STANDARD OPERATING PROCEDURE

TITLE: Contextual and Tone-cued Fear Conditioning

CATEGORY: Behavioral Assay

Introduction

Goal: This document aims to provide the reader information on how to conduct the Fear Conditioning Test. The test evaluates ability to learn tone-cued fear conditioning in novel contexts as well as contextual fear conditioning. Brain areas involved in tone-cued fear conditioning include the amygdala, hippocampus, frontal cortex, and cingulate cortex. As this is a general description of standard materials, test settings, and procedures, variations may be made to fit specific needs.

Materials

- *Subjects*: any strain of mice. No prior training is required, though subjects should be acclimated to testing environment and experimenter before testing.
- *Apparatus*: two fear conditioning chambers, each including overhead camera, light, small cup, small bottle of mint extract, animal shocker, and inner cage with metal floor grid and collecting pan. Chamber used for Days 1 and 3 has square inner cage and is lit with white light, while chamber used for Day 2 has U-shaped with blue plastic walls and floor and is lit with blue light.
- *Software*: fear conditioning software including motion detection (ex. FreezeFrame).
- *White noise machine*: used in Day 2 to create background noise.
- *10% Simple Green*: used between trials on Days 1 and 3 to eliminate visual and olfactory residue in arena.
- *20% ethanol:* used between trials on Day 2 to eliminate visual and olfactory residue in arena.

Test Settings

- *Lighting*: normal on Days 1 and 3, dim red on Day 2.
- *Test Area*: two rooms; one for Days 1 and 3, other for Day 2.

Detailed Standard Operating Procedure

Before testing:

- *Acclimation*: subjects in home cage are placed in testing room for at least 1hr before testing to minimize effects of stress on behavior during testing.
- *Subject training*: none required.

Testing procedures:

1. Day 1

- Two drops of mint extract are placed inside small cup in each fear conditioning chamber and animal is placed in inner cage. With two chambers, two animals may be tested at once. Once animal is in cage, cage and chamber doors are closed. Trial begins immediately and ends after defined procedure, including exposure to tone and mild shock, is complete.
- Animal is removed from chamber and returned to home cage, and apparatus is cleaned with 10% Simple Green between trials.

2. Day 2

- Two drops of vanilla extract are placed inside small cup in each fear conditioning chamber and animal is placed in inner cage. Trials are run as in Day 1, cleaning apparatus with 20% ethanol instead of 10% Simple Green between trials. Day 2 includes exposure to same tone as Day 1 but no shock.

3. Day 3

- Two drops of mint extract are placed inside small cup in each fear conditioning chamber and animal is placed in inner cage. Trials are run as in Day 1, cleaning apparatus with 10% Simple Green between trials. Day 3 does not include tone or shock.

Data Analysis

- The following parameters are collected for analysis:
    - Total freezing
    - Percent freezing on Days 1, 2, and 3
    - Percent freezing during individual stages
        - Day 1
            - Baseline period (first 200s before tone)
            - During tones
            - Intertrial intervals (ITIs)
        - Day 2
            - Pre-tone period
            - During tones
            - ITIs
        - Day 3
            - During each minute