Automated Measures of Fear Conditioning and Extinction in the Rat

Gina Forster, Ph.D.
Neuroscience Group,
Basic Biomedical Sciences
Sanford School of Medicine,
University of South Dakota
Fear Conditioning and Extinction

**Conditioning**
- CS (context or tone)
- US (foot shock)

**Extinction**
- CS (context or tone)

**Retrieval/Retention**
- CS (context or tone)
Why Study Fear Conditioning and/or Extinction?

- To study the cognitive, emotional and biological process underlying:
  - Pavlovian learning
  - Extinction processes
  - Fear learning/conditioning and extinction

- As a tool to determine whether dysfunction of a particular brain region observed in an animal model has functional consequences.
Why Study Fear Conditioning and/or Extinction using Foot shock?

• The paradigm is robust, and only requires one session-learning.

• Allows the study of different facets of learning and memory (e.g. retention, extinction).

• The cognitive, emotional and biological processes underlying fear conditioning and extinction have been extensively studied.
Commonly Used Systems to Measure Fear Conditioning and Extinction

**Manual scoring:**
- reliable but labor intensive and subject to biases.
- if conducted ‘live’, lack of ability to rescore and evaluate other behaviors.

**Photo beams:**
- automated, reducing labor and subjectivity.
- additional video footage needed to allow the ability to rescore and evaluate other behaviors.
- problems with the ability to differentiate immobility from freezing.
Commonly Used Systems to Measure Fear Conditioning and Extinction

Video tracking:
- automated, reducing labor and subjectivity.
- video footage allows the ability to rescore and evaluate other behaviors.
- problems with the ability to differentiate immobility from freezing.

Automated scoring systems need to be well validated to ensure accurate measures of freezing, e.g.:
- Pham et al., 2009 – Ethovision with mice
- Anagnostaras et al., 2010 – Video Freeze with mice
A System that Allows Both Automated and Hand-Scoring

A System that Allows Both Automated and Hand-Scoring

- Foot shock and stimuli (tone/light) controlled by Ethovision 3.1 (Noldus Technologies) and animals tracked by Ethovision 3.1.
- Keys for experimenter-scored behaviors can be programmed for current (or later) hand-scoring of behavioral states.
- Video footage recorded for later analysis.
- Ability to rework data into different time bins for analysis.
A System that Allows Testing of Multiple Facets of Fear Conditioning

• Reactivity to fearful stimuli – response to foot shock.
• Auditory/olfactory/visual cue conditioning – recall, retention and extinction.
• Context conditioning – recall, retention and extinction.
• General activity/locomotion and other behaviors of interest.
Validation of an Automated System for Fear Conditioning in Rats

Ethovision 3.1. immobility measure vs. hand-scored freezing

**Ethovision Parameters:**
- 5 frames per second
- Subtraction detection method
- 2.5% immobility threshold

**Conditioning Parameters:**
- 56 dB tone (5 sec duration)
- 0.5 mA scrambled foot shock (0.5 sec duration), coinciding with the last 0.5 sec of the tone
- tone + foot shock pairings every 2 min x 10 (20 min session duration total)
Validation of an Automated System for Fear Conditioning in Rats

Ethovision 3.1. immobility measure vs. hand-scored freezing

* significant differences between ethovision and hand-scoring

# significantly different from baseline

Retention and Extinction trials were conducted every 24 hours following conditioning – data represent 20 min time bins
Validation of an Automated System for Fear Conditioning in Rats

Ethovision 3.1. immobility measure vs. hand-scored freezing

Baseline, Training & Retention

\[
\begin{array}{c}
y \text{ intercept} = 1.492 \\
slope = 0.946 \\
r^2 = 0.994 \\
p < 0.001
\end{array}
\]
Validation of an Automated System for Fear Conditioning in Rats

Ethovision 3.1. immobility measure vs. hand-scored freezing

![Graph showing the relationship between hand-scored % time freezing and Ethovision % time immobile. The equation for the line is y = 56.591 - 0.209x, with r² = 0.020 and p = 0.656.](image)
Application of an Automated System for Fear Conditioning in Rats

Used to assess:
- acclimation to the environment
- behavioral and endocrine responses to foot shock
- cue (auditory) fear conditioning
- contextual fear conditioning
- extinction of conditioned fear responses

...in rat models of:
- early-life stress
- amphetamine withdrawal
- mild traumatic brain injury
Rat Model of Early-life Stress

**Isolation Rearing**
- PND 21 (pre-adolescence)
- Rats housed in isolation or in groups of 3-4 rats (controls) from day of weaning.

**Re-housing**
- PND 42 (mid-adolescence)
- Rats re-housed into groups of 3-4 and allowed to develop to adulthood.

**Testing**
- PND 56+ (early adulthood)
- Behavioral and/or neurochemical testing.
Early-life Stress alters Adult Anxiety States and CRF-Serotonin Interactions

Post-weaning social isolation of male rats:
- increases anxiety-like behaviors
- increases the expression of CRF$_2$ receptors in the dorsal raphe nucleus
- increases CRF$_2$-elicited serotonin release

Since CRF$_2$ receptor activation in the dorsal raphe nucleus elicits freezing via serotonin release in the limbic system:
...do isolates show altered freezing in response to foot shock?
.....how would this translate to the retention of conditioned fear behavior?

Lukkes et al., 2008, 2009; Forster et al., 2006; 2008
Early-life Stress and Adult Fear Conditioning

Days 1-3
- **Acclimation** to the foot shock box for 30 mins/day, distance moved recorded.

Day 4
- **Conditioning**: with foot shock (0.5 mA, 0.5 sec) paired with tone (56 dB, 5 sec) x 10 within the second 10 min of the 30 min session.

Day 5
- **Retention**: rats placed in foot shock boxes for 30 mins, and tone (x 10) presented in the second 10 minute block.

Lukkes et al., 2009
Cue but not Context Fear Conditioning

% time freezing

Baseline
Presentation
Retention

*
Early-life Stress and Adult Fear Conditioning

Lukkes et al., 2009
Early-life Stress and Adult Fear Conditioning

Conditioning Day

Duration of freezing (sec)

Time (min)

+P < 0.050 and \( \delta P < 0.050 \) compared to pre-shock or pre-tone levels for group-reared and isolation-reared rats, respectively.

Lukkes et al., 2009
Early-life Stress and Adult Fear Conditioning

Retention Test

*P < 0.050 between group-reared and isolation-reared conditions; #P = 0.056 between group-reared and isolation-reared conditions; +P < 0.050 and δP < 0.050 compared to pre-shock or pre-tone levels for group-reared and isolation-reared rats, respectively.

Lukkes et al., 2009
Early-life Stress and Adult Fear Conditioning

Lukkes et al., 2009
Summary

• Video-tracking automated scoring of immobility can provide valid measures of freezing behavior in rats and mice.
  • Substantially reduces labor involved.
  • Removes potential experimenter bias.
  • Provides additional measures to aid in interpretation.

• Automated measures should be validated with comparisons to hand-scored measures.

• Increased immobility over multiple testing sessions (e.g. extinction) poses a problem for automated measures.
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