# ARTICLE
# FraidyRat: A Virtual Module Examining the Neural Circuitry Underlying Fear Conditioning

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FraidyRat is a teaching tool that allows students to investigate the neural basis of fear conditioning and extinction using a virtual rat with a virtual brain. FraidyRat models well-known phenomena at both a behavioral and neural level. Students use virtual versions of tract tracing, systemic and intracerebrally infused drugs, neural recording, and electrical stimulation to understand the neural substrates underlying the observed behavior. This module helps students develop critical thinking skills in order to deduce immediate cause and effect as well as inductive reasoning to grasp the broader scheme. This module utilizes scaffolded instruction and formative assessment to shape the thinking of students as they unfold and discover the neural mechanisms responsible for fear conditioning and extinction in FraidyRat, which largely reflect what is found in real rats. Experience with this three-week module resulted in students showing significant gains in content knowledge as well as a trend toward gains in critical thinking. An attitudinal questionnaire showed that students had an overall positive experience. This module can be replicated at any institution with just a computer. All materials are available at: https://mdcune.psych.ucla.edu/modules/fraidy-rat.

*Key words: circuit neurophysiology; fear conditioning; virtual neuroscience tools; neural basis of fear conditioning; extinction and renewal; extinction mechanisms; remote learning; distance learning; online learning; higher education; neuroscience*

The purpose of this article is to describe an undergraduate laboratory exercise that gives students a taste of what it is like to do real behavioral neuroscience research. The goal is to get students to think like real scientists—to design, do, and interpret experiments that answer questions—not just grind through a cookbook lab. The exercise focuses on the behavioral properties and neurophysiological mechanisms of fear conditioning. We have chosen this subject matter because learning is a core topic in the study of psychology, and the understanding of the neural mechanisms of learning has undergone tremendous progress in the last 50 years. In particular, research on fear conditioning has played a central role in this progress. The objects of study here are not living organisms; rather, students do experiments on a computer-simulated "virtual" animal named "FraidyRat". Because of the virtual nature of FraidyRat, students are able to create and re-do experiments at very low cost without sacrificing animals or incurring the expense and care of a vertebrate animal colony. As with other virtual labs (Grisham et al., 2008; Diwakar et al., 2014), FraidyRat provides an effective learning experience.

Using FraidyRat, students are able to do virtual versions of the experiments that real researchers used to investigate the same questions. Students also experience experimental research design and interpretation analogous to actual research. In fact, FraidyRat provides an example of a case where the practice of science had consequences for STEM education. Co-author Franklin Krasne, who created the code for these modules, believed that extinction phenomena could be explained without resorting to inhibitory mechanisms that are usually postulated. In developing and writing the code for a model, he discovered that some of the phenomena alleged to involve inhibitory mechanisms do not demand such a construct, but others do. What ultimately emerged was a general model of fear conditioning (Krasne et al., 2011) and a great teaching tool.

FraidyRat was constructed to work on principles similar, *but not identical*, to those that are currently thought to underlie real fear conditioning. Krasne et al. (2011) provides an extensive review of the experimental literature on which FraidyRat is based. This review should provide adequate background material for introducing FraidyRat to students.

Students are told that they should think of FraidyRat as a mutant or alien rat that they have been given for analysis. Once they are introduced to the literature on real fear conditioning, they have some idea of the things they might find in FraidyRat, but they never know for sure what their own experiments are going to show. Thus, they learn something about real learning mechanisms. More importantly, they have an experience mirroring professional scientists working on the same problems. We feel that this sort of experience is a valuable part of their scientific education.

Although a very wide range of experiments is possible, only our recent use of FraidyRat will be detailed here. We have used FraidyRat as a three-week long segment of a larger laboratory course during which students attend one lecture and one 3-hour lab session per week. Thus, the time available to us is limited. This paper will focus on what we do during these three weeks—we believe that an exercise of this duration and scope is likely to be useful as a part of either laboratory courses in behavioral neuroscience or as a laboratory component of courses on the neuroscience of learning.

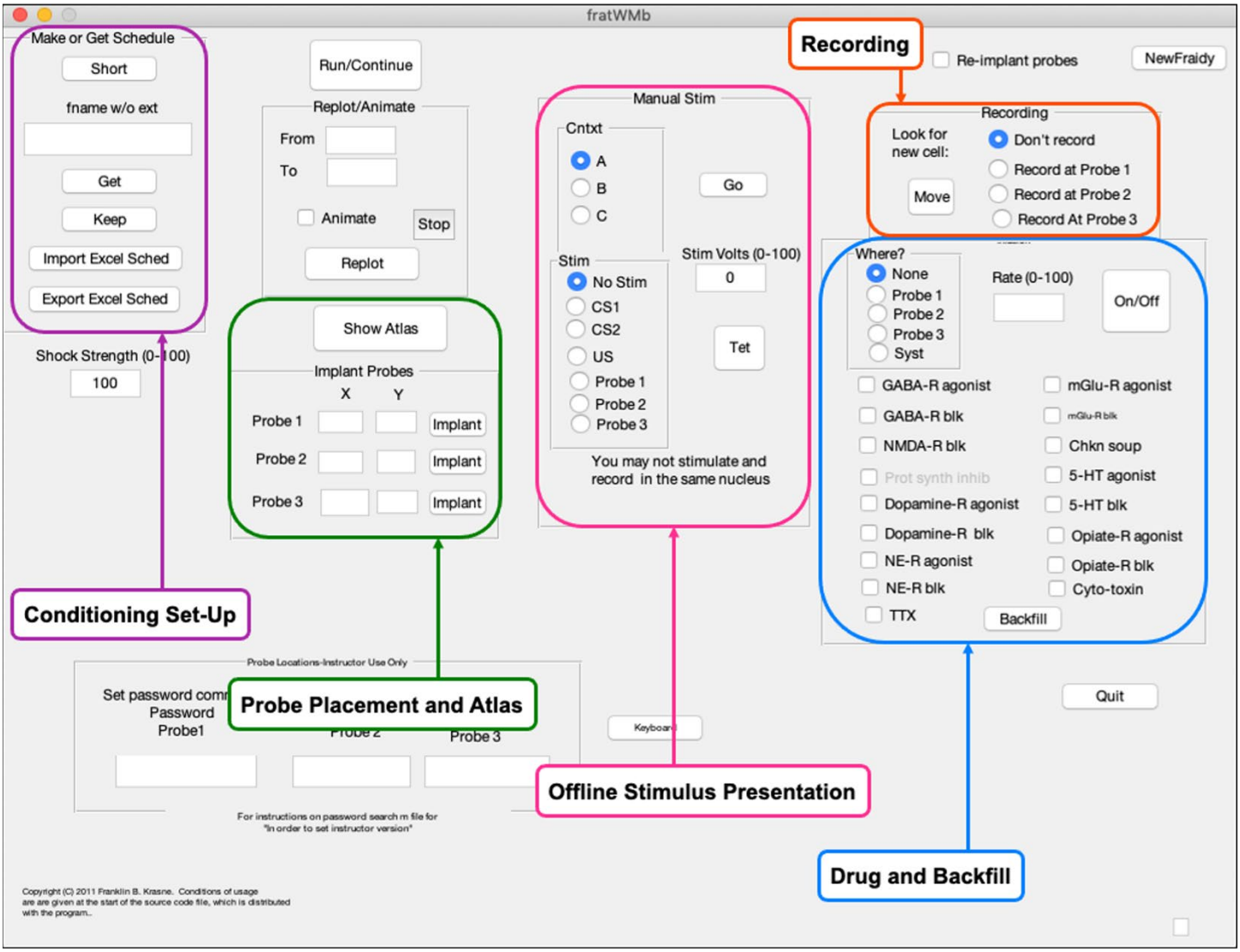


*Figure 1.* Control panel for FraidyRat showing the variety of things that can be done in various experiments. A brain atlas is provided so that students may direct probes. Manual stimulation allows students to test various states of FraidyRat's nervous system offline without changing its brain.

## MATERIALS AND METHODS

### Overview

We present and guide students in their "research" on FraidyRat in a way that is consistent with best practices in education. Our approach provides problem-based learning with scaffolded instruction and formative assessments, which are all considered strong pedagogical approaches (Belland et al., 2017). The scaffolded nature and formative assessment not only check students' progress with the material but also shape students' thinking so that they are capable of attacking the problems presented to them. To accomplish these goals, students are presented with prelab problems that provide an opportunity to engage in deep critical thinking and to develop deductive reasoning skills. Each week's lab session is preceded by a short reading assignment and some prelab "preparation questions" designed to prepare students for the lab work for that week. Answers to the questions are provided as soon as they are turned in. Grading on these is extremely lenient—their purpose is not evaluative but rather to prepare the students for the lab work they will do. The Final Report, currently six essay questions with multiple parts, poses problems of various levels of difficulty and provides a summative assessment of the students' mastery of what they learned during the three weeks of the FraidyRat module. (Formative and summative student assessments are provided in the supplementary documents, found at: https://mdcune.psych.ucla.edu/modules/fraidy-rat/.)

During the first lab session, students learn to use the FraidyRat program, map out connections in FraidyRat's brain, and determine which connections are important for expressing fear behavior. During the second week, they address the question of what regions of FraidyRat's brain: (1) contain the cells and plastic synapses that change their properties so that conditioned behavior results, and (2) whether the changes in those regions are due to Hebbian(NMDA-dependent) plasticity, which has been

| *Activity* | *Learning Outcome* |
|---|---|
| **Learn Program Basics** | Facilitates understanding of the rest of the unit. Understand classical conditioning basics. |
| **Unit Recording** | Learn about different types of neural activity (e.g., tonically active cells). |
| **Drug Infusion** | Learn how blocking receptor sites can alter neural firing/behavior. |
| **Circuit Mapping** | Understand how selective drug administration can reveal sites necessary for fear behavior. |

*Table 1.* Week 1

introduced in lecture and assigned reading. During the final week's session, students do experiments to learn about the nature of extinction in FraidyRat, particularly whether it is due to erasure of conditioned learning, or inhibition of conditioned responses, or both. At the end of each lab session, students must earn an "exit ticket" by explaining to an instructor what they have found and concluded for each of their experiments to make sure they understand what they did and what it meant. More detailed descriptions are below.

In the first week of the module (outlined in Table 1), students learn how to download the FraidyRat application and get familiar with the control panels by which FraidyRat can be manipulated in behavioral experiments (behavioral readouts and animations) or with a wide range of classic tools in neuroscience. These tools include retrograde tracing, unit recording, electrical stimulation and systemic and intracerebral drug infusion. The control panel can be seen in Figure 1.

Experiments can be set up to occur in any of 3 contexts, and the resultant freezing behavior observed as a measure of fear. Initially, students conduct a simple fear conditioning and extinction study. Freezing behavior results from fear conditioning, which we can show graphically (Figure 2) or via an animation. Since we focus on cued fear conditioning, we often extinguish FraidyRat in a different context from that in which it was conditioned.

Students are then introduced to our 2-D stereotaxic atlas (a single sagittal section, Figure 3). This stereotaxic atlas guides the implantation of probes used for electrical stimulation, recording, infusion of drugs, making lesions in specific brain regions, or infusing retrograde-transporting dyes that allow anatomical pathways to be mapped. Students first use this atlas to place probes and configure them as extracellular single unit recording electrodes. They are asked to record the activity of cells in Grisham's Nucleus, which fire tonically at a high rate, making it easy to see when one is recording from a cell. (Grisham's Nucleus is the only nucleus in FraidyRat that does not have a homolog in real rats).

Students advance their electrodes and keep doing so until they see unit activity as in Figure 4. Students also see that (as in reality) one doesn't always encounter a cell with a given electrode placement, so they may have to advance their electrode several times before they successfully record

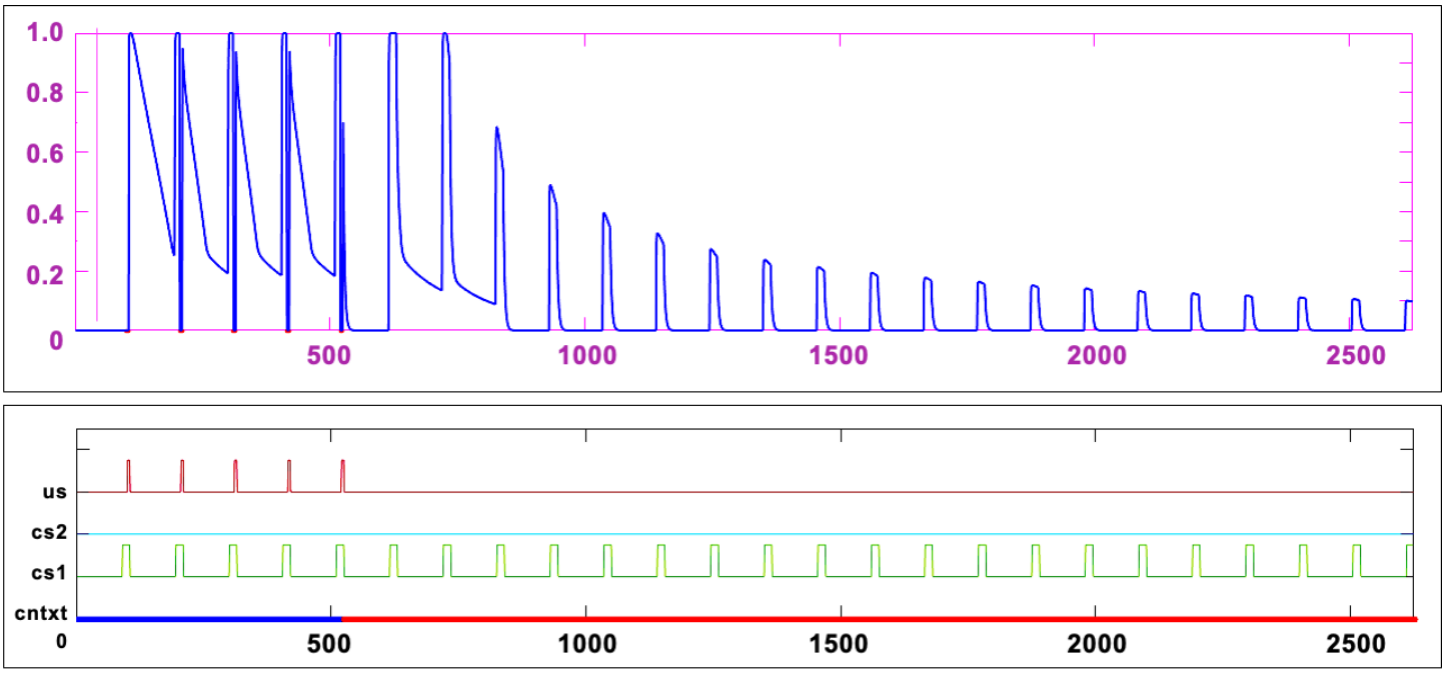


*Figure 2.* Behavior graph of FraidyRat in a session with 4 conditioning and 30 extinction trials. The top panel reflects freezing as a function of time (freezing—stillness—is up). Bottom panel shows the CSs (thin green line) and USs (thin red line) as they were delivered in time. The bottom-most line reflects the context in which FraidyRat was placed. In this example, he was conditioned in context A (blue line) and extinguished in B (red line).

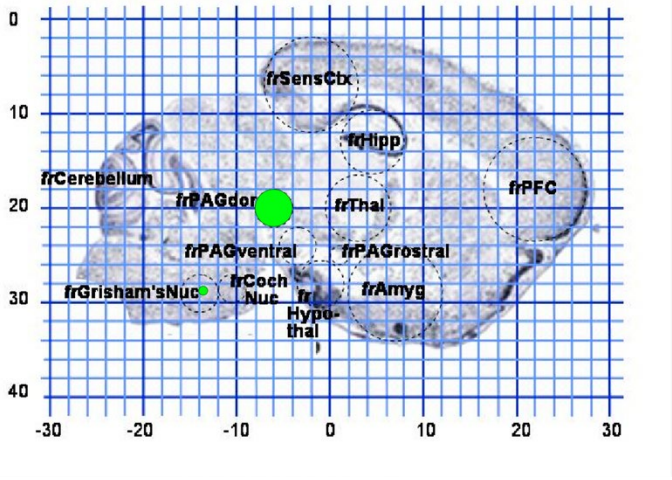


*Figure 3.* 2-D atlas of FraidyRat's brain displays the results of a retrograde tracing study showing that PAGdorsal neurons project to Grisham's Nucleus. Although Fraidy's brain shows a great deal of homology to an actual rat's brain, all areas are prefaced by "*fr*" to disabuse students of the notion that they are absolutely like a real rat's. Abbreviations: *fr*SensCtx—sensory cortex; *fr*Hipp—hippocampus; *fr*PFC—prefrontal cortex; *fr*Cerebellum—cerebellum (the only area that can't be manipulated, but it gives students a sense of rostral and caudal); *fr*PAGrostral, *fr*PAGdorsal, and *fr*PAGventral—rostral periaqueductal gray, dorsal periaqueductal gray, and ventral periaqueductal gray, respectively; *fr*Thalamus—thalamus; *fr*Amyg—amygdala; *fr*Hypothal—hypothalamus; *fr*CochNuc—cochlear nucleus; and *fr*Grisham's nucleus, which has no homologue in a real rat's brain and awaits discovery.

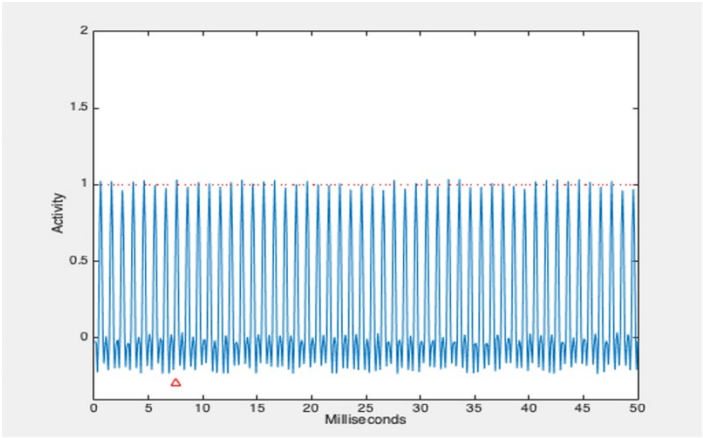


*Figure 4.* Sustained tonic activity of Grisham's nucleus in an unconditioned FraidyRat.

from a cell.

Students are told that neurons in Grisham's Nucleus are spontaneously active and that they drive exploratory behavior. When these cells stop firing, FraidyRat freezes.

Students then do an experiment in which they see that conditioning causes Grisham's cells to stop firing. It is sometimes convenient to use the slowing of Grisham's cells' firing as a proxy for freezing because such slowing causes FraidyRat's freezing behavior.

Students examine the effects of infusing drugs both systemically and intracerebrally. Initially, they use the effect of a GABA receptor agonist on the firing of Grisham's cells to give them practice with this technique. Drugs being infused intracerebrally need to cover the given nucleus, and we give students appropriate direction—as in reality, overly large infusion rates will affect other nuclei and give results that are uninterpretable.

Students then introduce a probe in the PAGdorsal to provide electrical stimulation while recording from Grisham's nucleus. When the stimulation is applied in PAGdorsal, the firing rate of Grisham's Nucleus drops for a few milliseconds (Figure 5) because PAGdorsal cells inhibit Grisham's cells. They then can conclude that FraidyRat's freezing to a conditioned CS occurs because the PAGdorsal cells fire during the CS, and in turn inhibit Grisham's cells causing FraidyRat to freeze.

The next activity is retrograde tracing, which can be accomplished via the drug panel—on the very bottom of this panel there is a button for "backfill" (Figure 1). If the backfill is applied via a probe implanted in the coordinates given by the atlas (Figure 3), this retrograde marker will reveal brain areas that project to the region of interest. We demonstrate by backfilling Grisham's nucleus and showing that only the PAGdorsal lights up (Figure 3). The backfill is intended to mimic HRP tracing (McDonald, 1992). This backfill study provides a teaching moment to talk about retrograde and orthograde axoplasmic flow.

Having demonstrated this technique, small groups of students are assigned a given nucleus to find what projects to it. Students report out the connections along with the radius and coordinates of their region. These data are then displayed for the entire class for later use (Figure 6A). (Some regions, such as the cochlear nucleus, do not receive afferents from any region on the atlas.)

The last activity of this first week is to have students use some of the methods that they have learned to map out the regions needed to express previously conditioned fear (freezing) behavior (what we call the "fear expression pathway"). Students first condition FraidyRat and then shut down various nuclei one at a time via infusions of GABA agonists. As mentioned above, students are urged to use the activity in Grisham's Nucleus as a proxy for behavioral fear in this experiment. (Activity in Grisham's Nucleus will be suppressed in response to a conditioned CS if the fear expression circuit is intact.) If the inactivation of any given nucleus prevents the CS presentation from diminishing activity in Grisham's Nucleus, they can conclude that the suppressed region is part of a fear expression pathway. They find, for example, that the fear expression pathway for cued fear includes the cochlear nucleus (because the CS is a tone) and the amygdala (the locus of the engram). Further, students will find the hippocampus is involved in contextual fear but not cued fear. We usually split up this task among students and have groups examine a given region and then report their results to the class. Students arrive at a complex-looking diagram like that in Figure 6B. But we then show them that, portrayed differently, the pathway is remarkably simple as shown in Figure 6C (and more or less like what is thought to be the case for real animals). Although students do not know all of the details of

| *Activity* | *Learning Outcome* |
|---|---|
| **Use NMDA Blocker** | Understand LTP. |
| **Differentiate Cells' Electrophysiological Activity** | Learn about different types of neural activity (e.g., tonically active cells). |
| **Antidromic Stimulation** | Learn that axons can be "backfired" and principles of axonal conduction. |

*Table 2.* Week 2 objectives.

the circuit at this juncture, they are working with a circuit akin to that diagrammed in Figure 6D.

During the Week 2 lab session (outlined in Table 2), students determine the locus of the engram in FraidyRat's brain underlying the conditioned response and whether the learning is due to Hebbian LTP (NMDA dependent LTP).

In preparation for the Week 2 lab session, there is a small amount of reading on synaptic plasticity, including Hebbian potentiation. We also present arguments suggesting that the regions of the brain that underlie fear conditioning are likely to be within the cued fear expression pathway itself, which they already know. Students are also asked to read a short background study in which various regions in the fear expression pathway were shut down with a GABA agonist during conditioning, and think about the consequent changes in behavior or the lack thereof. The data from this study are presented without any conclusions being drawn and students are asked, as one of their preparation questions, to infer what the data imply about the locus of the engram. After some thought, they should conclude that as long as neural activity is shut down upstream from the learning mechanism, there would be no learning because information about the CS could not pass through to the region where learning can occur. In the end, students conclude (with some help from us) that learning can occur within the amygdala, but this technique does not rule out sites downstream from the amygdala as possible loci of a learning mechanism. These experiments are not easy to think about, but it is good exercise for their critical thinking skills, and it prepares them for the sort of thinking they will have to do in this week's lab session.

Now realizing that learning must be able to occur in FraidyRat's amygdala, students are asked to see if neural representations in that locus would allow Hebbian LTP. If such representations exist, cells in the amygdala should be strongly excited by the US even before conditioning has occurred. (LTP occurs only if the postsynaptic cell is strongly depolarized when input from the CS occurs.) Students are asked to predict whether amygdala cells should be excited by the US prior to any conditioning and then are asked to do an experiment to see what actually happens (Figure 6D). (At this point, most students make the correct prediction and can do the experiment on their own.)

During the Week 2 lab session, students, through group discussion, determine logical paths to: (1) further test the hypothesis that conditioning in FraidyRat is due to Hebbian LTP, and (2) further establish where the changes that underlie conditioning occur. With some guidance, they decide to see whether infusing an NMDA receptor blocker into the amygdala would prevent conditioning, as it should if the mechanism is truly Hebbian.

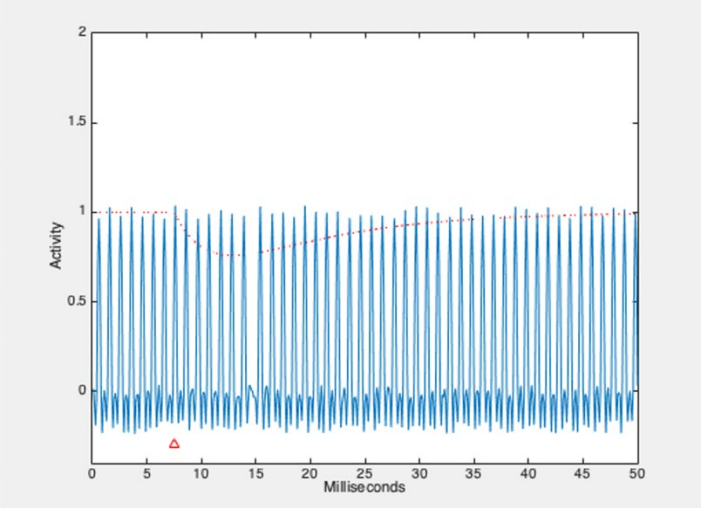


*Figure 5.* Effect of stimulating the PAGdorsal on the firing rate in Grisham's nucleus. Notice that the average firing rate, which is indicated by a dashed red line, dips down for a few milliseconds after the red triangle, which indicates the onset of an event such as stimulation or CS presentation.

Infusing the NMDA blocker into the amygdala yields a FraidyRat that is unable to be conditioned. This provides good evidence that conditioning is due to Hebbian LTP and further evidence that the engram is in the amygdala. This result is also good evidence that there are no engrams downstream in the PAGdorsal or Grisham's Nucleus (Figure 6C). NMDA blockers prevent new LTP but don't disable other neural functions like GABA agonists do. Thus, if the downstream regions contained a learning mechanism, an NMDA blocker in the amygdala would not prevent these downstream regions from changing during conditioning and FraidyRat would still exhibit learning. But the amygdalar NMDA block absolutely prevents conditioning. Thus, students can conclude that the engram in FraidyRat is probably only in the amygdala. This experiment also provides a good teaching moment with regard to controls—when testing the effect of the NMDA receptor blocker, students often do not run a no-drug control and thus cannot conclusively demonstrate that the drug affects conditioning. They also tend to continue the drug infusion during the CS-alone test trials that they give after conditioning. When they do this, we point out that the NMDA blocker they are using is similar to certain street drugs (PCP and ketamine), and it might be that FraidyRat did learn that the CS predicts shock, but was so stoned during acquisition that either acquisition or retrieval mechanisms were impaired! Students also sometimes mistake the post-shock UR for a CR, but careful examination of the event bar shows them that the response is temporally associated with the US rather than the CS.

Students next explore how cells in the amygdala respond to the CS before and after conditioning. The expectation at

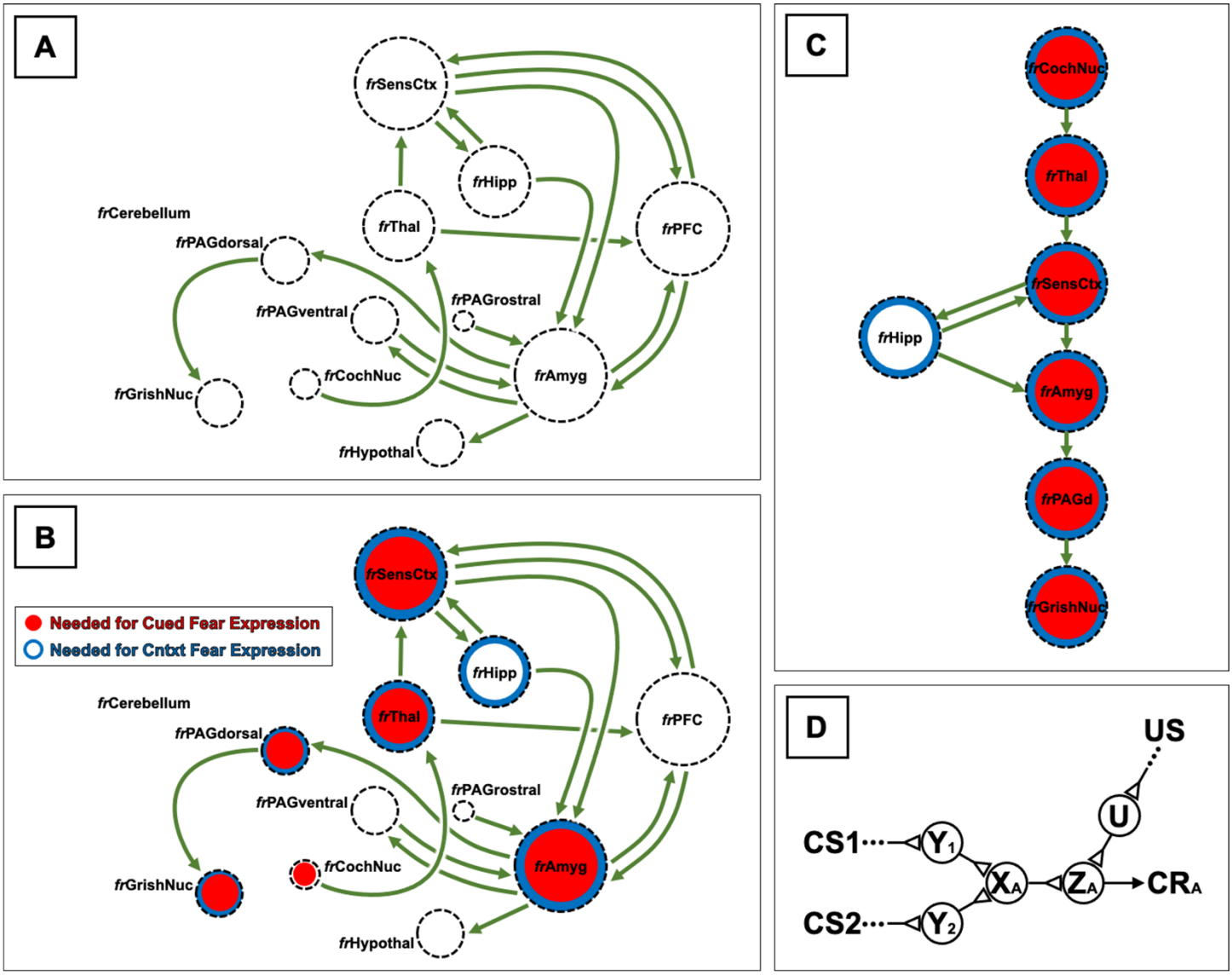


*Figure 6*. *(A)* Connections among FraidyRat's brain regions found by tract tracing. *(B)* Map of fear expression pathways generated by results of GABA agonist suppression study. *(C)* Re-drawn version of 6B, in which the simple nature of the pathway is revealed. *(D)* Circuit somewhat similar to FraidyRat's. In this circuit, The cells representing the two CSs (Y1 and Y2) have excitatory synapses on neuron $X_A$, which in turn excites neuron $Z_A$. Besides receiving depolarizing input ultimately resulting from CS presentation, $Z_A$ also receives strong depolarizing input via the U cells, which represent the US (*fr*PAGrostral in FraidyRat). Neuron $Z_A$ has the NMDA receptors responsible for LTP. Neuron $Z_A$ likely resides in the amygdala. (Notably, FradyRat's amygdala is a gross simplification of a real rat's amygdala—Duvarci and Pare, 2014).

this point is that there should be cells that do not respond much to the CS before conditioning and respond a lot after conditioning. (We refer to such cells as "conditioning cells".) Students discover that there are two cell types in the amygdala: one type responds dramatically to the conditioned CS by making a flurry of action potentials (Type 1), and the other responds rather minimally but does increase its firing rate after conditioning (Type 2); neither type responds at all to the CS prior to conditioning. Firing rates of Type 1 and Type 2 cells are shown in Figure 7. At this point, it seems pretty likely that the Type 1 cells are "conditioning cells" (i.e., are ultimately responsible for FraidyRat's freezing response to conditioned CSs), but what the Type 2 cells are doing is something of a mystery. Are they just "bad" conditioning cells or something else?

Students know from their earlier anatomical tracing that the amygdala has cells projecting down to PAGdorsal. But now that they know there are two types of cells, it becomes of interest to try to find out which type of amygdala cells project to the PAGdorsal because they would likely affect freezing behavior. At this point, we discuss a technique often employed in real neurophysiological research, which is whether an antidromic spike can be elicited by stimulating the region where the putative axon terminals would be. Students examine both amygdala cell types and find that they can produce an antidromic spike in amygdala Type 1 cells but not in Type 2 cells. This result suggests that Type 1 cells project to the PAGdorsal as they would expect

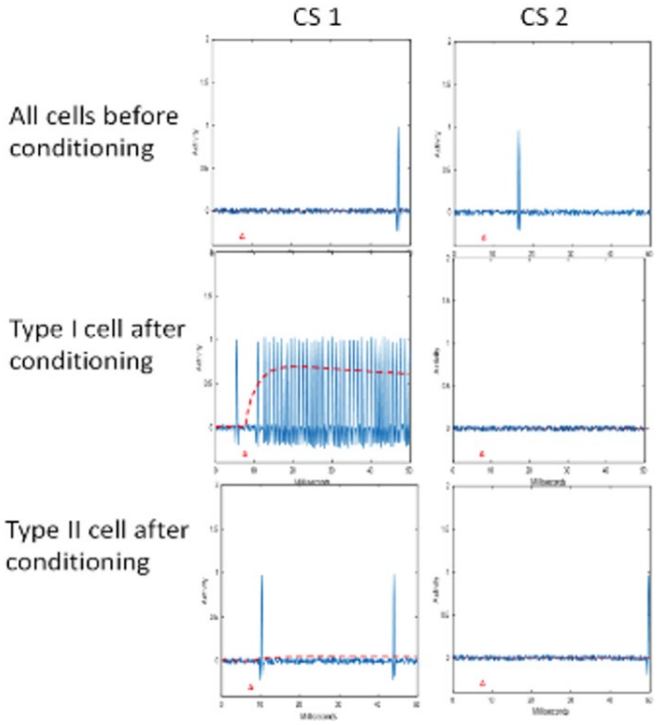


*Figure 7.* Firing rates of amygdala cells to two different CSs. When responses are elicited by the CS, two cell types are revealed—one cell type that responds a lot (Type 1 cells), and another cell type that only increases responding a little (Type 2 cells). Only CS1, which was conditioned, evokes a response in the amygdala, which establishes that the cell responses are not reflecting pseudoconditioning.

conditioning cells to do, but Type 2 cells do not. During the following week they will get evidence that the Type 2 cells are inhibitory neurons involved in extinction.

During the Week 3 lab session, students do experiments aimed at determining whether extinction in FraidyRat is due to "erasure", which is a reversal of the neural changes during conditioning, or whether those changes persist but are masked by some sort of learned inhibition. Rather than simply ask that question in the abstract, we take this opportunity to illustrate science's hypothetico-deductive method. We propose four specific (not necessarily mutually exclusive) hypotheses that are similar to ones that have been considered during research on real animals. Then students do experiments to evaluate their validity (Figure 8). Specifically, the hypotheses given to students to explain extinction are outlined in Table 3.

In hypotheses II-IV, synaptic input onto inhibitory neurons is strengthened if the fear expression pathway is activated but there is no US presented. Synapses on the inhibitory neurons are strengthened much like synapses of cerebellar granule cells on cerebellar Purkinje neurons, which involve postsynaptic metabotropic glutamate receptors (see Hirano, 2013, for a review). (Students may discover this fact using appropriate drugs available in FraidyRat.)

Notably, the three inhibitory hypotheses differ with regard to where the inhibition originates or is applied. In the end, it will turn out that most of their experiments will be consistent with inhibitory Hypothesis III and will rule out Hypotheses II and IV. They will, however, do one experiment that shows that there is also a small bit of erasure (Hypothesis I).

| ***Hypothesis*** | ***Description*** |
|---|---|
| **I** | Erasure Hypothesis: A weakening of the potentiated synapses in acquisition. |
| **II** | Inhibitory Hypothesis: Potentiation of synapses onto the inhibitory PFC neurons—inhibitory action is on PAGdorsal and amygdala Type 1 neurons. |
| **III** | Inhibitory Hypothesis: Synapses onto inhibitory interneurons in the amygdala, which inhibit Type 1 neurons. |
| **IV** | Similar to Hypothesis III, except that the inhibitory site of action is in PAGdorsal. |

*Table 3.* Week 3 extinction hypotheses.

Again, the week (outlined in Table 4) begins with some reading introducing students to experiments on renewal, the return of extinguished cued fear when the context is changed in extinction (Bouton, 2004)—this result is found in real animals and provides strong evidence of inhibition in extinction. Their reading also explains exactly how inhibitory Hypotheses I-IV predict the renewal phenomena. Students are given a prelab quiz that should help guide their thinking for this final week of the module. They are also asked to do a renewal experiment on their own (the results of a typical such experiment are shown in Figure 9). We make sure at this point that they understand that this phenomenon rules out erasure as the *sole* mechanism at work during extinction. There must be some inhibitory processes at work to get the renewal phenomenon.

Given that students understand that extinction must involve inhibition, the next logical step is to determine the origins and targets of the inhibition. Hypotheses II-IV provide three specific hypotheses to test. The third lab session begins with experiments to try to discriminate among these hypotheses. A popular hypothesis is that the PFC is involved in extinction (Izquierdo et al., 2016; Figure 8—Hypothesis II). To test this hypothesis in FraidyRat, PFC neurons should be inactivated with a GABA agonist. It turns out that inactivating PFC neurons does *not* reverse extinction in FraidyRat—thus providing evidence against Hypothesis II. Students then go on to use a GABA receptor blocker to determine where inhibition occurs in the process of extinction. They discover that blocking GABA receptors reverses extinction when infused into amygdala but has no effect in PAGdorsal, thus providing evidence against Hypothesis IV.

The students' findings so far indicate that the neural basis

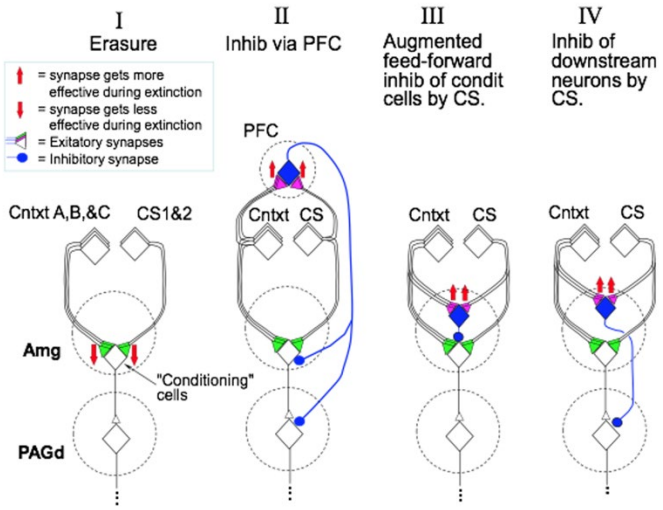


*Figure 8*. Schematic diagram of the four hypotheses of extinction given for consideration. Cell bodies are represented by diamonds, excitatory synapses are triangles, inhibitory synapses are small blue circles. Green- and fuchsia-colored terminals represent plastic synapses—green are NMDA-mediated plastic "Hebbian" synapses whereas the fuchsia are plastic synapses using mGluRs. Arrows accompanying neuron terminals show the postulated direction of potentiation during extinction only. Although we present the regions as hypothetical places, at this juncture the students know that the learning mechanism must be in the amygdala, so it is apparent to them that region 1 is amygdala and region 2 is PAGdorsal.

of extinction in FraidyRat is most consistent with Hypothesis III. The next obvious step is to record from the two cell types of the amygdala during conditioning and extinction to see how these cells behave. They find that Type 1 cells increase their firing rate during acquisition and decrease their rate of firing during extinction, while Type 2 cells do the opposite. They can also see that infusing a GABA receptor blocker into the amygdala after extinction restores the Type 1 cells to its original firing rate whereas the activity of Type 2 cells does not change. Students should be able to infer that the Type 2 cell is likely an inhibitory interneuron and the Type 1 cell is excitatory. Since Type 1 cells have also been demonstrated to project downstream to the PAGdorsal by the antidromic stimulation experiment described above, it is likely that they are the conditioning neurons.

Although the renewal experiment established that erasure is not the sole process involved in extinction, the final in-class experiment deals with whether or not erasure contributes at all to extinction. If there were no erasure, then blocking GABA receptors should reverse extinction completely, and extinction must be entirely due to an inhibitory process. Whereas if there is some erasure, an infusion of GABA blocker should not completely reverse extinction. However, designing an experiment to reveal erasure is a bit tricky and provides another teaching moment regarding the importance of proper control procedures.

If students compare freezing after acquisition to freezing after extinction when they administer a GABA receptor blocker in the amygdala, they find freezing returns to roughly the pre-extinction level. The facile conclusion is that there is no erasure. The correct experiment, however, entails comparing freezing before and after extinction with GABA receptors blocked in both conditions. If this is done, students get the results similar to Figure 10, which clearly indicates that extinction did in fact cause some erasure. It may seem paradoxical that GABA receptor blockage increases the before-extinction level of fear, but, as we discuss with the students, one possibility is that the synapses on the inhibitory neurons may be potentiated a little in acquisition. Notably, the Type 2 cells (the inhibitory neurons) do potentiate slightly during acquisition (Figure 7C).

| ***Activity*** | ***Learning Outcome*** |
|---|---|
| **Examine Successive Extinctions with Different Contexts** | Understand the phenomenon of renewal; understand that extinction is new learning—at least in part. |
| **Probe Neural Bases of Extinction** | Understand that both inhibition and erasure underlie extinction in FraidyRat. |
| **Examine Different Hypotheses about Renewal** | Understand how hypotheses can be tested and ruled out. |

*Table 4.* Week 3 objectives.

At the end of the in-class exercises, the only viable inhibitory hypothesis is Hypothesis III (Figure 8). Nonetheless, as part of the students' final reports, a renewal experiment is proposed whose outcome they are asked to predict assuming Hypothesis III. If they have a good understanding of the material, they should realize that Hypothesis III predicts that there will be no renewal with the proposed experiment. They are then asked to do the experiment, but when they do, they find that renewal is in fact still present. We then ask, given the outcome of this experiment, what should be the next step in the analysis of FraidyRat? The "right" answer is something like "go back to the drawing board and try to construct a hypothesis that is consistent with all that is now known about how FraidyRat behaves". Some students seem disturbed when all hypotheses fail to explain a phenomenon even though this is the process of science. Perhaps they cannot conceive that all of their professor's hypotheses are wrong!

**Student Misconceptions**

In addition to some discomfort about all posited hypotheses being unable to explain renewal, conceptual obstacles that we have encountered are few, but notable. In Weeks 1 and 2, students frequently confuse the effects of GABA agonists versus the effect of NMDA receptor blockers. The GABA agonist completely shuts-down the actions of the neurons whereas the NMDA blocker merely blocks the acquisition of new learning but leaves the other neural activities intact. Students also seem sometimes confused about what is meant by an inhibitory neuron. Some students believe that

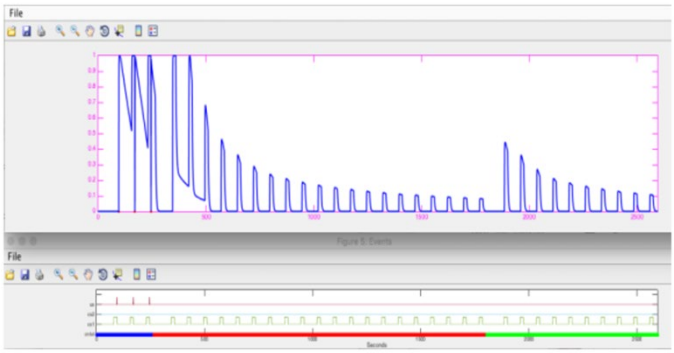

*Figure 9*. Renewal experiment. The top graph shows freezing (freezing is up on this graph; exploring is down). The bottom graph is an event graph. Upswings in the thin green line indicate CS1 presentations, and those in the thin red one show US presentations; the thick lines at the bottom indicate different contexts (blue = context A, red = context B, green = context C). Note that extinguished behavior "renews" when extinction context is changed from B to C.

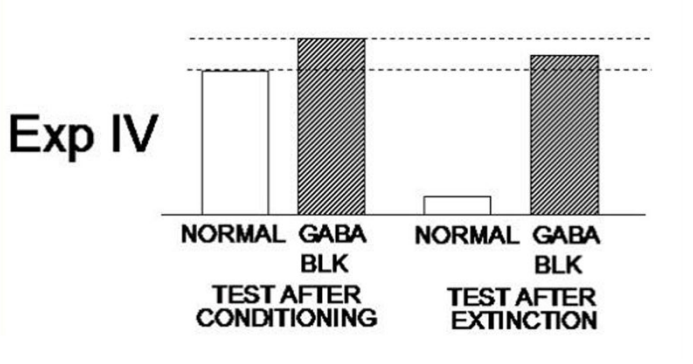


*Figure 10*. Bars indicate amount of "fear" as indexed by rate of amygdala conditioning cell firing. Crucial comparison is between GABA blocker infused into amygdala after conditioning and after extinction. Because GABA blockade does not reach the same level after extinction as after conditioning, students can infer that although extinction does involve GABAergic mechanisms, there is also some erasure involved.

an inhibitory neuron itself is hyperpolarized and inhibited rather than releasing GABA onto another neuron, which is then inhibited. In Week 3, some students confuse a GABA agonist and a GABA antagonist. Further, in the third week, when examining extinction, some students stumble with the idea of renewal; some think that it is either a reacquisition phase or spontaneous recovery. Finally, there are synapses that are potentiated in the course of extinction (and are responsible for extinction in FraidyRat), but these synapses involve an mGluR receptor mechanism rather than an NMDA-mediated mechanism (Toth et al., 2012). Students sometimes have trouble considering an mGluR-mediated mechanism in extinction when an NMDA-mediated mechanism worked so well in acquisition.

### Pedagogical Assessment

*Subjects*

Data were obtained from 146 UCLA students in two different Fall terms. Nearly all students were senior psychobiology majors although there were a few neuroscience, psychology, and cognitive science majors. An IRB exemption was obtained for this research.

*Methods and Materials*

We assessed the efficacy of FraidyRat with a quiz, using a pre-post design. The pretest consisted of 11 content items and 12 critical thinking items. Some of the critical thinking test came from the Cornell Critical Reasoning Test, Form X (Ennis, 1993; Ennis et al., 1964) and others from various open-access sources. The post-test was identical to the pretest but also included affective Likert scale measures of attitudes about the experience, free response items to discern what the students perceived the purpose was, and what the students liked and didn't like about the module.

## PEDAGOGICAL RESULTS

The full scale of the pre-post quiz showed excellent reliability when considering the post-test scores (Cronbach's alpha = 0.798—which is in the range of the SAT). The full scale with all 23 content/critical thinking items showed a significant difference between the post-test and the pre-test, $t(145) = 4.391$, $p < 0.001$, Cohen's $d = 0.363$ (Figure 11). The items selected were difficult because we wanted to rigorously discern genuine gains—thus the pre-test to post-test difference was not large, albeit highly significant with a substantial effect size as shown by the Cohen's *d*. An examination of the content items alone yielded a larger post-test to pre-test difference, $t(145) = 5.119$, $p < 0.001$, Cohen's $d = 0.430$. An analysis of the critical thinking items showed a strong trend toward positive gains, albeit just short of significance, $t(145) = 1.818$, $p = 0.06$, Cohen's $d = 0.157$ (Figure 11).

Affective items as well as free-response items generally showed that students had a positive experience with the FraidyRat module (Figure 12). The only aspects of the experience that students did not rate highly were the manual and occasional instability of the FraidyRat application, which have subsequently been revised.

Three open-ended qualitative questions were posed, and the responses categorized after examining the responses. The first was: (1) "Describe the purpose of the module." Most of the categorizations are self-evident, but the "Simulate Experiments" category included statements such as "useful simulation of animal study without using real animals which may be more expensive, time consuming, and impractical", and "the purpose of using the FraidyRat is for students to see firsthand how small changes in experimental design can affect a rat's behavior" (Figure 13A). We also asked: (2) "What did you like most about using the Fraidy Rat program?" The "Easily Change Experimental Parameters" category included some responses such as "I liked most that you could mess up once but simply do another experiment whereas a real experiment would have cost you weeks or months", and "I liked having access to the different drugs and seeing their effects on circuits", and "I liked creating my own experiments and being able to easily change what I was looking at" (Figure 13B). Further, we asked students: (3) "Tell us more

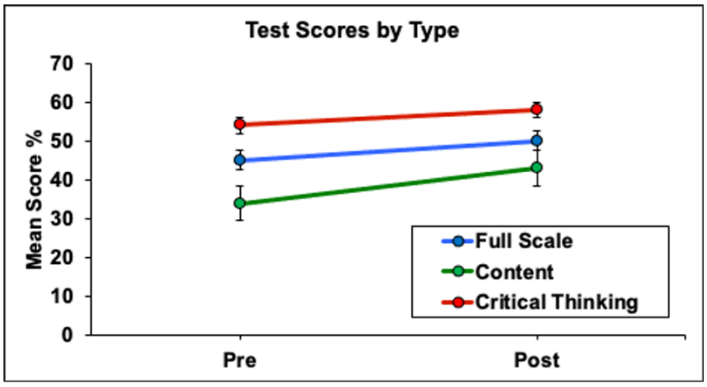


*Figure 11*. Results of the pre-post tests as full scale and broken down into content and critical thinking items. Error bars are the standard error of the mean.

about what you did and did not like about the Fraidy Rat module, and what did you like most about using the Fraidy Rat program." Since the responses were both negative and positive, we split them into likes and dislikes, which are represented in Figure 13C and 13D.

## DISCUSSION

The pedagogical aims of the FraidyRat module were not only to teach something about classical conditioning and neural circuits, but also to buttress their critical thinking skills (Ennis et al., 1964; Ennis, 1993). Content items in our pre-post tests tapped both facts and reasoning about the neural bases of learning and extinction and also posed reasoning problems using the vocabulary of the module. When content items were separated from critical thinking items, the t-tests showed that students made significant gains in content.

We were also encouraged by a healthy trend toward gains in critical thinking. Critical thinking as an independent psychological construct has been questioned, and some maintain that it is only domain-specific. But there is some evidence that it exists as a general construct (for a review, see Huber and Kuncel, 2016). Teaching critical thinking skills has not met with great success. Nursing programs are mandated to teach critical thinking skills as a part of their curriculum. Yet, efficacy assessments of gains in critical thinking in nursing programs have produced mixed results at best, yielding the conclusion that there has been no clear effect (Huber and Kuncel, 2016; Jones and Morris, 2007). Given that the module was only three weeks in length, the possibility that we enhanced students' approach to critical thinking is exciting.

The affective responses were generally positive as seen in Figure 12. Items on the affective measures with which students tended to disagree reflected their opinions about the lab manual and instability of the application. Since then, we have modified the application to improve stability, and we have made substantial revisions to the lab manual for clarity.

We have successfully taught this module a number of times, and we continue to refine both the FraidyRat application as well as the accompanying manual. In this article, we have detailed a single module that students accomplish in three 3-hour labs with three additional

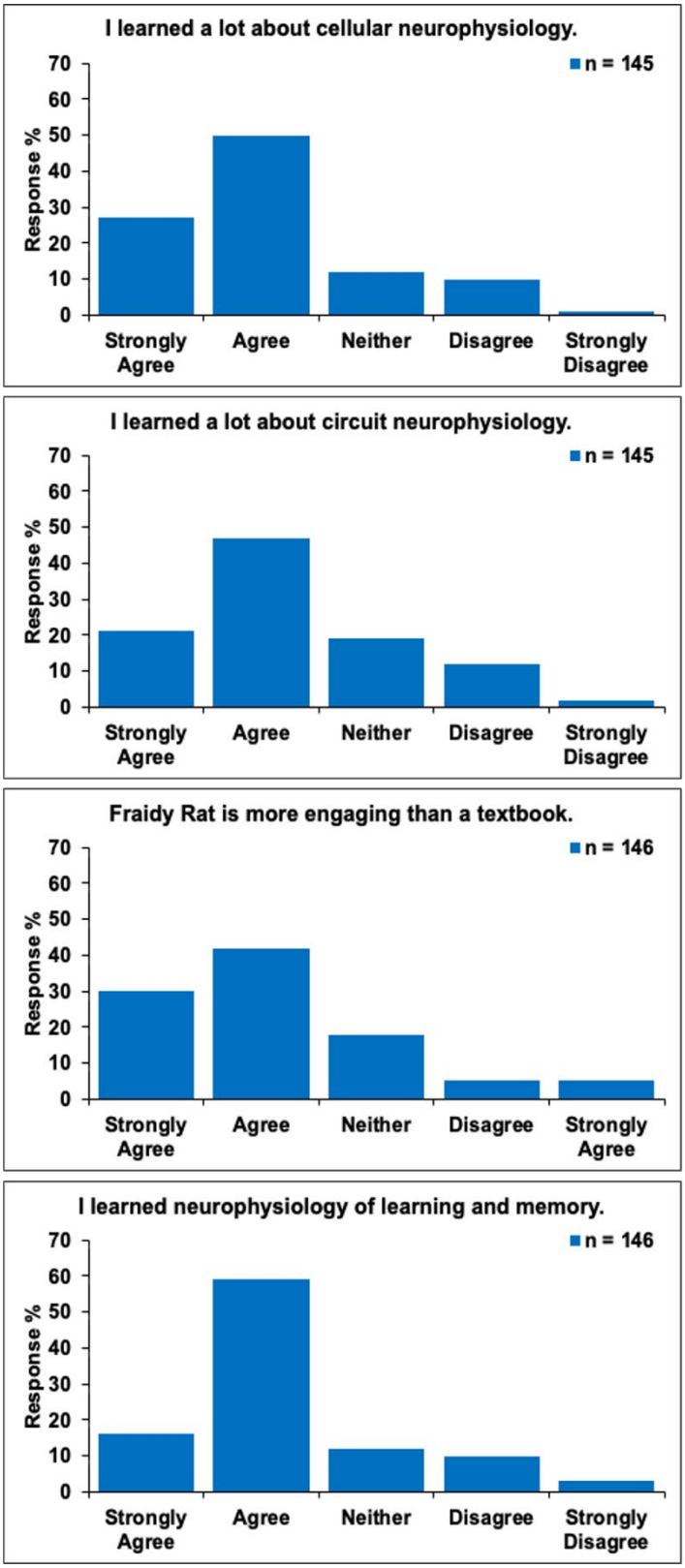


*Figure 12.* Results of selected Likert scale evaluative/affective items.

lectures, but the module could be expanded to encompass more weeks by having students perform the background studies instead of just reading about them, and/or by using FraidyRat to explore more phenomena. Alternatively, if

instructors have less time to devote to FraidyRat, they could simply not present the materials in Week 3. FraidyRat is ideal for any class size, even small sections, because each student can derive conclusions from their own data without the necessity of combining their data with others—although group efforts can also be encouraged.

FraidyRat is deeply and richly programmed so that other modules are possible. These other modules also mirror findings in the behavioral neuroscience of fear conditioning. These modules include not only the cued fear learning mechanisms considered here but also: (1) mechanisms of context fear conditioning (and its relation to hippocampus and systems consolidation), and (2) reinforcement mechanisms (including blocking mechanisms and the role of neuromodulators in controlling synaptic plasticity). Further,imaginative instructors can construct modules of their own device because of the broad range of tools available. Materials, including a Zoom-recorded tutorial, are available and describe a range of experiments that can be done with FraidyRat as well as a description of the

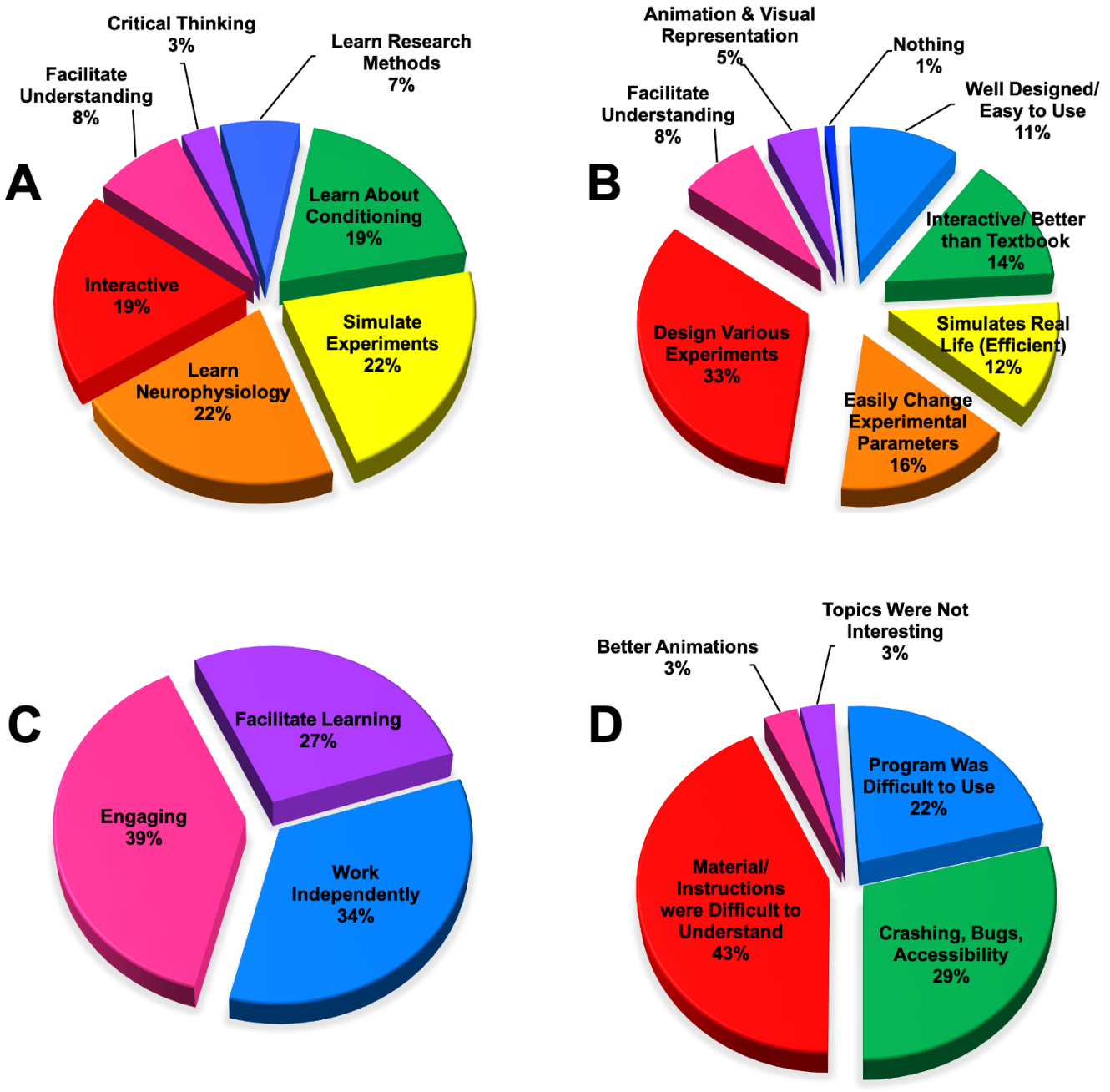


*Figure 13*. Categorization of responses to open-ended programs. *Chart A* relates to students' perceived purpose of FraidyRat. *Chart B* displays what students liked most about using the Fraidy Rat program. *Charts C* and *D* display what students liked versus what they did not about FraidyRat, respectively. All responses were characterized and the percent in the various categories are displayed. (A given response could appear in more than one category).

neurophysiological mechanisms and neural circuitry that the computer program simulates. Instructors should request faculty materials at https://mdcune.psych.ucla.edu.

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