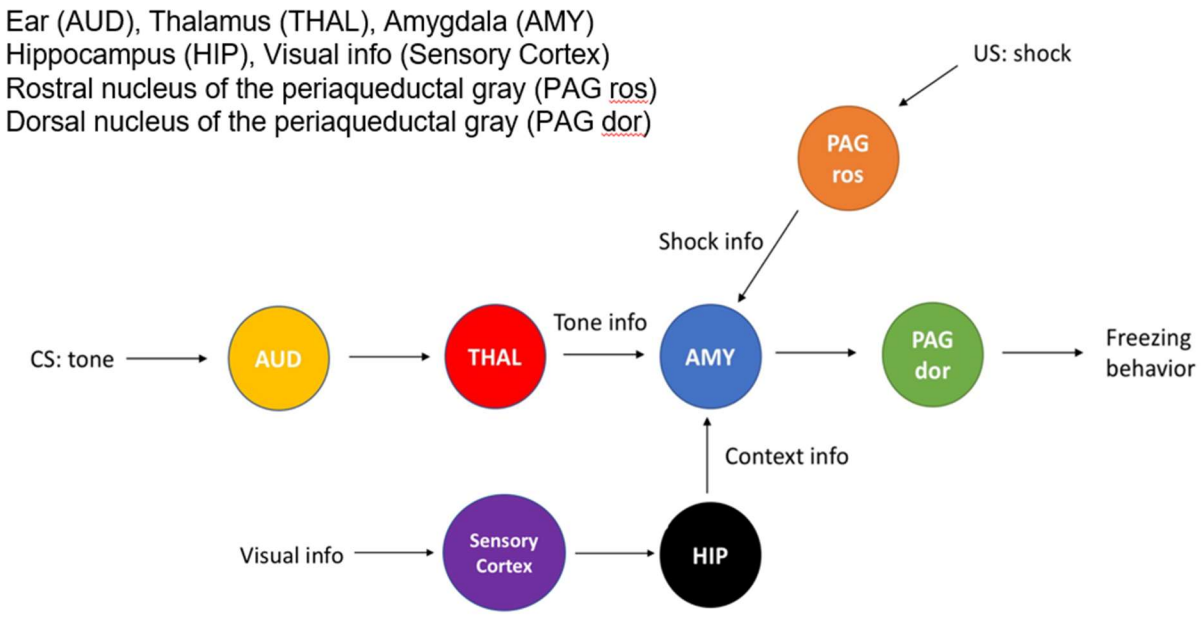
Lab 7: Brain Circuits with Fraidy Rat

You may be familiar with Pavlov's experiment with dogs, where he trained dogs to salivate to the sound of a metronome (or bell, as the false stories say), in what has been called classical conditioning. Today, the standard way to measure classical conditioning or associative learning is through fear conditioning in rodents. This teaches a rat to display fear behavior based on past experience. Thus begins a two-part lab where we can train a virtual rat (Fraidy Rat!) to fear things. Today we will focus on how brain areas within Fraidy Rat are connected in a circuit that help promote both innate and learned behaviors related to fear.

In fear conditioning, rodents learn to associate things in their environment with a fear-inducing stimulus, electric shock. *Electric shock is referred to as an* ***unconditioned stimulus (US),*** because on its own, it automatically elicits a behavioral response. The behavioral response of fear for a rodent is freezing (complete lack of movement). When a sensory signal, like an auditory tone, is consistently played before the US (shock), it serves as a predictive signal that shock is coming. Through associative learning, rodents will start freezing to the tone, in preparation for the shock to come. *The tone at this point is called a conditioned stimulus (CS) because it has become a learned signal*, associated with the US. Thus, in the process of fear learning, a tone (CS) is paired with a shock (US), which eventually produces freezing (fear behavior) to the tone.

We can use Fraidy Rat not only to train a virtual rat to freeze to a tone, but we can also mess around with the neuronal circuits that underlie this learned behavior to see what brain areas are important for producing this learning. Below is a simplified circuit of the brain areas involved in this learning:



1. In the above circuit, tone information is sensed by cochlear nuclei neurons of the ear (AUD), which routes that information to the thalamus (THAL). The thalamus routes that auditory information to the amygdala (AMY), an important brain area for learned fear, as we'll see.

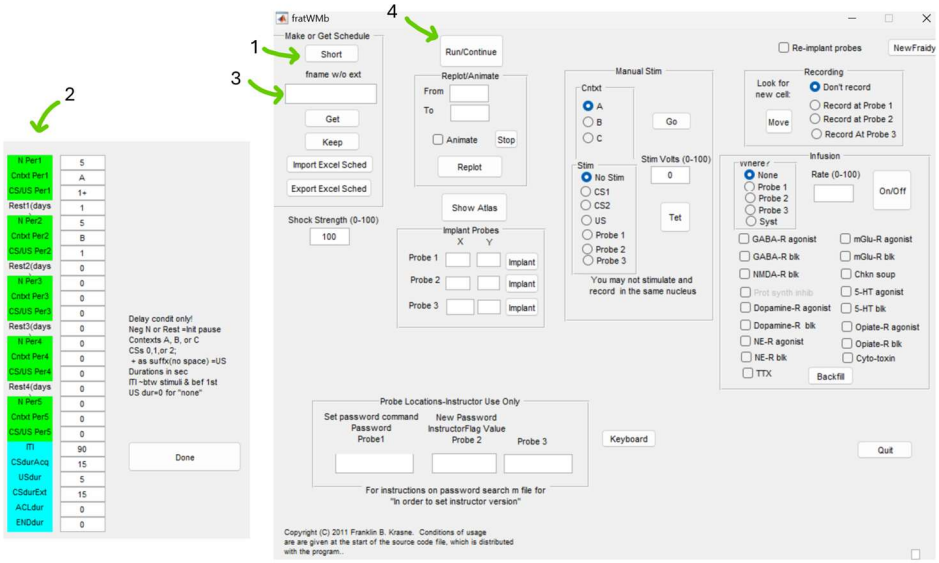
2. The amygdala receives direct information about electric shock from the rostral nucleus of the periaqueductal gray (PAG ros).

3. The amygdala also receives broad context information (about the environment) from the hippocampus (HIP), which builds the environment through visual info (Sensory Cortex).

4. **The amygdala takes in all of this information** and sends information to the dorsal nucleus of the periaqueductal gray (PAG dor) to produce freezing.

Experiment 1: Teach Fraidy Rat to freeze to cues and contexts

1) In the top left corner of the Fraidy Rat control panel, click on **Short** to create a conditioning schedule.



2) You'll want to set up the below schedule, that we'll walk through

N Per1	5
Cntxt Per1	A
CS/US Per1	1+
Rest1(days)	1
N Per2	5
Cntxt Per2	B
CS/US Per2	1
Rest2(days)	0
N Per3	0
Cntxt Per3	0
CS/US Per3	0
Rest3(days)	0
N Per4	0
Cntxt Per4	0
CS/US Per4	0
Rest4(days)	0
N Per5	0
Cntxt Per5	0
CS/US Per5	0
ITI	90
CSdurAcq	15
USdur	5
CSdurExt	15
ACLdur	0
ENDdur	0

Let's say you want to train Fraidy Rat with 5 conditioning trials (tone+shock) in Context A. Then the next day, you want to place Fraidy Rat in a new context (Context B) and play just the tone 5 times to see if Fraidy Rat freezes.

In the first green block, put in the information for the conditioning trials

- N Per1 – the number of trials – put 5
- Cntxt Per1 – the context for the trials – put A
- CS/US Per1 – the tone played (1 or 2) and shock (with a +). Put 1+ for tone 1 followed by a shock

For Rest1 – put 1 for 1 rest day (we'll test Fraidy one day later)

In the second green block, put in the information for the testing trial

- N Per2 – 5 trials again
- Cntxt Per1 – we want a new context – put B
- CS/US Per1 – put 1 to say we're playing tone 1 ***without*** the shock

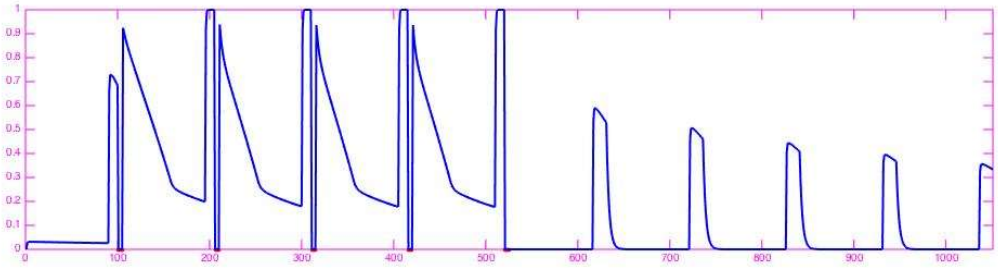
For the turquoise box, we put additional details for the training

- ITI is the inter-trial interval, or how much time between tones. Put 90 for 90 seconds
- CSdurAcq is the duration of the CS during acquisition, or how long the tone plays. Put 15 for 15 seconds
- USdur is the duration of the shock. Put 1 for 1 second
- CSdurExt is the duration of the CS during testing, or how long the tone plays in the freeze test. Again put 15 for 15 seconds

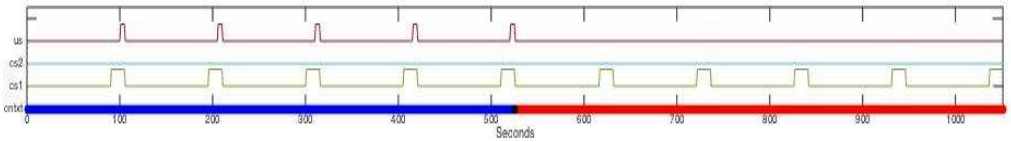
Click Done

3) You should save your experiment by entering a name in the box **fname w/o ext**. Put the name "cue" because this conditioning teaches fear learning to a cue (the tone) and press **Keep** to save it to your program. At any point in the future, if you want to pull up this training schedule, you can type in "cue" and press **Get** to retrieve. Pressing **Short** will pull it up again so you can check it.

4) To the right of this box, press **Run/Continue** to run the conditioning experiment. You will get two graphs. The first (below) will show freezing behavior over time. High values indicate high freezing (fear) behavior. Take a screenshot for the report



5) The second will show your experimental paradigm. Notice the colors that dictate context (A vs. B) and the bumps in us/cs lines that show when the tone is played, and the shock occurs. You can overlay these graphs on each other to see when Fraidy Rat froze to what stimuli.



6) Answer questions 1-5 in the worksheet below.

7) Press **NewFraidy (right top corner)** to "wipe Fraidy's brain clean" of any previous stuff. You'll end up doing this multiple times to start a new.

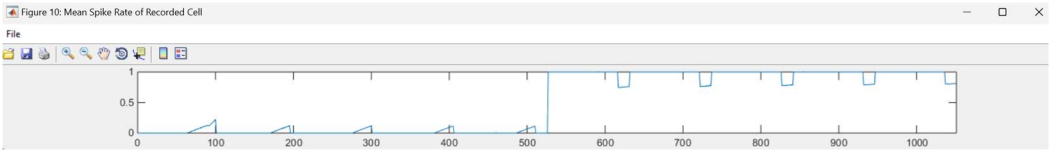
8) Create a new conditioning schedule with the following conditions.
   a. During conditioning**, we want 10 shock trials, with no tone (this would be 0+)** in Context A, followed by a day of rest
   b. Fill in one testing session, putting 5 trials in Context B with no tone or shock (this would be 0), with no rest
   c. Add one more testing session by putting 5 trials in Context A again with **no tone or shock (this would be 0)**
   d. Set the ITI to 30, CSdurAcq to 15, USdur to 5, and CSdurExt to 90
   e. Click **Done** then save this experiment as "context"

9) Answer questions 6-9 in the worksheet below

Experiment 2: Determine Brain Areas of Importance for Fear Conditioning

1) Click **NewFraidy** to start fresh.
2) Reload the first "**cue**" experiment by typing "cue" into the **fname w/o ext** box and pressing **Get**. You can check to make sure it loaded by clicking **Short**.
3) Click **Show Atlas** to open a sagittal slice of Fraidy Rat's brain with coordinates for different brain areas in our network. You have to navigate this atlas in order to learn about Fraidy's rat's brain.
4) First, place Probe 1 in Grisham's nucleus (X = -14; Y = 29). This nucleus is a "freezing center" and if we record from this nucleus, it will tie directly into behavior. **Implant** this probe, then in the upper right corner, click to **Record at Probe 1**. This will conduct extracellular recording to determine the rate of action potentials from this brain area.

5) Now run the conditioning experiment to see how the recording from Grisham's nucleus matches up with freezing behavior. It ***should*** be inversely related, so high activity in Grisham's nucleus means high exploration (*not freezing, then*).



**** If you cannot see any spike here, click the move button to find a new neuron.**

6) Click **NewFraidy** to start fresh. When you do this, you'll have to click on the **Move** button to re-find a neuron to record from.

7) Based on your lab worksheet answers, you probably have a hypothesis as to what brain areas are necessary to produce freezing to a tone. One brain area at a time, implant a new probe into that brain area, then infuse a **GABA-R agonist** into **Probe 2** (your brain area of interest). Set the Rate according to what is said below and press **On/Off** so that it turns red. *This will pharmacologically inhibit the brain area* where you drop Probe 2.

8) Now you can re-run the experiment and still record from Probe 1 while inhibiting your brain area to see what happens. **To test multiple brain areas, you will likely need to refresh everything (NewFraidy)** but remember to reimplant and infuse probes each time to make sure they're implanted correctly. (Do not forget to include a screenshot of Probe 1 at each location in your lab report.)

** An important note about infusing drugs into the brain. How much to infuse depends on the size of the brain region. A general rule of thumb in Fraidy Rat for how much to set the infusion rate: calculate the radius of the brain area you want to infuse into and multiply by 6 to make sure that you fill the entire brain area (all neurons) without spreading to other areas (thus being a confound)

9) Answer questions 10-14 in the worksheet below

Your name:
Your group members' names:

Lab 7 Questions: Answers

1) For the first experiment, how does Fraidy Rat's behavior change over the course of the conditioning trial? Add a screenshot here. How does Fraidy Rat's behavior change during the testing trial?

2) In the new environment (Context B), does Fraidy Rat still show fear? Explain the reasoning behind your answer.

3) Why does Fraidy Rat's freezing steadily decrease with each tone in Context B?

4) Based on the colored brain circuit, which brain areas seem most important for learning to fear the tone?

5) In this experiment, Fraidy Rat **learns to fear the tone that is paired with the shock.** Which type of synaptic plasticity best explains this kind of learning: **associative LTP or homosynaptic LTP**? Explain the reasoning behind your answer

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6) Fraidy Rat got **shocks with no tone.** After running the second experiment, explain Fraidy Rat's behavior this time. Add a screenshot here

7) When Fraidy Rat is placed in **Context B**, does it still act scary?
What happens when it goes **back to Context A**?
Why does the amount of freezing change over time in Context A?

8) Since there is **no tone** in this experiment, what does Fraidy Rat use to predict that a shock might come? This is called **contextual fear conditioning**, learning that depends on the environment. Based on the colored brain circuit, which brain areas are most important for this type of learning?

9) In the **context fear conditioning** experiment, Fraidy Rat learns to fear **Context A** even though there is **no tone**. If this type of learning is based on **associative LTP**, what are the two stimuli that act as the **CS (conditioned stimulus)** and **US (unconditioned stimulus)** in this experiment?

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10) Why does infusing the GABA-R agonist inhibit your brain region? Explain your answer using your knowledge gained from the previous unit.

11) Pick 3 brain areas to inhibit. Remember, a good scientist will choose some areas they think will affect fear conditioning and some areas they think won't affect it, to properly test their theory. For each of the 4 brain areas you choose, explain what happens when you inhibit them one at a time

12) For each of the 4 brain areas you tested above, it affects freezing differently. Add a screenshot of **Probe 1 recordings** for each area (you will need three screenshots here). Make a conclusion on what each brain area may be doing in fear conditioning based on the freezing data.

13) Repeat questions 11-12 using the "context" fear paradigm. Write your results and conclusions below.

14) Based on your experiments, what is the difference between cued and context fear conditioning, and how the brain regulates both, based on your experiments with Fraidy Rat?