Protein Synthesis Inhibition Modifies Duration and Acquisition of Loser Effect-Related Behaviors

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Abstract

The loser effect (LE) is the propensity of an organism to lose subsequent resource contests after experiencing an initial loss. In this study, we test whether ongoing protein synthesis maintains and modulates the intensity of LE behaviors in the broad-horned flour beetle, *Gnatocerus cornutus*. We find that CHX-treated losers took longer to retreat from fights and did not enter LE shutdown as rapidly as untreated beetles; however, CHX-treated losers presented with familiar opponents in a second fight entered LE more quickly than those given a novel opponent. This supports a model in which a) ongoing protein synthesis is required for the typical LE presentation in this beetle and b) LE involves components of both opponent recognition and memory consolidation which may work independently. **Investigating the molecular mechanisms of LE in this system may also clarify potential genetic influences on stress-related psychopathologies like PTSD.**

Introduction

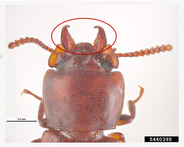
LE is observed across many organisms and may range in severity and duration; competition loss often involves subsequent changes in mating or fighting tactics by loser (Rutte et al. 2006; Okada and Miyatake 2010)

Insect systems' fast generation time, stereotyped aggressive behaviors, and more well-characterized nervous systems make them ideal for studying LE establishment

STUDY GOAL: Test whether protein synthesis is required for LE-related behaviors in *Gnatocerus cornutus*.
HYPOTHESIS: Protein synthesis inhibition eliminates loser behaviors,
RATIONALE: Protein synthesis inhibitors like cycloheximide (CHX) have been experimentally associated with memory impairment in mice and *Drosophila* (Tully et al. 1994, Yin et al. 1994; Trannoy et al. 2016)

Methods

Identifying Competitors
- 2 weeks after eclosion
- Only males have mandibular horns
- Weighed and given odd/even ID#



Initial Fight
- Fights in dark under red lamp, recorded; 20 min-1h
- Female perfumed 2.5cm arena in 6-well plate + filter paper, then one odd# and one even# male beetle added
- Losers showed LE shutdown initiation

CHX Treatment of Losers
- Fresh CHX solution dropped onto filter paper
- +CHX group incubated 16h

Behavior Trials
- 29 unfamiliar (+CHX, –CHX), 12 familiar (+CHX)
- Time of first retreat, time to LE shutdown quantified via observation of ~40-min videos

Figures and Results

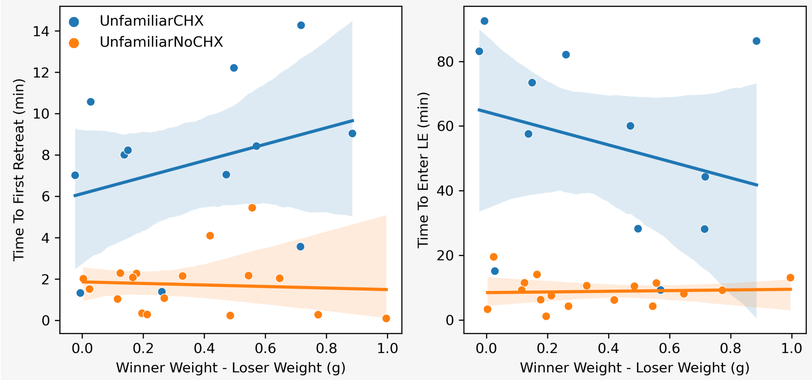


Figure 1. Effects of CHX treatment and weight difference on the time to (L) first retreat and (R) start of LE shutdown. Shaded area is a 95% CI.

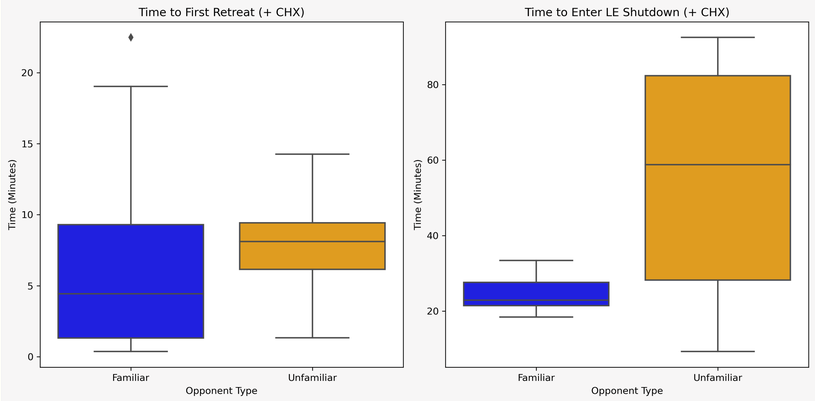


Figure 2. The time for males exposed to CHX to (A) First Retreat or (B) LE Shutdown when paired with a male they previously lost to (blue: familiar) or a male they had no prior experience fighting (orange: unfamiliar).Shaded area is a 95% CI.

Conclusion

***Ongoing protein synthesis is likely required for the typical LE presentation in G. cornutus*:** CHX-treated, losing *G. cornutus* delay both time to first retreat and transition into LE shutdown

***LE may involve independent components of both opponent recognition and memory consolidation*:** +CHX losers in familiar trials entered LE shutdown much more rapidly than their +CHX unfamiliar counterparts, but more slowly relative to -CHX competitors in unfamiliar trials

Future directions: Ruling out contribution of winner effects; **RNAi/pharmaceutical KD of genes that may be involved in different chronological aspects of LE response**

Further characterizing molecular mechanisms of LE establishment in insects facilitates ID of functional homologs to vertebrate stress pathways, more rapid screening of pharmaceutical interventions

References

Okada K, Miyatake T. 2009. Effect of losing on male fights of broad-horned flour beetle, Gnatocerus cornutus. Behavioral Ecology and Sociobiology. 64(3):361–369. doi:https://doi.org/10.1007/s00265-009-0852-0.

Pest and Diseases Images Library. broadhorned flour beetle (Gnatocerus cornutus) (Fabricius, 1798) [Image]. Bugwood.org. [updated 2012 March 9; accessed 26 March 2024]. https://www.insectimages.org/browse/detail.cfm?imgnum=5460395

Rutte C, Taborsky M, Brinkhof MWG. 2006. What sets the odds of winning and losing? Trends in Ecology & Evolution. 21(1):16–21. doi:https://doi.org/10.1016/j.tree.2005.10.014. [accessed 2021 Mar 25]. https://www.sciencedirect.com/science/article/pii/S0169534705003332.

Trannoy S, Penn J, Lucey K, Popovic D, Kravitz EA. 2016. Short and long-lasting behavioral consequences of agonistic encounters between male Drosophila melanogaster. Proceedings of the National Academy of Sciences. 113(17):4818–4823. doi:https://doi.org/10.1073/pnas.1520953113. [accessed 2021 Dec 7]. https://www.pnas.org/content/pnas/113/17/4818.full.pdf.

Tully T, Preat T, Boynton SC, Del Vecchio M. 1994. Genetic dissection of consolidated memory in Drosophila. Cell. 79(1):35–47. doi:https://doi.org/10.1016/0092-8674(94)90398-0.

Yin JCP, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, Tully T. 1994. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell. 79(1):49–58. doi:https://doi.org/10.1016/0092-8674(94)90399-9.

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