# The Accurate Measurement of Fear Memory in Pavlovian Conditioning: Resolving The Baseline Issue

N.S. Jacobs, J.D. Cushman, & M.S. Fanselow

*Department of Psychology, University of California Los Angeles.*

## INTRODUCTION

•Auditory fear conditioning has become an essential behavioral task in neuroscience and biomedical research.

•The most common fear conditioning task involves pairing a discrete stimulus (such as a tone) with an aversive footshock.

•During training, fear of the tone is acquired as well as fear of the conditioning chamber itself, a phenomenon which relies on different neurobiological substrates[1].

•Tone fear memory is probed during a tone test, where freezing is observed throughout a three minute baseline period followed by several non-reinforced tone presentations.

•Ideally, freezing during the tone is observed against a low baseline level of fear.

•Baseline fear is rarely reduced to zero, however, and researchers have come up with a variety of ways to correct for high baseline fear[2-7] or simply do not report baseline fear levels[8-12].

•Critical assumptions used to justify correcting and interpreting tone fear data remain untested.

•How to appropriately adjust tone fear data that is confounded by high or non-equivalent baseline fear levels remains a significant methodological issue

•Here we sought to characterize the interaction between baseline fear and tone fear by discretely manipulating baseline fear using unpaired footshocks.

•In addition, the efficacy of four simple post-hoc data manipulations were analyzed for their ability to consistently report tone fear despite differences in baseline fear.

HYPOTHESIS: We hypothesize that baseline fear interacts significantly with conditional responses during tone presentation in a way that can be corrected using a simple post-hoc data manipulation.

## MATERIALS & METHODS

- Subjects: 109 three month old, C57/BL6 male mice from Taconic were used and kept on a 12hr regular light-dark cycle.
- Fear conditioning: all experiments were done using MedAssociates VideoFreeze conditioning equipment. Two distinctly different contexts were used: Context A (wintergreen scent, cleaned with isopropyl alcohol, level grid floor rods) and Context B (windex scent, cleaned with ethanol, alternating grid floor rods). A detailed description of the training and testing protocol is described in Figure 1.
- Tone fear reporting methods: (1) Absolute tone freezing: average percent freezing across 5 tone presentations; (2) Ratio: absolute tone freezing divided by the sum of baseline and tone freezing, similar to Annau and Kamin[6]; (3) Subtraction: difference between absolute tone freezing and average freezing during the 180s baseline period; (4) Baseline covariate: baseline scores were used as a covariate in an ANCOVA during statistical analysis of absolute tone freezing.
- Statistical Analyses: An ANOVA (or ANCOVA for the covariate method) was used for all analyses with baseline manipulation group as a factor. A priori planned LSD post-hoc tests were performed for individual comparisons, when justified.

## DESIGN

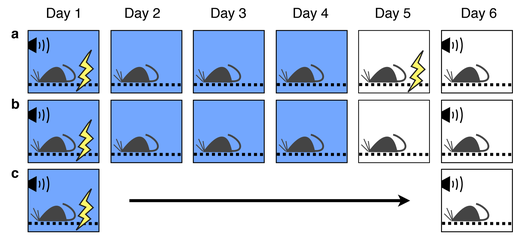


**Figure 1.** Experimental design. On Day 1 all animals were trained in Context A (blue boxes) with either 1 (weak) or 5 (strong) tone-shock pairings. Context fear extinction sessions were given in Context A on Days 2-4 for 0, 1, 2, and 6 Sh groups. On Day 5, animals received 0, 1, 2, or 6 unpaired footshocks in Context B (white boxes), a distinctly different chamber used for the tone test. **(a)** 1, 2, and 6 Sh groups. **(b)** 0 Sh groups. Note that since these groups received no aversive foot shocks on Day 5, these animals were essentially pre-exposed to the testing chamber. **(c)** No-extinction (NE) groups remained in their homecages for Days 2-5.

## RESULTS

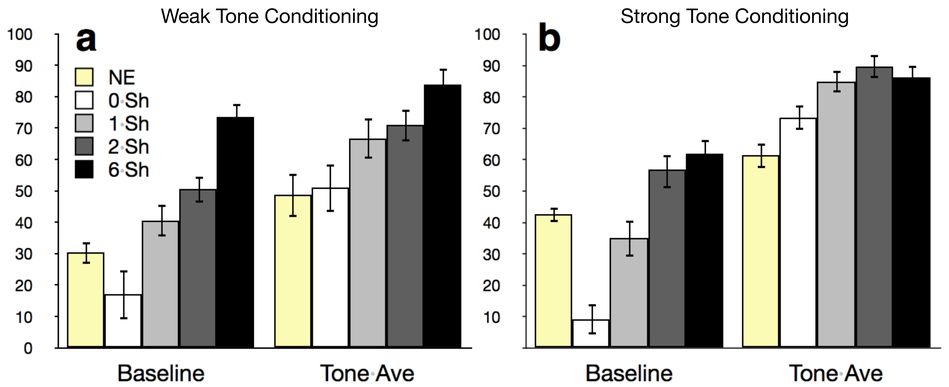


**Figure 2.** Percent freezing during the baseline period and tone presentations. **(a)** Weak tone conditioning. Both baseline and tone freezing increased as a function of the number of unpaired footshocks in the 0, 1, 2, and 6Sh groups. Compared to the 0Sh group, the NE group had higher baseline freezing but equivalent tone freezing. **(b)** Strong tone conditioning. Again, both baseline and tone freezing increased as a function of the number of unpaired footshocks given. Note that the NE group had significantly *higher* baseline freezing and *lower* tone freezing than the 0Sh group.

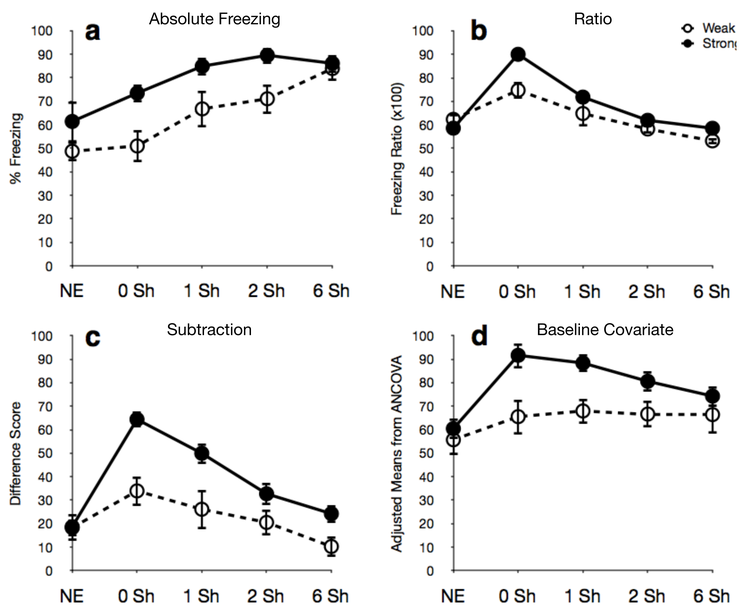


**Figure 3.** Common methods of reporting tone fear. **(a)** Absolute freezing (percentage time spent freezing) averaged over all tone presentations. **(b)** Freezing ratio of percentage freezing during the CS divided by the sum of percentage freezing during the CS and the percentage freezing during baseline. **(c)** Subtraction score calculated by subtracting percentage freezing during the 180s baseline period from percentage freezing during the tone. **(d)** Baseline covariate (adjusted means and standard error from ANCOVA). *Open circles, weak tone conditioning groups; closed circles, strong tone conditioning.*

## CONCLUSIONS

- There is a clear interaction between baseline fear and tone fear
- However, this interaction was inconsistent and none of the four reporting methods effectively corrected for differences in baseline fear.
- In light of these findings, it seems unacceptable to neglect baseline fear when interpreting measures of tone fear during discrete CS's
- Even equivalent but non-zero levels of baseline fear may still confound tone fear data.
- Reducing baseline fear to a small fraction of cued fear responses may be the only viable option for resolving the baseline issue.
- We propose a specific methodological solution: multiple days of context extinction to the conditioning chamber followed by at least one day of pre-exposure to the testing chamber.
- Reducing baseline fear in this way would avoid uninterpretable data and potentially augment differences in tone fear between experimental groups.

## LIMITATIONS/ FUTURE RESEARCH

- Baseline fear was only discretely manipulated using one technique (footshocks).
- Our results raise the possibility that the interaction between baseline and tone fear may differ depending on the exact training protocols used.
- In future projects it would be interesting to identify any such performance rules that could mediate the extent and type of baseline-tone fear interaction.

## ACKNOWLEDGEMENTS

Supported by MIMH grant # RO1 MH62122

## REFERENCES

1. Kim, J.J. and Fanselow, M.S. Science 256(5057), 675-7 (1992).
2. Reijmers, L.G., Perkins, B.L., Matsuo, N., and Mayford, M. Science 317(5842), 1230-3 (2007).
3. Huerta, P.T., Sun, L.D., Wilson, M.A., and Tonegawa, S. Neuron 25(2), 473-80 (2000).
4. Bangasser, D.A., Waxler, D.E., Santollo, J., and Shors, T.J. J Neurosci 26(34), 8702-6 (2006).
5. Shors, T.J., et al. Hippocampus 12(5), 578-84 (2002).
6. Gewirtz, J.C. and Davis, M. Nature 388(6641), 471-4 (1997).
7. Monfils, M.H., Cowansage, K.K., Klann, E., and LeDoux, J.E.. Science 324(5929), 951-5 (2009).
8. Han, J.H., et al. Science 316(5823), 457-60 (2007).
9. Marsicano, G., et al. Nature 418(6897), 530-4 (2002).
10. Monfils, M.H., Cowansage, K.K., Klann, E., and LeDoux, J.E.. Science 324(5929), 951-5 (2009).
11. Schafe, G.E., et al. Eur J Neurosci 22(1), 201-11 (2005).
12. Goggola, N.., Caroni, P., Luthi, A., Herry, C. Science. 325 (5945), p1258-1261 (2009).