

Conditioned Freezing Instructions

This is the procedure for running a 2- or 3-day conditioned freezing experiment, in which the first day consists of a single Training session. The second day may consist of both a Context Test session and a Cue Test session an hour later, or one may choose to do the Context Test on the second day and the Cue Test on the third day. Needless to say, it should be done exactly the same way for every mouse.

Arguably, the most important component of the conditioned freezing test is the context(s). The context during the Context Test must be identical to the context during the Training session, and the context during the Cue Test must be different from the context during the Training session. Other than the tone and the shock, **everything that impinges on the mouse's senses should be considered a part of the context**—the conditioning chamber, lighting in the holding room and experimental rooms, the background noise, people talking or laughing, ultrasonic vocalizations from other mice, smells from perfume or shampoo or clothing adulterated with tobacco smoke, the way the mice are handled, ambient temperature, nature of the holding cage etc. The experimenter is also part of the context. White and black lab coats are available to wear for the purpose of creating consistent or different training and test contexts. If more than one experimenter is involved, one experimenter should handle the mice for the Training and Context Testing sessions, and the other handle the mice for the Cue Testing session. When planning the experiment, walk through the experimental and holding rooms the day before and decide exactly how things should stay the same for the Context Test, and how they should be different for the Cue Test.

The day before testing, bring the mice to the animal room (PRB 860) for overnight housing. (Remember to sign out on the census sheet of the room their leaving and in on the PRB 860 sheet for the following day). This prevents the animal care staff from changing the bedding or otherwise disrupting the mice on the morning of the experiment. Weigh all mice and place indelible marks on their tails for easy and quick identification the next day (scruffing a mouse to get an ID is stressful and we don't want to stress the mouse immediately before the test).

Day 1 (Training):

Sessions should be conducted only in the morning. Bring the mice to a quiet holding room at least one hour before the first session. Turn off the white noise generator in the room and leave the fluorescent lights on.

Conditioned Freezing protocol:

Do the following things in this order:

1. Turn on the MED Associates interface/power supply (blue box with white & yellow faced cards w/ LEDs and green switch).
2. Turn on shock generator
3. Open the MED-PCIV program on the PC computer
4. Use the WIZARD to select the program (e.g. Freez1) and enter your subject ID, group, experiment names and the location of the folder where you want to save your data.
(Use the programs named "CONTEXT" and "CUE" on the appropriate days)
5. Under "File" choose "Open Session" and load Freez1 program into Box 1.
6. Under "Options" choose "Signals" and prepare to issue a start signal to Box 1. **Don't click on "Issue" yet!**
7. Turn on the video monitor and adjust the camera if necessary, so that the sides of the shock chamber are parallel with the sides of the monitor
8. Turn on the fluorescent light inside the shuttlebox
9. **ON THE MAC**, Copy the conditioning (or Context or Cue) folder and move to desktop. Give the folder a appropriate name. Open Image OF 1.11.
10. Choose "Start Capturing" under "Special", and click once on the picture to get a still frame
11. Choose the rectangle tool from the tools menu bar
12. Choose "Set Field 1" under "Special"; using the rectangle tool, draw a box around the inside of the grid floor, i.e., anywhere the mouse is likely to be detected
13. Choose "Set Field 2" under "Special"
14. Place an artificial mouse (small black object) in the shuttlebox. Place the top acrylic insert in position, then close the doors to the sound-attenuating chamber. **If running cue test, make sure the acrylic walls and floor are in place too!!!**
15. Choose "Start Capturing" under "Special", and click once on the picture to get a still frame
16. Choose "Threshold" under "Options", and adjust the threshold so only the artificial mouse remains as a silhouette, and most everything else within the confines of the shock grid has turned white; **remember the threshold number**
17. Quit Image of 2.11 and open Image FZC 2.22sr1
18. Remove the artificial mouse from the chamber and close the shuttlebox doors
19. Under "Special" choose "Capture trials" and give the session a unique name.
20. Set all the parameters that are not already set (**see next page**); most should be done already except for level, which is the threshold level you obtained in step 16
21. For the sequence, enter "**conditioning**" on the training day, "**context**" on the context day, and "**cued**" on the cue day

22. When the software asks to "**click to get background**", make sure all the lights are on, shuttlebox doors are closed, and the floor is in place, then click.
23. Before clicking the next time, place the mouse in the shock chamber
24. Simultaneously press "I" on the PC keyboard and click the Macintosh mouse

PARAMETERS (select number to check/change)

Rate = 1.0

Dur = 480 (i.e. 8 min)

Bin = 30

level = Threshold obtained in step 16

Tone Dur = 30.0 (Must be set even though this is controlled by the other computer)

Shock Dur = 2.0 (Must be set even though this is controlled by the other computer)

Subj

min = 50

max = 500

Frame

width = 16

height = 18

Trace = 0 (off)

Fz. Criterion = 10

Min Fz Dur = 2

Human = 0 (off)

Trial name = subject id no. (**MUST BE AT LEAST 3 CHARACTERS!!!**)

seq1 = conditioning (on training day)

context (on context test day)

cued (on cue test day)