It is noted that various concentrations of ethanol do produce an effect on the VNC of the fruit fly. In addition to this, the various behavioral parameters observed, and their corresponding results, provide an overview of a few mechanisms by which ethanol acts upon the VNC of the fruit fly.

Further studies that can be conducted in addition to the behavioral assessments include estimation of ethanol concentration in whole fly extracts and development of larval to adult fly after ethanol exposure of the larva.

These tests, along with the behavioral assays can help in elucidating the complex mechanisms responsible for ethanol induced behaviors in flies and also validate the use of the fruit fly as effective model to assess behavioral paradigms for future research.

Acknowledgments: The authors thank the Department of Biomedical Sciences for permitting us to use the *Drosophila* facility and other laboratory equipment.

References: Arthur, B.I., and M.S. Magnusson 2005, *Microanalysis of* Drosophila *Courtship Behavior*. The Hidden Structure of Interaction: From Neurons to Culture Patterns, 8-15; Boris, T.P.D., P.D. Paula, and L. Hoffman 2000, Alcohol Research & Health 24: 8-13; Devineni, A.V., and U. Heberlein 2010, Communicative & Integrative Biology 4: 3-7; Jefferis, G.S., 2002, Current Opinion in Neurobiology 12: 6-10; Kaun, K.R., A.V. Devineni, and U. Heberlein 2012, Human Genetics 131: 975-978; Kohsaka, H., 2012, Development, Growth & Differentiation 54: 12; Kvitsiani, D., and B.J. Dickson 2006, Curr. Biol. 16: 355-56; Malherbe, Y., 2005, J. Evol. Biol. 18: 811-19; Nichols, C.D., J. Becnel, and U.B. Pandey 2012, Journal of Visualized Experiments 61: 5-13; Olivier, B., 2011, Curr. Top. Behav. Neurosci. 8: 15-36; Piercea, R.C., and V. Kumaresana 2006, Neuroscience and Biobehavioral Reviews 30: 23-27; Seggio, J.A., B. Possidente, and S.T. Ahmad 2012, Chronobiology International 29: 6-12; Steven, E.H.M.D., 2005, American Journal of Psychiatry 12: 162-167; Ulrike, H.P., 2000, Alcohol Research and Health 24: 3-6.

Genetic suppression of *Netrin* adult behavioral defects by *Notch*$^{l1N\text{-}ts1}$.

Alavi, Maryam, and Thomas Kidd*. Biology/ms 314, University of Nevada, 1664 N. Virginia St., Reno, NV 89557, USA; *Corresponding author (email: tkidd@unr.edu).

Abstract

Deletion of both *Netrin* genes (*NetAB*) in adult *Drosophila* leads to behavioral defects that can be suppressed by inhibiting cell death pathways. The *Notch* locus has been shown to play a role in modulating apoptosis, so we tested the ability of a *Notch* temperature sensitive allele (*Notch*$^{l1N\text{-}ts1}$) to alter *NetAB* phenotypes. Surprisingly, *Notch*$^{l1N\text{-}ts1}$ was able to suppress *NetAB* locomotor and negative geotaxis defects at the permissive temperature. These results suggest that the *Notch*$^{l1N\text{-}ts1}$ allele may have subtly impaired function even at permissive temperatures and that *Notch*$^{l1N\text{-}ts1}$ and *NetAB* mutations display positive epistasis in the adult.

Introduction

The Netrins are secreted proteins that guide developing axons over short and long distances and are best known for attracting axons to the central nervous system (CNS) midline (reviewed in Lai Wing Sun *et al*., 2011). In *Drosophila*, the two *Netrin* genes (*NetA* and *NetB*) are largely redundant (Brankatschk and Dickson, 2006), although *NetB* has a neurotrophic activity that *NetA* lacks (Newquist *et al*., 2013a). An adult viable stock lacking both *Netrin* genes (*NetAB*$^{\Delta GN}$) was created by removing a lethal mutation from a *NetAB* chromosome by recombination of the proximal portion of the chromosome. Genetic analysis suggested the existence of a distal mutation near the *white* locus that enhanced viability of the *NetAB* deletion (Newquist *et al*., 2013b). We wished to test the hypothesis that the *Notch* locus might be the gene responsible for the suppressor effect.

Notch encodes a cell surface receptor required for many developmental decisions including cell fate, proliferation, and apoptosis (Hori *et al*., 2013). *Notch* also plays a role in axon guidance and is required for development of the longitudinal connectives in the embryonic CNS (Kuzina *et al*., 2011). Longitudinal axon guidance also requires *Netrin* activity (Mitchell *et al*., 1996; Harris *et al*., 1996), and *Notch* and *Netrin* enhance defects in longitudinal axon guidance (Kuzina *et al*., 2011). Apoptotic signaling plays a role in *Netrin* mediated axon guidance (Newquist *et al*., 2013a), and *Notch* is capable of modulating apoptosis (Ye and Fortini, 1999; Lundell *et al*., 2003) suggesting that *Notch* could suppress *Netrin* phenotypes in other contexts. We constructed a recombinant chromosome carrying both the temperature sensitive *Notch* allele ($Notch^{l1N\text{-}ts1}$; Shellenbarger and Mohler, 1978) and the adult viable *NetAB* deletion ($NetAB^{\Delta GN}$) and tested the stock in adult behavioral assays, predicting that intermediate temperatures between the permissive (18°C) and restrictive (29°C) temperatures for the allele might yield results. Surprisingly, the recombinant chromosome was indistinguishable from wild type at the permissive temperature.

Materials and Methods

Drosophila stocks and Genetics

The Oregon R and $Notch^{l1N\text{-}ts1}$ stocks were obtained from the Bloomington *Drosophila* stock center. The $NetAB^{\Delta GN}$/*FM7actin-lacZ* stock is a Kidd laboratory stock (Newquist *et al*., 2013a). Recombination was carried out at 18°C and candidate recombinant stocks were screened for the *NetAB* phenotypes of uncoordination and wing posture defects, and confirmed by PCR to detect the *NetAB* deletion. The presence of the $Notch^{l1N\text{-}ts1}$ allele was detected by raising stocks at the restrictive temperature of 29°C and screening for the absence of hemizygous male progeny.

Behavioral assays

Negative geotaxis and locomotor activity were assayed by slight modifications of previously published protocols (Newquist *et al*., 2013b). Negative geotaxis was carried out in graduated cylinders with ten males individually tested three times with a one-minute rest in between. Locomotor assays were carried in vials the day after flies were born between 10 am and noon, and activity during a 45 second interval was recorded. For both assays, the experimenter was blind to genotype, and statistical analysis was performed using a Tukey HSD test within a one-way ANOVA using Statistica software.

Results and Discussion

We recombined the temperature sensitive $Notch^{l1N\text{-}ts1}$ allele onto the viable $NetAB^{\Delta GN}$ chromosome. The presence of both mutations was confirmed by assaying for temperature sensitive lethality ($Notch^{l1N\text{-}ts1}$) and a lack of coordination and altered wing position ($NetAB^{\Delta GN}$) in hemizygous males. We also confirmed the presence of *NetAB* using polymerase chain reaction detection of the deletion. We tested the recombinant stock using flies raised at 18°C, the permissive temperature for the $Notch^{l1N\text{-}ts1}$ mutation, comparing the flies to Oregon R and *NetAB* flies as positive and negative controls, respectively. Surprisingly, in both locomotor (Figure 1A) and negative geotaxis (Figure 1B) assays, the $Notch^{l1N\text{-}ts1}$ *NetAB* recombinant flies resembled wild type, being statistically different from the *NetAB* control flies. These results suggest that *Notch* can suppress certain *NetAB* phenotypes, while enhancing others such as the longitudinal axon guidance defects (Kuzina *et al*., 2011), as well as leaving other phenotypes such as altered wing positioning unchanged. *Notch-Netrin* genetic interactions are, therefore, likely to be highly dependent on developmental context, which is not surprising given the pleiotropy of Notch signaling.

The results obtained also suggest that the $Notch^{l1N\text{-}ts1}$ allele, although capable of supporting wild type development, is not completely wild type at the permissive temperature. The $Notch^{l1N\text{-}ts1}$ mutation is a missense mutation in an extracellular Epidermal Growth Factor repeat that leads to altered Notch protein distribution at the restrictive temperature (Xu *et al*., 1992; Heitzler *et al*., 1996). The mutation could possibly alter specific Notch functions at the permissive temperature while retaining overall activity. An alternative explanation for our results is that the $Notch^{l1N\text{-}ts1}$ chromosome carries an independent suppressor mutation.

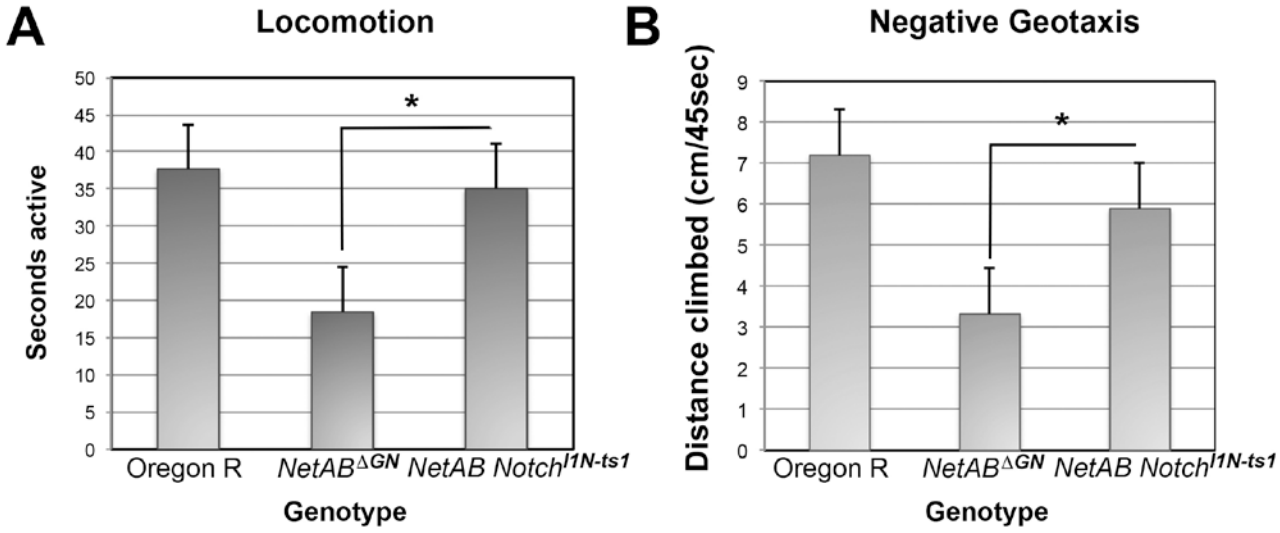

Figure 1. Behavioral assays of *Notch*$^{l1N\text{-}ts1}$ *NetAB* flies. Oregon R was used as a wild type control, and the behavior of *Notch*$^{l1N\text{-}ts1}$ *NetAB* flies was compared to *NetAB* mutants. A**,** Locomotor activity. Adult males were placed in fly food vials, tapped to the bottom of the vial, and the amount of time spent walking during a 45 second time span recorded. There was a statistically significant difference (* p = 0.01, Tukey HSD within a one-way ANOVA) between the *Notch*$^{l1N\text{-}ts1}$ *NetAB* and *NetAB* genotypes suggesting that the *Notch*$^{l1N\text{-}ts1}$ mutation rescues the behavioral defects of the *NetAB* deletion. B**,** Negative geotaxis behavior of *Notch*$^{l1N\text{-}ts1}$ *NetAB* flies. 1-2 day old flies were placed in a graduated cylinder, mechanically pushed to the bottom by a mechanical disturbance and their upward walking distance was recorded after 1 minute, with the flies being pushed back down if it neared the top of the cylinder. Ten male flies were each tested three times with a 1-minute rest between tests. There was a statistically significant difference (* p = 0.01, Tukey HSD within a one-way ANOVA) between the *Notch*$^{l1N\text{-}ts1}$ *NetAB* and *NetAB* genotypes suggesting that the *Notch*$^{l1N\text{-}ts1}$ mutation rescues the negative geotaxis defects of the *NetAB* deletion.

This mutation would have to be linked to Notch and given the large number of studies using the *Notch*$^{l1N\text{-}ts1}$ allele, it seems unlikely that such a mutation would have gone undetected. Additional temperature sensitive alleles of *Notch* could be tested, although these may be restricted to specific tissues (Shellenbarger and Mohler, 1975). Finally, we believe that the suppression is likely to be developmental in nature, but could reflect the ability of *Notch* alleles to affect behavioral functions acutely after development is complete (Presente *et al*., 2004). Our data support our original hypothesis that a *Notch* mutation could be the unidentified modifier that promotes the overall viability of the *NetAB*$^{\Delta GN}$ stock.

Acknowledgments: We thank Gunnar Newquist, Adam Bousum, and Kidd laboratory members for advice on genetics and behavioral assays. This project was supported by grants from the National Science Foundation IOS-1052555, National Center for Research Resources (P20RR016464, 5P20RR024210), and the National Institute of General Medical Sciences (8 P20 GM103554) from the National Institutes of Health. Stocks obtained from the Bloomington *Drosophila* Stock Center (NIH P40OD018537) were used in this study.

References: Brankatschk, M., and B.J. Dickson 2006, Nature Neuroscience 9: 188-294; Harris, R., L.M. Sabatelli, and M.A. Seeger 1996, Neuron 17: 217-228; Heitzler, P., M. Bourouis, L. Ruel, C. Carteret, and P. Simpson 1996, Development 122: 161-171; Hori, K., A. Sen, and S. Artavanis-Tsakonas 2013, J. Cell Sci. 126: 2135-2140; Kuzina, I., J.K. Song, and E. Giniger 2011, 138: 1839-1849; Lai Wing Sun, K., J.P. Correia, and T.E. Kennedy 2011, Development 138: 2153-2169; Lundell, M.J., H.K. Lee, E. Perez, and L. Chadwell 2003, Development 130: 4109-4121; Mitchell, K.J., J.L. Doyle, T. Serafini, T.E. Kennedy, M.

Tessier-Lavigne, C.S. Goodman, and B.J. Dickson 1996, Neuron 17: 203-215; Newquist, G., J.M. Drennan, M. Lamanuzzi, K. Walker, J.C. Clemens, and T. Kidd 2013a, Cell Reports 3: 595-606; Newquist, G., J. Hogan, K. Walker, M. Lamanuzzi, M. Bowser, and T. Kidd 2013b, PLoS ONE 8: e72524; Presente, A., R.S. Boyles, C.N. Serway, J.S. de Belle, and A.J. Andres 2004, Proc. Natl. Acad. Sci. USA 101: 1764-1768; Shellenbarger, D.L., and J.D. Mohler 1978, Dev. Biol. 62: 432-446; Shellenbarger, D.L., and J.D. Mohler 1975, Genetics 81: 143-162; Xu, T., L.A. Caron, R.G. Fehon, and S. Artavanis-Tsakonas 1992, Development 115: 913-922; Ye, Y., and M.E. Fortini 1999, J. Cell Biol. 6: 1351-1364.

Genomic localization of two public gal80ts transgenes.

Siági, Fruzsina, Nóra Zsindely, and László Bodai*. Department of Biochemistry and Molecular Biology, University of Szeged, 6726 Szeged, Közép fasor 52., Hungary; *e-mail: bodai@bio.u-szeged.hu; keywords: gal80^{ts}, transposone, genomic position, *Drosophila*

The application of thermo-sensitive *S. cerevisiae* GAL80 protein as an experimental tool was introduced to *Drosophila melanogaster* research more than a decade ago (Davis *et al.*, 2003a). These mutant proteins can be used to regulate GAL4 driven transcription enabling temporal regulation of UAS containing transgenes. The goal of this study was to determine the genomic position of GAL80ts transgenes in the P{tubP-GAL80^{ts}}10 and P{tubP-GAL80^{ts}}7 lines available from the Bloomington Drosophila Stock Center (stock #7108 and #7018, respectively). Both stocks carry a P{tubP-GAL80^{ts}} element (Davis *et al*., 2003b) expressing a temperature-sensitive Scer\GAL80 under the control of the αTub84B promoter. To determine the insertion site of the P{tubP-GAL80^{ts}} elements we applied inverse PCR followed by capillary sequencing. The 5' end of the P{tubP-GAL80^{ts}} construct has a FspBI site (CTAG) 373 bp from the end of the element. We designed inverse PCR primers (forward: TGC ACC TGC AAA AGG TCA GA, reverse: CGA CGG GAC CAC CTT ATG TT) specific for the 5' end of the P element before the FspBI site and used them in PCR reactions to generate amplicons from FspBI digested genomic DNA fragments circularized by ligation. Agarose gel electrophoresis showed single ~500 bp and ~700 bp bands in the lanes of samples prepared form stocks #7108 and #7018, respectively. There was no amplification in the control samples in which DNA ligation was omitted. We determined the sequence of the amplicons by capillary sequencing then identified the positions of the sequences on the r6.08 release of the *D. melanogaster* genome [Dos *et al*., 2015] by BLAST. The sequence recovered from stock #7108 corresponds to an intergenic genomic region (2R:14884330-14884713, inferred cytogenetic location 51D1) between the *Cyp6a20* and *Cyp6a21* genes. The sequence recovered from stock #7018 contains sequences (3R:29806159-29806760, inferred cytogenetic location 99C2) from the *non-claret disjunctional* (*ncd)* gene. The transposon is inserted at position 3R:29806760 in the 5' UTR of the ncd-RB transcript, 13 bp upstream of the transcriptional start site of the ncd-RA transcript variant.

Acknowledgments: Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. This work was supported by the Hungarian Scientific Research Fund (OTKA) grant K-112294 to LB.

References: Dos, S.G., A.J. Schroeder, J.L. Goodman, V.B. Strelets, M.A. Crosby, J. Thurmond, D.B. Emmert, W.M. Gelbart, the FlyBase Consortium 2015, Nucleic Acids Res. 43(Database issue): D690-D697; Davis, R.L., S.E. McGuire, P.T. Le, A.J. Osborn, and K. Matsumoto 2003a, Science 302: 1765-1768; Davis, R.L., S.E. McGuire, P.T. Le, A.J. Osborn, and K. Matsumoto 2003b, A. Dros. Res. Conf. 44: 153.