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# Fear Conditioning

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Sabina Marciano[1,2], Roberta Marongiu[1,2]

[1]Department of Neurological Surgery, Weill Cornell Medical College, New York, NY 10065;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA

ASAP Collaborative Rese…

Eileen Ruth Torres
Weill Cornell Medicine



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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Behavioral assay to measure fear learning and memory in mice.

## Materials

- MedAssociates fear conditioning chambers
- white floor inserts
- black ceiling inserts
- pepperminet and limonene spray

## Fear Acquisition

1 Habituate mice to the room. Set up fear conditioning chambers as well as protocols (listed below) in VideoFreeze software.

2 Place individual mice in a lit fear conditioning apparatus scented with peppermint spray.

3 Protocol: 3m 20s

- 00:02:00 baseline followed by 5 tone-shock pairings where the shock follows the tone
- shock = 0.5mA, 00:00:01 is delivered after the end of the tone (75dB, 2kHz, 00:00:19 )
- empty trace intervals between the shocks last 00:01:00

4 After the protocol runs for each mouse, place the mouse back in the home cage and clean the fear conditioning chamber thoroughly.

5 Contextual test is performed 24:00:00 after the fear acquisition test. Cued Test is performed at least 02:00:00 after the contextual test. 1d 2h

## Contextual Test

6 Goal: Measure the subjects' fear response to the environment where fear conditioning was received

7 24 hours after fear acquisition: Mice are placed in a lightened FC apparatus scented with peppermint spray.

8 Protocol: Neither cue nor shock are delivered during this test session (Duration 00:07:30 ) 7m 30s

## Cued Test

9 Goal: Measure the subjects fear response to the CS, and ideally no other features of the training environment. The key is to alter the chamber so the subjects are exposed to a new set of environmental cues.

10 Mice are placed in a lightened FC apparatus which is altered by placing a smooth floor insert on shock grid and black triangles on top.

Limonene spray is used, instead of peppermint scent.

11 Protocol:

19s

- Baseline: 2 min followed by five tone-cues
- No shock is delivered after the end of the tone (75dB, 2 kHz, 00:00:19 )

## Primary Analyses

12 Fear conditioning acquisition

- % Component freezing for baseline, each tone (if used), shock, and interstimulus interval
- Graphs for each component should be graphed separately
- Average motion

Context

- % Component freezing per minute and total
- Per minute and total will be on separate graphs
- Average motion per minute and total

Cued

- %Component freezing for baseline, each tone, and interstimulus interval
- Graphs for each component should be graphed separately
- Average motion for baseline, each tone, and interstimulus interval