



STANFORD
SCHOOL OF MEDICINE

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| Title: | Fear Conditioning in C57BL/6J Male Mice using Scopolamine |
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| Procedure | Fear Conditioning |
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Fear Conditioning

Fear Conditioning (FC) is a type of associative learning task in which mice learn to associate a particular neutral Conditional Stimulus (CS; often a tone) with an aversive Unconditional Stimulus (US; often a mild electrical foot shock) and show a Conditional Response (CR; often as freezing). After repeated pairings of CS and US, the animal learns to fear both the tone and training context. FC is learned rapidly, and after one conditioning session, a very stable and long-lasting behavioral change is produced which is useful for neurobehavioral, genetic, and pharmacological studies. In Trace FC the CS and the US are separated by a certain time interval. It has been shown that the frontotemporal amygdala has an important role in both acquisition and expression of conditional fear and that the hippocampus is necessary for contextual and trace FC.

Two different contexts, Context A and B, are used in FC. In the first day, the subjects are placed in Context A, and after 3 min, they receive five tone-shock pairings. The shock (0.5 mA, 2 sec) is delivered 18 sec after the end of the tone (75dB, 2 kHz, 20 sec). An empty trace interval interposes between the tone and the shock in each CS-US pairing. In the second day, mice are placed in Context B (new olfactory cue, floor texture, and visual cues) for 3 min and subsequently given three tone presentations without any shocks. In the last day of the experiment, mice are placed in Context A for 5 min without any CS and US. Freezing is defined as the complete lack of motion for a minimum of 0.75 second and the percent of freezing in each period of time are reported

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| Project Title | Fear Conditioning in C57BL/6J Male Mice using Scopolamine |
| Species | Mouse |
| Strain | C57BL/6J |
| Sex | Male |
| Age | 6-7 Months |

| Subjects | | |
|----------|-----------|--|
| Group | # of mice | Treatment |
| C57BL/6J | 10 | Saline |
| C57BL/6J | 10 | Scopolamine (1.0 mg/kg body weight) dosed 20 min. before day 3 (contextual memory) trial |

Day 1 (Acquisition): % Freezing During ITI's

| Animal ID | Dosing Group | Average % Freezing ITI 1 | Average % Freezing ITI 2 | Average % Freezing ITI 3 | Average % Freezing ITI 4 | Average % Freezing ITI 5 |
|-----------|--------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 2 | Saline | 10.3875 | 27.3325 | 20.3325 | 45.445 | 45.52 |
| 3 | Saline | 6.365 | 26.6675 | 32.6675 | 42.1075 | 55.31 |
| 6 | Saline | 12.7225 | 13.665 | 25.9975 | 26.79 | 31.195 |
| 8 | Saline | 3.3325 | 22.3325 | 28.665 | 32.3925 | 49.2175 |
| 9 | Saline | 1.3325 | 8.3325 | 10.3325 | 14.0125 | 15.09 |
| 11 | Saline | 7.345 | 30.6675 | 9 | 22.1775 | 30.7875 |
| 14 | Saline | 4.3575 | 5.6675 | 0 | 33.52 | 33.87 |
| 15 | Saline | 8.355 | 20.6675 | 26.665 | 44.5075 | 30.4225 |
| 17 | Saline | 20.3775 | 27.3325 | 44 | 44.205 | 51.835 |
| 20 | Saline | 23.74 | 31.3325 | 40.3325 | 61.62 | 65.1975 |
| 1 | Scopolamine | 16.68 | 30.6675 | 22.6675 | 21.8925 | 20.21 |
| 4 | Scopolamine | 2 | 9.6675 | 19.335 | 19.7025 | 31.785 |
| 5 | Scopolamine | 5.71 | 8 | 34.3325 | 51.21 | 25.0725 |
| 7 | Scopolamine | 12.695 | 18.3325 | 29.665 | 52.135 | 40.47 |
| 10 | Scopolamine | 15 | 24.6675 | 34.0025 | 45.4975 | 52.9125 |
| 12 | Scopolamine | 4 | 17 | 28 | 35.78 | 54.225 |
| 13 | Scopolamine | 1 | 2.3325 | 8.665 | 10.6675 | 13.36 |
| 16 | Scopolamine | 3.0125 | 16.6675 | 26.335 | 45.8375 | 40.46 |
| 18 | Scopolamine | 5.02 | 17.3325 | 37.335 | 33.8375 | 25.3875 |
| 19 | Scopolamine | 7.035 | 32.665 | 49 | 54.1125 | 77 |

Day 1 (Acquisition): % Freezing During Pre-Tone and Tones

| Animal ID | Dosing Group | Average % Freezing Pre-Tone | Average % Freezing Tone 1 | Average % Freezing Tone 2 | Average % Freezing Tone 3 | Average % Freezing Tone 4 | Average % Freezing Tone 5 |
|-----------|--------------|-----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| 2 | Saline | 0.4 | 0 | 18.67 | 2.67 | 14.67 | 12 |
| 3 | Saline | 0 | 0 | 0 | 8.11 | 0 | 1.33 |
| 6 | Saline | 0 | 6.67 | 25.33 | 26.67 | 4 | 5.33 |
| 8 | Saline | 0 | 0 | 14.67 | 17.33 | 30.67 | 6.67 |
| 9 | Saline | 0 | 0 | 1.33 | 25.33 | 6.67 | 4 |
| 11 | Saline | 0 | 0 | 13.33 | 5.33 | 21.33 | 18.67 |
| 14 | Saline | 0 | 0 | 0 | 0 | 6.67 | 0 |
| 15 | Saline | 1.067 | 0 | 0 | 10.67 | 0 | 14.67 |
| 17 | Saline | 3.333 | 12 | 10.67 | 12 | 22.67 | 18.67 |
| 20 | Saline | 0.533 | 6.67 | 17.33 | 24 | 17.33 | 28 |
| 1 | Scopolamine | 1.6 | 4 | 0 | 6.67 | 16 | 0 |
| 4 | Scopolamine | 0 | 0 | 16 | 13.51 | 2.67 | 2.67 |
| 5 | Scopolamine | 0.533 | 0 | 5.33 | 16 | 14.67 | 49.33 |
| 7 | Scopolamine | 0 | 0 | 0 | 0 | 14.67 | 0 |
| 10 | Scopolamine | 0 | 0 | 0 | 14.67 | 9.33 | 0 |
| 12 | Scopolamine | 0.8 | 0 | 0 | 0 | 0 | 0 |
| 13 | Scopolamine | 0 | 0 | 0 | 0 | 0 | 10.67 |
| 16 | Scopolamine | 0 | 0 | 5.41 | 9.33 | 12 | 14.67 |
| 18 | Scopolamine | 0.533 | 0 | 10.67 | 13.33 | 16 | 8 |
| 19 | Scopolamine | 0 | 0 | 9.33 | 30.67 | 14.67 | 33.33 |

Day 2 (Cued Memory): % Freezing During ITI's

| Animal ID | Dosing Group | Average % Freezing ITI 1 | Average % Freezing ITI 2 | Average % Freezing ITI 3 |
|-----------|--------------|--------------------------|--------------------------|--------------------------|
| 2 | Saline | 35.17 | 38.6675 | 49.735 |
| 3 | Saline | 71.5725 | 49.3325 | 19.7 |
| 6 | Saline | 24.405 | 35.3325 | 31.4 |
| 8 | Saline | 39.7375 | 42.665 | 14.3475 |
| 9 | Saline | 25.3925 | 9.6675 | 22.3325 |
| 11 | Saline | 70.585 | 64.335 | 60.8775 |
| 14 | Saline | 28.3975 | 15.9975 | 14.035 |
| 15 | Saline | 62.5425 | 41.665 | 13.0125 |
| 17 | Saline | 68.2375 | 64.3325 | 39.0325 |
| 20 | Saline | 75.9425 | 82 | 16.7325 |
| 1 | Scopolamine | 70.5975 | 69 | 70.545 |
| 4 | Scopolamine | 52.9275 | 52.3325 | 16.6875 |
| 5 | Scopolamine | 49.2375 | 26.665 | 21.6675 |
| 7 | Scopolamine | 74.5375 | 40.0025 | 43.4775 |
| 10 | Scopolamine | 73.2525 | 53.6675 | 30.3625 |
| 12 | Scopolamine | 41.4525 | 30.665 | 31.74 |
| 13 | Scopolamine | 37.1375 | 32 | 1 |
| 16 | Scopolamine | 35.855 | 28.9975 | 27.665 |
| 18 | Scopolamine | 56.2125 | 40 | 13.6675 |
| 19 | Scopolamine | 54.505 | 45.3325 | 33 |

Day 2 (Cued Memory): % Freezing During Pre-Tone and Tones

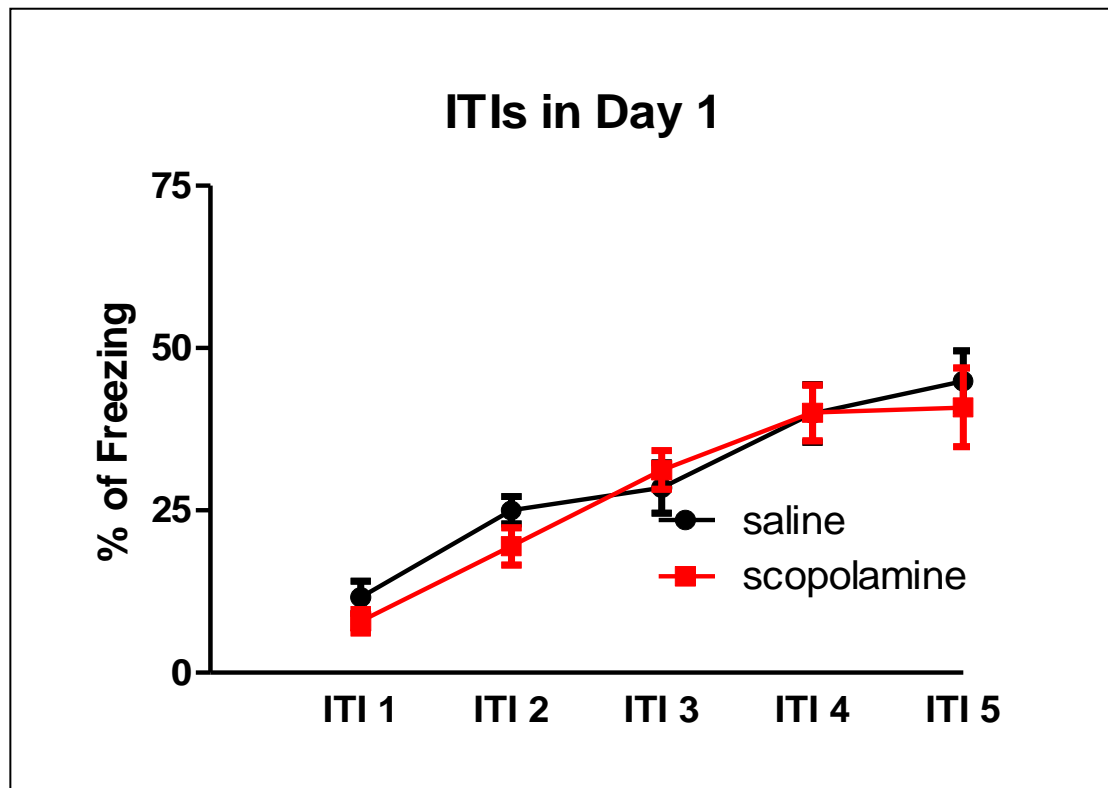
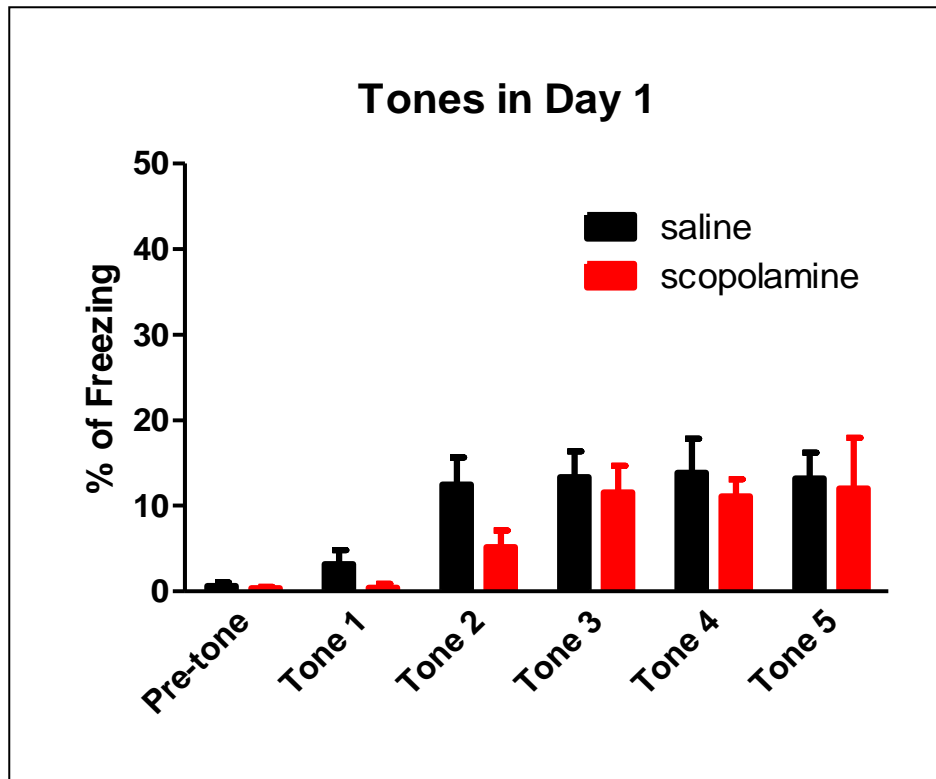
| Animal ID | Dosing Group | Average % Freezing Pre-Tone | Average % Freezing Tone 1 | Average % Freezing Tone 2 | Average % Freezing Tone 3 |
|-----------|--------------|-----------------------------|---------------------------|---------------------------|---------------------------|
| 2 | Saline | 16.8 | 30.67 | 13.33 | 20 |
| 3 | Saline | 19.067 | 42.67 | 10.67 | 18.67 |
| 6 | Saline | 11.213 | 26.67 | 46.67 | 24 |
| 8 | Saline | 8.533 | 29.33 | 21.62 | 17.33 |
| 9 | Saline | 11.888 | 29.33 | 13.33 | 13.33 |
| 11 | Saline | 16.55 | 33.33 | 22.67 | 13.33 |
| 14 | Saline | 2.8 | 0 | 12 | 0 |
| 15 | Saline | 12.133 | 36 | 36 | 22.67 |
| 17 | Saline | 21.346 | 40 | 26.67 | 1.33 |
| 20 | Saline | 23.202 | 33.33 | 25.33 | 16 |
| 1 | Scopolamine | 17.509 | 41.33 | 18.67 | 29.33 |
| 4 | Scopolamine | 8.266 | 49.33 | 17.33 | 0 |
| 5 | Scopolamine | 11.866 | 65.33 | 25.33 | 30.67 |
| 7 | Scopolamine | 10.532 | 29.33 | 24.32 | 10.67 |
| 10 | Scopolamine | 20 | 50.67 | 13.33 | 16 |
| 12 | Scopolamine | 13.6 | 13.33 | 5.33 | 0 |
| 13 | Scopolamine | 1.733 | 16 | 0 | 0 |
| 16 | Scopolamine | 0.667 | 14.67 | 29.33 | 26.67 |
| 18 | Scopolamine | 9.6 | 14.67 | 12 | 4 |
| 19 | Scopolamine | 6.552 | 29.33 | 17.33 | 0 |

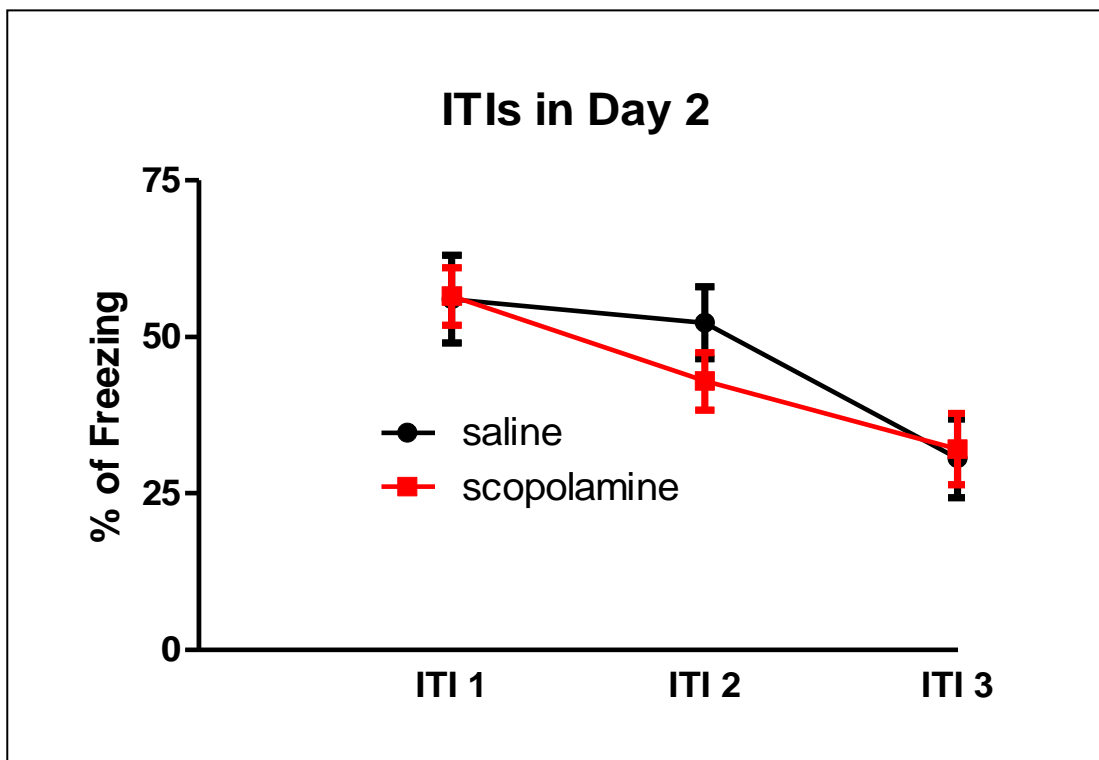
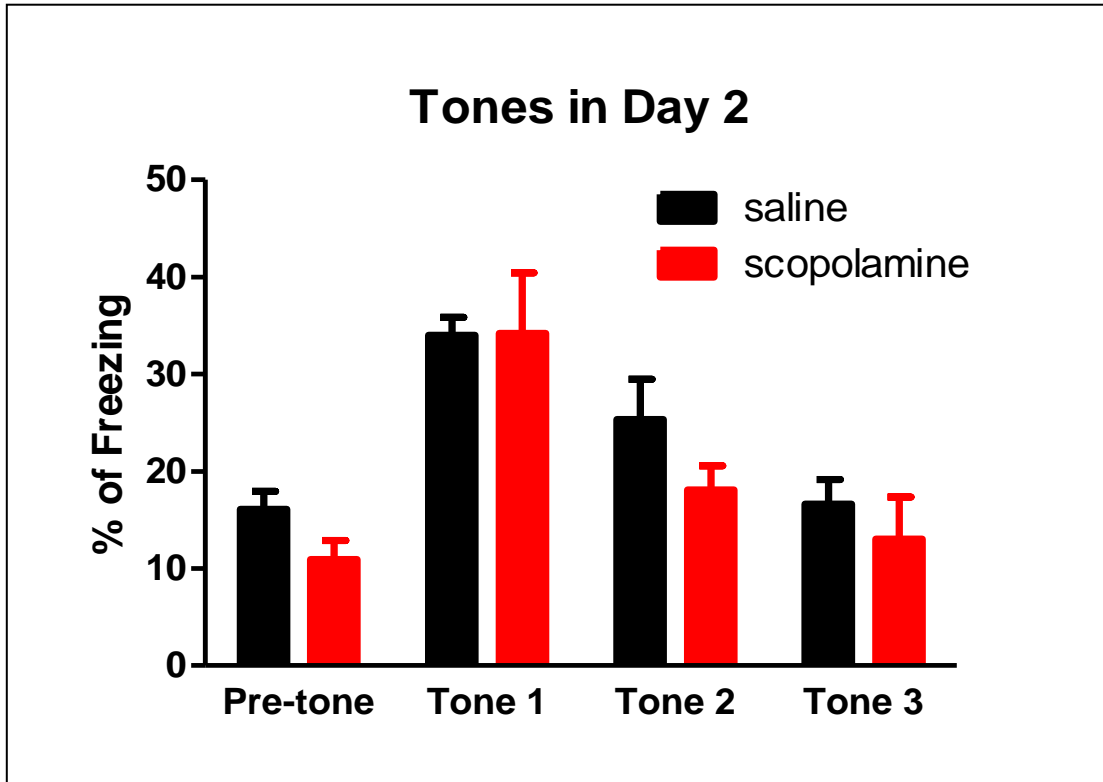
Day 3: % Freezing in Context

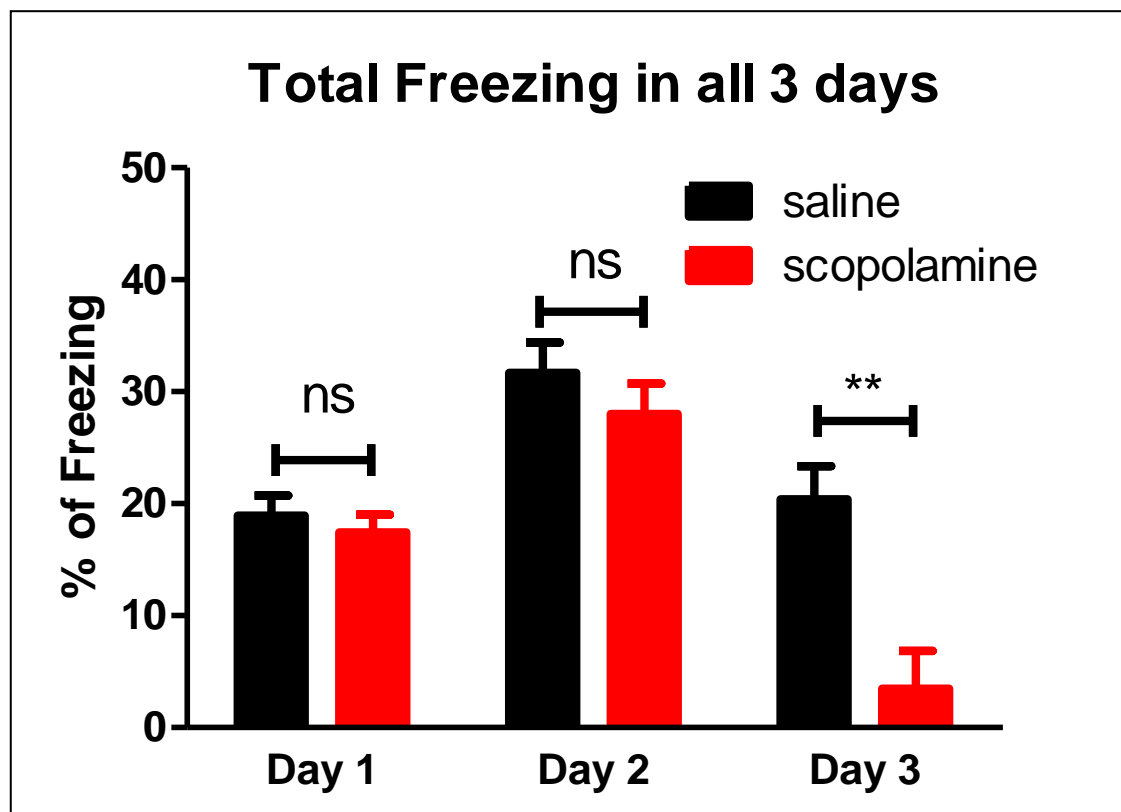
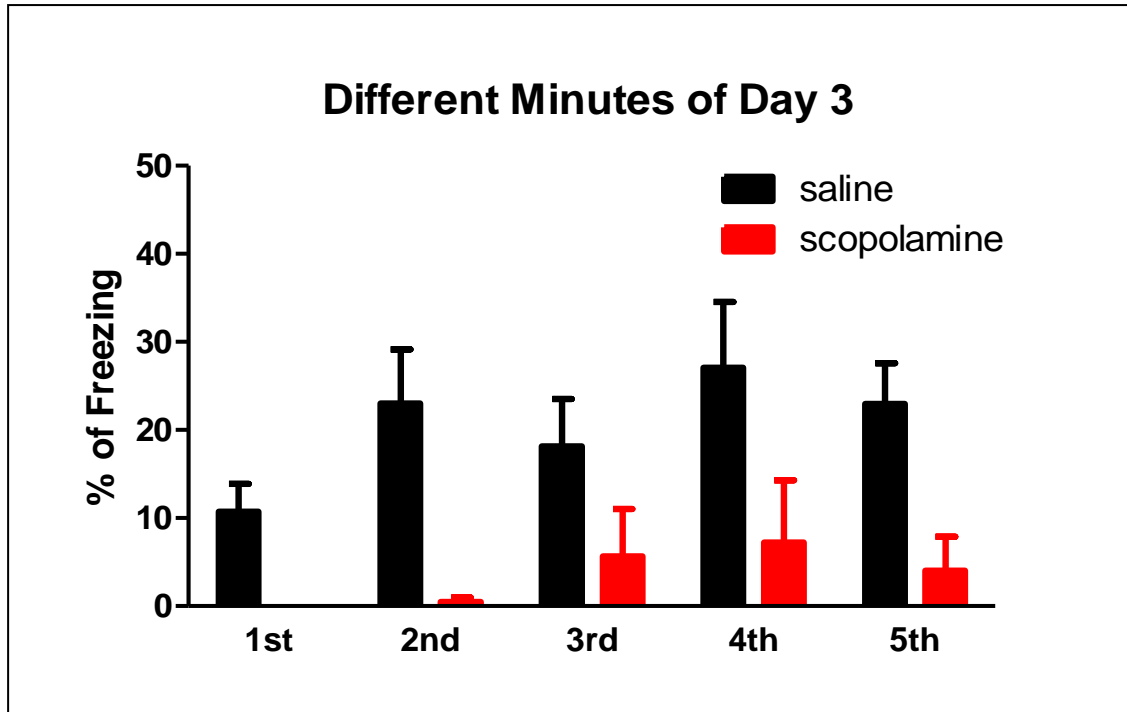
| Animal ID | Dosing Group | % Freezing Min. 1 | % Freezing Min. 2 | % Freezing Min. 3 | % Freezing Min. 4 | % Freezing Min. 5 |
|-----------|--------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 2 | Saline | 2.68 | 60.89 | 12 | 72 | 36.77 |
| 3 | Saline | 0 | 12.89 | 48.44 | 16.89 | 43.5 |
| 6 | Saline | 18.3 | 10.67 | 9.78 | 17.33 | 24.66 |
| 8 | Saline | 0 | 9.33 | 8.89 | 12 | 12.11 |
| 9 | Saline | 4.46 | 4 | 1.33 | 1.78 | 1.35 |
| 11 | Saline | 11.61 | 16.89 | 5.33 | 11.11 | 30.94 |
| 14 | Saline | 1.79 | 8.44 | 5.78 | 9.78 | 11.66 |
| 15 | Saline | 24.11 | 14.22 | 21.78 | 15.11 | 13.45 |
| 17 | Saline | 14.29 | 24.89 | 32 | 43.56 | 7.17 |
| 20 | Saline | 14.73 | 34.22 | 7.11 | 28.44 | 15.25 |
| 1 | Scopolamine | 0 | 0 | 0 | 1.33 | 1.35 |
| 4 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 5 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 7 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 10 | Scopolamine | 0 | 0 | 1.33 | 0 | 0 |
| 12 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 13 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 16 | Scopolamine | 0 | 0 | 0 | 0 | 0 |
| 18 | Scopolamine | 0 | 0 | 1.33 | 0 | 0 |
| 19 | Scopolamine | 0 | 4.44 | 48.44 | 63.56 | 34.98 |

Day 1-3: Total Freezing

| Animal ID | Dosing Group | Total Freezing Day 1 | Total Freezing Day 2 | Total Freezing Day 3 |
|-----------|--------------|----------------------|----------------------|----------------------|
| 2 | Saline | 18.5 | 29.04 | 36.87 |
| 3 | Saline | 18.92 | 33 | 24.31 |
| 6 | Saline | 14.53 | 22.96 | 16.12 |
| 8 | Saline | 17.51 | 21.63 | 8.46 |
| 9 | Saline | 6.67 | 16.18 | 2.58 |
| 11 | Saline | 13.08 | 40.68 | 15.23 |
| 14 | Saline | 9 | 11 | 7.48 |
| 15 | Saline | 15.99 | 27.39 | 17.81 |
| 17 | Saline | 24.6 | 38.71 | 24.4 |
| 20 | Saline | 28.22 | 40.2 | 19.95 |
| 1 | Scopolamine | 16.4 | 44.15 | 0.53 |
| 4 | Scopolamine | 10.45 | 25.47 | 0 |
| 5 | Scopolamine | 16.78 | 25.2 | 0 |
| 7 | Scopolamine | 17.96 | 32.05 | 0 |
| 10 | Scopolamine | 20.37 | 36.36 | 0.27 |
| 12 | Scopolamine | 16.09 | 22.8 | 0 |
| 13 | Scopolamine | 4.42 | 12.55 | 0 |
| 16 | Scopolamine | 16.29 | 17.89 | 0 |
| 18 | Scopolamine | 15.1 | 22.64 | 0.27 |
| 19 | Scopolamine | 27.65 | 25.73 | 30.28 |







The total freezing on each day was compared with an unpaired t-test. Mice showed no significant differences in freezing on days 1 and 2. The difference in freezing was significant for day 3 ($p < 0.01$).

Conclusion:

- The Fear Conditioning Test was performed on BL6 mice dosed with saline (n=10) and on BL6 dosed with scopolamine (n=10).
- Both groups showed equal levels of learning on day 1. Both groups of mice were able to learn to associate the tone with the shock, as demonstrated by an increase in percent freezing over each subsequent tone-shock pairing.
- On day 2, mice were placed in a novel environment and were given three tone presentations. Both groups were able to remember the tone from the previous day and froze in response to it at comparable levels.
- On day 3, one half of the mice were injected with scopolamine and the other half were injected with saline. While saline injected mice were able to recall the context of the shocks and froze in response, scopolamine injected mice demonstrated significantly lower freezing when placed in the chamber from day 1. This suggests that scopolamine is able to induce impairments in recall of contextual memories.