Communicative & Integrative Biology 6:2, e23501; March/April 2013; © 2013 Landes Bioscience

Drosophila larvae as a model to study physiological alcohol dependence

Brooks G. Robinson, Sukant Khurana and Nigel S. Atkinson*

Section of Neurobiology and Waggoner Center for Alcohol and Addiction Research; The University of Texas at Austin; Austin, TX USA

Keywords: *Drosophila*, larvae, alcohol, ethanol, physiological dependence

Alcohol addiction is a disease that includes a diverse set of phenotypes. Functional alcohol tolerance is an adaptation to the effects of alcohol that restores neuronal homeostatic balance while the drug is present. When the drug is suddenly withheld, these adaptations unbalance the nervous system and are thought to be the origin of some withdrawal symptoms. Withdrawal symptoms, which can be a motivating factor for alcoholics to relapse, are taken as evidence of physiological ethanol dependence. Both tolerance and withdrawal symptoms are diagnostic criteria for alcoholism. Recent studies have demonstrated that the larvae of *Drosophila* show conserved alcohol tolerance and withdrawal phenotypes indicating that *Drosophila* genetics can now be used in studying this endophenotype of alcohol addiction.

In humans, alcoholism can be thought of as having two distinct stages. Physiological tolerance and dependence make up the first stage and the second stage encompasses an unknown number of psychological events that promote the transition to uncontrolled and compulsive alcohol consumption.[17] While these phenomena have proven difficult to reproduce in animals, the physiological responses of functional alcohol tolerance and dependence have lent themselves to study in animal model systems. Understanding these processes is important because they arise from neural changes that occur during the early stages of alcohol addiction. These changes contribute to the psychological dysregulation observed in alcoholics, producing continued drinking despite serious family, health, or legal problems.

Physiological alcohol dependence is a core endophenotype of alcoholism. According to Koob and LeMoal,[11] dependence is defined by the manifestation of withdrawal symptoms that originate from the physiological adaptations that occur in response to the drug. This definition is rooted in the counter-adaptive theory of drug addiction,[14] which postulates that dependence arises from the same neuroadaptive mechanisms that produce drug tolerance. These adaptations oppose the pharmacological effects of the drug, but once the drug is cleared, their persistence is counter-adaptive and produces symptoms of withdrawal. Thus, withdrawal symptoms serve as an indicator of physiological drug dependence. The underlying counter-adaptive changes are believed to directly contribute to the motivational aspects of drug addiction. A framework for the psychological interrelationship between tolerance and dependence was outlined in the opponent process theory.[22] Here, both tolerance to the positive affective state and the negative consequences of withdrawal lead to the motivational changes that escalate drug use.[12]

Alcohol tolerance, withdrawal-induced seizure, and the rewarding effects of alcohol have all been modeled in the fruit fly *Drosophila melanogaster*.[4,6,9,21] These studies show not only a between-species conservation in the fly and mammalian behavioral responses to alcohol but also a conservation of the role of genes in these responses. While the neural circuitry of the fly and the mammalian brain do not resemble one another, there is substantial conservation of genes and signaling pathways in neurons. This conservation is sufficiently high that fly genes that modulate neural excitability or that regulate animal behavior have been used to identify their mammalian counterparts where they perform similar functions.[1,3,5,8,13,20,24] Thus, it is likely that alcohol responses that are mechanistically conserved from flies to mammals arise from the conserved cellular effects of alcohol. Behavioral outputs of flies can serve to magnify the effects of small changes in neural function.

One variant of alcohol dependence, which has been little studied in animal models, is the adaptation that allows high-functioning alcoholics to appear behaviorally normal and to be productive members of society for much of their lives.[2,15] During alcohol abstinence, their addiction becomes more noticeable because of alcohol-withdrawal symptoms. In extreme cases, symptoms can include alcohol-withdrawal seizures, but abstinence can also produce an inability to concentrate, remember, or learn.[16,23]

We have recently shown that acute alcohol treatment impairs the performance of *Drosophila* larvae in a simple associative learning and memory assay.[19] In our learning assay,[10] we leveraged the capacity of larvae to associate a noxious heat stimulus with an otherwise attractive odor. Once the association is made, memory retention can be tested by observing how the larvae respond to the odorant. Avoidance of the previously attractive odor is indicative of memory. Larvae are ideal for this purpose because large numbers of animals can be simultaneously tested in a single Petri dish. Similar learning and memory paradigms are becoming popular in larvae due to the additional model system

*Correspondence to: Nigel S. Atkinson; Email: nsatkinson@austin.utexas.edu
Submitted: 01/03/13; Accepted: 01/04/13
http://dx.doi.org/10.4161/cib.23501
Citation: Robinson BG, Khurana S, Atkinson NS. Drosophila larvae as a model to study physiological alcohol dependence. Commun Integr Biol 2013; 6:e23501

advantages of speed and economy of both behavioral assays and genetic manipulations.

In addition to acute alcohol effects, we have also seen striking effects of chronic alcohol exposure on larval behavior. Using the same learning and memory paradigm, we have shown that when larvae chronically feed on alcohol food, they adapt to it (acquire tolerance) and then can learn as well as animals that have never been exposed to alcohol. Concurrent with the development of this chronic tolerance, physiological alcohol dependence was apparent as evidenced by withdrawal symptoms. Specifically, larvae chronically treated with alcohol that underwent a subsequent abstention showed a learning deficit. Alcohol reinstatement restored normal learning in withdrawn larvae, further verifying the presence of dependence. Larvae in withdrawal also had an increased sensitivity to the convulsant drug picrotoxin indicating an underlying nervous system hyperexcitability.[18]

In our study, the larvae become dependent on alcohol after maintaining internal alcohol concentrations around 10 mM for 6 d. In a human, this would correspond to a blood alcohol concentration below the legal limit for driving in the United States. It would be unusual to observe a similar consumption pattern in humans, however in mice, alcohol withdrawal hyperexcitability has been seen following persistent low blood-alcohol levels over a period of days.[7]

Our findings indicate that the larvae of *Drosophila* can adapt to alcohol and display similar tolerance and withdrawal phenotypes as mammals. This conservation advances *Drosophila* larvae as an additional instrument to study the adaptations that lead to physiological alcohol tolerance and dependence. Alcohol addiction is a multifaceted disease that has yet to be comprehensively modeled in a non-human system. Thus, many models systems are needed because the experimental advantages and disadvantages of each allow focus to fall on a specific set of questions. The distinct toolset of the *Drosophila* model system may provide insight into aspects of alcohol-related behaviors that are difficult to study in mammals.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding Sources

This work was supported by National Institute of Health Grant R01AA018037 to N.S.A. and T32AA007471 awarded to B.G.R.

References

1. Atkinson NS. Tolerance in *Drosophila*. J Neurogenet 2009; 23:293-302; PMID:19180359; http://dx.doi.org/10.1080/01677060802572937.
2. Benton SA. Understanding the high-functioning alcoholic: breaking the cycle and finding hope. 2010; 224.
3. Brodie MS, Scholz A, Weiger TM, Dopico AM. Ethanol interactions with calcium-dependent potassium channels. Alcohol Clin Exp Res 2007; 31:1625-32; PMID:17850640; http://dx.doi.org/10.1111/j.1530-0277.2007.00469.x.
4. Cowmeadow RB, Krishnan HR, Ghezzi A, Al'Hasan YM, Wang YZ, Atkinson NS. Ethanol tolerance caused by slowpoke induction in *Drosophila*. Alcohol Clin Exp Res 2006; 30:745-53; PMID:16634842; http://dx.doi.org/10.1111/j.1530-0277.2006.00087.x.
5. Ghezzi A, Atkinson NS. Homeostatic control of neural activity: a *Drosophila* model for drug tolerance and dependence. Int Rev Neurobiol 2011; 99:23-50; PMID:21906535; http://dx.doi.org/10.1016/B978-0-12-387003-2.00002-1.
6. Ghezzi A, Krishnan HR, Atkinson NS. Susceptibility to ethanol withdrawal seizures is produced by BK channel gene expression. Addict Biol 2012; In press; PMID:22734584; http://dx.doi.org/10.1111/j.1369-1600.2012.00465.x.
7. Goldstein DB, Pal N. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. Science 1971; 172:288-90; PMID:5102255; http://dx.doi.org/10.1126/science.172.3980.288.
8. Hardin PE. Molecular genetic analysis of circadian timekeeping in *Drosophila*. Adv Genet 2011; 74:141-73; PMID:21924977; http://dx.doi.org/10.1016/B978-0-12-387690-4.00005-2.
9. Kaun KR, Azanchi R, Maung Z, Hirsh J, Heberlein U. A *Drosophila* model for alcohol reward. Nat Neurosci 2011; 14:612-9; PMID:21499254; http://dx.doi.org/10.1038/nn.2805.
10. Khurana S, Robinson BG, Wang Z, Shropshire WC, Zhong AC, Garcia LE, et al. Olfactory conditioning in the third instar larvae of *Drosophila melanogaster* using heat shock reinforcement. Behav Genet 2012; 42:151-61; PMID:21833772; http://dx.doi.org/10.1007/s10519-011-9487-9.
11. Koob GF, Le Moal M. What is addiction? In Neurobiology of Addiction. Koob GF, Le Moal M (eds), Elsevier/Academic Press, Amsterdam/Boston, 2006; 1-22.
12. Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. Science 1997; 278:52-8; PMID:9311926; http://dx.doi.org/10.1126/science.278.5335.52.
13. Mackay TF, Anholt RR. Of flies and man: *Drosophila* as a model for human complex traits. Annu Rev Genomics Hum Genet 2006; 7:339-67; PMID:16756480; http://dx.doi.org/10.1146/annurev.genom.7.080505.115758.
14. Martin WR. XVI. A homeostatic and redundancy theory of tolerance to and dependence on narcotic analgesics. Res Publ Assoc Res Nerv Ment Dis 1968; 46:206-25; PMID:5749385.
15. Moss HB, Chen CM, Yi HY. Subtypes of alcohol dependence in a nationally representative sample. Drug Alcohol Depend 2007; 91:149-58; PMID:17597309; http://dx.doi.org/10.1016/j.drugalcdep.2007.05.016.
16. Parsons OA, Nixon SJ. Neurobehavioral sequelae of alcoholism. Neurol Clin 1993; 11:205-18; PMID:8441371.
17. Ripley TL, Stephens DN. Critical thoughts on current rodent models for evaluating potential treatments of alcohol addiction and withdrawal. Br J Pharmacol 2011; 164:1335-56; PMID:21470204; http://dx.doi.org/10.1111/j.1476-5381.2011.01406.x.
18. Robinson BG, Khurana S, Kuperman A, Atkinson NS. Neural adaptation leads to cognitive ethanol dependence. Curr Biol 2012; 22:2338-41; PMID:23200990; http://dx.doi.org/10.1016/j.cub.2012.10.038.
19. Robinson BG, Khurana S, Pohl JB, Li WK, Ghezzi A, Cady AM, et al. A low concentration of ethanol impairs learning but not motor and sensory behavior in *Drosophila* larvae. PLoS One 2012; 7:e37394; PMID:22624024; http://dx.doi.org/10.1371/journal.pone.0037394.
20. Salkoff L, Baker K, Butler A, Covarrubias M, Pak MD, Wei A. An essential 'set' of K+ channels conserved in flies, mice and humans. Trends Neurosci 1992; 15:161-6; PMID:1377421; http://dx.doi.org/10.1016/0166-2236(92)90165-5.
21. Scholz H, Ramond J, Singh CM, Heberlein U. Functional ethanol tolerance in *Drosophila*. Neuron 2000; 28:261-71; PMID:11086999; http://dx.doi.org/10.1016/S0896-6273(00)00101-X.
22. Solomon RL, Corbit JD. An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol Rev 1974; 81:119-45; PMID:4817611; http://dx.doi.org/10.1037/h0036128.
23. Stavro K, Pelletier J, Potvin S. Widespread and sustained cognitive deficits in alcoholism: a meta-analysis. Addict Biol 2012; In press; PMID:22264351; http://dx.doi.org/10.1111/j.1369-1600.2011.00418.x.
24. Treistman SN, Martin GEBK. BK Channels: mediators and models for alcohol tolerance. Trends Neurosci 2009; 32:629-37; PMID:19781792; http://dx.doi.org/10.1016/j.tins.2009.08.001.