Leaky Neuron, Homework questions) addressed each goal.

An important feature of the activities is that they can be easily adapted to include more or less detail. For example, having the resting membrane potential (red line) a few centimeters above the bottom of the leaky neuron cup with the holes on the side of the cup just above this line would be a better representation of a neuron. However, with holes on the side of the cup, the water that leaks out covers a wide area, and it is more difficult to collect this water. Thus, the activities done by the students described above use holes in the bottom of the cup.

The activities above were concerned with just reaching threshold in the target neuron. Equally important is the concept that depolarization at the axon hillock above threshold

increases the frequency of action potentials. The activities could be adapted to show an increase in the frequency of action potentials with a supra-threshold depolarization by drawing additional lines on the large cup above the threshold line. The action potentials in the target neuron subsequently would be propagated down the axon of the target neuron producing temporal summation at the next neuron in the circuit.

The Lesson could be expanded to include the effects of inhibitory post-synaptic potentials (IPSPs). The simplest mechanism to produce an IPSP is a hyperpolarization of the postsynaptic membrane such that the postsynaptic membrane becomes more polarized and more negative after the neurotransmitter binds to its postsynaptic receptor. Such a hyperpolarization opposes any depolarization and keeps the target neuron from reaching threshold. In a modification of the Lesson, a student could withdraw water from the large plastic cup at some rate at the same time that another student was adding water to the large cup with holes. Alternatively, a large plastic syringe (without a needle) could be used to withdraw water. One could also combine temporal and spatial summation by have the student who was adding water have 2 cups that were both repeatedly filled and dumped. For summation of IPSPs, the person withdrawing water could use 2 cups. The more students and the more cups used, the messier the Lesson becomes, and moving the Lesson outdoors may be advisable.

In some cases, an IPSP may not cause a hyperpolarization of the postsynaptic membrane; this can occur if the reversal potential for the IPSP is close to the resting membrane potential. Nonetheless, activation of such a synapse is still inhibitory because it increases the leak of any excitatory depolarization that is trying to spread from a more distal location to the axon hillock. Putting additional (and large) holes on the side of the large cup between the resting potential (red line) and threshold (black line) and observing the additional leak of water once this reversal potential is reached could illustrate this difficult concept. A line added to the cup where these additional holes are placed would represent the reversal potential for the IPSP.

Not all EPSPs and IPSPs are the same amplitude even at the same synapse; examples include sensory adaptation, synaptic fatigue where the amplitude decreases and sensitization and potentiation where the amplitude increases. The instructor could model this by using different sizes of cups. The instructor could also vary the distance from a synapse to the axon hillock (large plastic cup) and have students carry water to the large plastic cup from different locations. One can also demonstrate the effects of changing threshold (the position of the black line on the large cup), for example demonstrating why more excitation is needed during the relative refractory period.

A greatly scaled down demonstration was tried the following year in a neurobiology class. Five students who sat near the front of the room were asked to participate in the demonstration; one held a large plastic cup with lines for the resting potential and threshold. This large cup did not have a hole. The other four students were each given a small cup of water. To demonstrate spatial summation, the students dumped water into the large cup at the same time, and threshold was reached. To demonstrate leakage, a student sitting near the back of the room was given a small cup with a hole in it. I instructed the student to walk to the front of the room to add his water (EPSP) to the large cup. Water obviously leaked out, but it was fairly easy to clean up with a rag. I did not evaluate whether this short demonstration increased student understanding of the decrease in the amplitude of a local potential as it spreads passively along the membrane. I did not attempt to demonstrate temporal summation.

## SUPPORTING MATERIALS

- S1: Leaky Neuron, Blank data sheet
- S2: Leaky Neuron, Blank worksheet
- S3: Leaky Neuron, Representative data sheet
- S4: Leaky Neuron, Worksheet with key
- S5: Leaky Neuron, Homework questions
- S6: Leaky Neuron, Sample test questions
- S7: Leaky Neuron, Required materials
- S8: Leaky Neuron, Introductory slides

## ACKNOWLEDGMENTS

This activity was developed at a Northstar Institute at the University of Minnesota in July 2015. Participants and staff provided many useful comments on the activities.

The Northstar team that developed the leaky neuron teachable unit included: Arun Anantharam, Ph.D., Department of Pharmacology, University of Michigan; Josh Benoit, Department of Biological Sciences, University of Cincinnati; Sally Harmych, Department of Biological Sciences, University of Toledo; Paul Nodzak, Department of Biological Sciences, University of Cincinnati; John Plenefisch; Department of Biological Sciences, University of Toledo; Mark VanBerkum, Department of Biological Sciences, Wayne State University.

I would like to thank Josh Benoit and John Plenefisch for their comments on an earlier version of this manuscript.

The use and analysis of the student response data was approved by the IRB at the University of Cincinnati, Study ID: 2016-9486

## REFERENCES

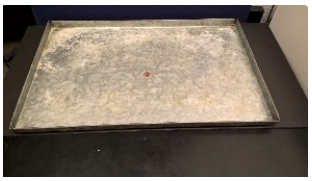
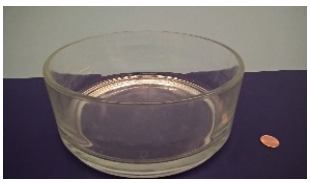
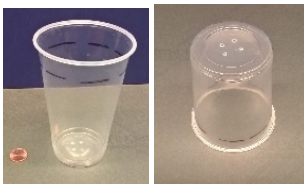
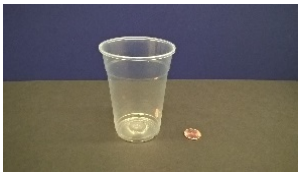
1. Kerchner M, Hardwick JC, Thornton JE. 2012. Identifying and using 'Core Competencies' to help design and assess undergraduate neuroscience curricula. J Undergrad Neurosci Educ 11: A27-A37.
2. Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia A-S, White LE. 2002. Neuroscience. Sunderland MA:Sinauer. pp 25-40.
3. Reiss MJ, Tunnicliffe SD. 2001. Students' Understandings of Human Organs and Organ Systems. Res Sci Ed 31: 383-399.
4. Appleton K. 2006. Science Pedagogical Content Knowledge and Elementary School Teachers. In: Elementary Science Teacher Education Mahwah, NJ:Association for Science Teacher Education. pp 31-34.
5. Silverthorn DU. 2002. Uncovering misconceptions about the resting membrane potential. Adv Physiol Educ 26: 69-71.
6. Montagna E, de Azevedo AMS, Romano C, Ranvaud R. 2010. What is transmitted in "synaptic transmission"? Adv Physiol Educ 34: 115-116.
7. Slominski TN, Momsen JL, Montplaisir LM. 2017. Drawing on student knowledge of neuroanatomy and neurophysiology. Adv Physiol Educ 41: 212-221.
8. Hamilton SB, Knox TA. 1985. The Colossal Neuron: Acting Out Physiological Psychology. Teach Psychol 12: 153-156.
9. Felsten G 1998. Propagation of Action Potentials: An Active Participation Exercise. Teach Psychol 25: 109-111.
10. Reardon R, Durso FT, Wilson DA. 1994. Neural Coding and Synaptic

Transmission: Participation Exercises for Introductory Psychology. Teach Psychol 21: 96-99.
11. Griff ER. 2006. How neurons work: An analogy and demonstration using a sparkler and a frying pan. Am Biol Teach 68: 412-417.
12. Mason KA, Losos JB, Singer SR. 2011. Biology. New York, NY:McGraw-Hill. pp 899-900.
13. Amerman EC. 2016. Human Anatomy and Physiology. San Francisco, CA:Pearson pp 412-413.
14. Kandell ER, Schwartz JH, Jessell TM. 1885. Essentials of Neural Science and Behavior. Norwalk, CT:Appleton & Long. pp 222-224.
15. Stuart AE. 2009. Teaching Neurophysiology to Undergraduates using Neurons in Action. J Undergrad Neurosci Educ 8: A32-A36.
16. Zao P, Stabler T, Smith L, Lokuta A, Griff E. 2012. PhysiolEx 9.0: Laboratory Simulations in Physiology. San Francisco, CA:Pearson Education. pp 33-56.
17. Sayer RJ, Friedlander MJ, Redman SJ. 1990. The time course and amplitude of EPSPs evoked at synapses between pairs of CA3/CA1 neurons in the hippocampal slice. J Neurosci 10: 826-836.
18. Henze DA, Buzsaki G. 2001. Action potential threshold of hippocampal pyramidal cells *in vivo* is increased by recent spiking activity. Neurosci 105: 121-130.
19. Kolb H. 2003. How the Retina Works. Sci Am 91: 28-35.

**Table 1. Leaky Neuron - Teaching Timeline**

| Activity | Description | Estimated Time |
|---|---|---|
| Purchase or gather materials | Gather or purchase cups of various sizes; gather or purchase trays and large bowls (see text and Table 2 for details) | 1-3 hours |
| Prepare materials | Determine appropriate size of holes; put holes and lines on clear large cups | 2-3 hours |
| Introductory slides | On the day before the lesson, discuss the important concepts with class (Supporting material S7: Leaky Neuron, Introductory slides) | 15-55 minutes |
| Pre-lesson information | Instructor previews activity, describes the materials, and demonstrates the resting membrane potential analogy | 5-10 minutes |
| Read directions | Students read directions, examine the materials | 5 minutes |
| Demonstration | Instructor demonstrates adding small cups of water to large cup with holes to reach threshol | 5 minutes |
| Activities 1-4 | Students do Activities 1-4, record the data on the datasheet (Supporting material S1: Leaky Neuron, blank data sheet), and fill out the worksheet (Supporting material S2: Leaky Neuron, Blank worksheet) | 30 minutes |
| Activity 5 | Students answer questions for homework | variable |

**Table 2. Leaky Neuron -** This table provides a picture, description, and the use of the materials needed for the Lesson. Note that none of the dimentions or volumes are critical to this lesson.

| Item | Picture | Use |
|---|---|---|
| Large tray (about 50 x 100 cm) |  | keep water contained |
| 1000 - 2000 mL bowl |  | resevoir for filling cups |
| 1000 mL large plastic cup or beaker |  | collect water that leaks out |
| 50 mL plastic cup or beaker |  | add water |
| 500 mL clear plastic cup with holes and lines<br>* note that this is the only container that must be **clear** |  | leaky neuron |
| 500 mLlastic cup without holes |  | stop activity |
| 120 mL paper or plastic cup |  | add water |
| 200 mL paper or plastic cup |  | add water |