HHS Public Access

Author manuscript

J Neurogenet. Author manuscript; available in PMC 2018 May 29.

Published in final edited form as:

J Neurogenet. 2016 ; 30(3-4): 155–158. doi:10.1080/01677063.2016.1218489.

How the Ganetzky Lab Drove Me to Alcohol

Nigel S. Atkinson

Department of Neuroscience and The Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin

Abstract

A brief reminescence of my time in the Ganetzky lab from 1986–1990 and its effect of my scientific tragectory.

Main Text

In 1986 I moved from yeast to Drosophila. All of my education and research experience was with single-celled organisms or phage. I had never worked with an animal and had seen but not really noticed fruit flies. But I had become infatuated with Seymour Benzer's treatment of flies as particles of behavior and with the idea that genetics could be used to identify the genes and proteins that produced complex behaviors (Benzer, 1967; Benzer, 1973). I was a postdoc in Barry's lab during an exciting time—we were in or near the center of the first cloning of genes for ion channels and synaptic proteins.

One can say that I literally ascended into the ether of the Ganetzky genetics lab. The lab was on the fifth floor of the genetics building and reeked of ether. I remember thinking that since ether was heavier than air I should hold my breath when I bent down to pick up a pencil or risk anesthetizing myself. By the end of my first month, I could not smell ether unless I put my nose on top of the can.

I have often thought about what it was that made Barry so successful. There are numerous equally smart and hard-working people whose labs do not produce the level of important work that Barry's has. I think that two things made Barry Barry. First was a complete and unshakeable belief in what we today call classical genetics. I think that Barry's generation were the apex inheritors of a story of genetics that dated back about a hundred years and that was replete with profound success after success. In principle, old-school genetics is so simple that experiments based on it have the opportunity for poignant elegance. The geneticists that inherited this legacy differed from religious zealots only in that the geneticist's fervor had a foundation of concrete achievements. I learned from Barry to view behavior as a magnifying glass that amplified tiny defects in the performance of single neurons into crystal-clear and often odd changes in behavior. Single-gene mutants that produced seizures or paralysis were our bread and butter (Ganetzky and Wu, 1985; Elkins & Ganetzky, 1988; Stern & Ganetzky, 1989; Loughney, Kreber & Ganetzky, 1989; Warmke,

Corresponding Author: Nigel Atkinson, Department of Neuroscience, College of Natural Sciences, 1 University Station C7000, Austin, TX 78712, Phone: 512-232-3404, Fax: 512-471-9651, nsatkinson@austin.utexas.edu.

Drysdale & Ganetzky, 1991; Drysdale, et al., 1991; Kernan, et al. 1991; Atkinson, Robertson, & Ganetzky, 1991; Gho & Ganetzky, 1992). It was obvious that these mutations had identified genes important for the normal function of the nervous system. And it was obvious that genetics would let us identify the genes and tell us how it all worked.

The second attribute that I think helped drive Barry's success was a gift for recruiting people who were independent and who also viewed the world through the prism of genetics as all powerful. In his lab, Barry maintained an environment that cultivated these attributes. I wish I knew how this was done. Barry's approach to managing his lab was devoid of the pressures of today. While Barry wrote grants and fought to get papers published, his postdocs were mostly free of this pressure. We had the freedom to discover things on our own, to chart the direction of our projects, and to make mistakes and recover from them. I remember that I clearly understood that I had been handed the opportunity to fail. We could waste tons of time (my modes of time wasting included dissecting flies with razor blades to see for myself what was inside, hangovers, and writing programs that had no useful purpose). But we also had time to think and learn, and with the opportunity to fail came the opportunity to own any success we might have. It is the only time in my life that I have read review journals cover to cover (Trends in Genetics, Trends in Neuroscience). I had no clock. The only pressure to perform was internal, and the only reward was having something to say at lab meeting in front of my lab mates. Barry must have realized that when given the choice between sinking or swimming, we would probably swim. Barry would give you advice if you went to him, but he did not seem to have any personal need to give you advice. I know that I felt that he trusted us to make good decisions, which made me try harder to do so.

Our usual daily interaction with Barry was when he came into the lab to do his fly work or talk on the phone. He would push some flies and then return to his writing. The other time he came to the lab was when someone called him. The phone was in the fly pushing lab and Barry would do quite a bit of his phone business pacing, tethered by the long cord to the wall phone. This openness was interesting, because we overheard who was doing what, heard what Barry thought, and picked up names of people we had not met.

In my time, the strength of the lab's belief in genetics pushed us to do things that no one would undertake today. For example, my project was to clone the *slowpoke* (*slo*) gene (Atkinson, Robertson, & Ganetzky, 1991). We had a single mutant allele and nothing else. My entire plan for how to clone *slo* was to isolate more mutant alleles with the expectation that one of them would provide a molecular entry point to the gene. P-element mutations did not work—the insertions always seemed unstable. I just kept isolating gamma ray–induced chromosome rearrangements and cytologically mapping them. The sticky feet phenotype of *slo* mutants could be tested for by briefly heat pulsing a vial of flies, banging them out onto the bench, and then pushing on them with a pencil point. Mutant *slo* animals could not release their grip on the bench. The sticky-feet phenotype caused the animals to hang on to the surface, leaning over as I pushed until they toppled over. New *slo* mutants were easily captured. I am going to disavow any knowledge of where the nonmutants went. I knocked over about 20,000 flies with a number 2 pencil with a Pink Pearl eraser in order to generate four new *slo* alleles. One of them was a chromosomal inversion that juxtaposed a piece of *slo* next to a previously cloned gene (*E*(*spl*)). This gave me my "in" to the *slo* gene. Even in

that world without PCR or sequenced genomes, cloning it was then child's play. This approach gave us the *slo* gene—the first example of a BK-type Ca^{2+}-activated K^+ channel from any organism. Based on *slo*, BK channel genes were quickly cloned from a variety of mammals. In today's research climate, one could not convince anyone that the above approach was sensible, and a grant application based on it would be an instant triage casualty. The approach is clearly all based on chance and is absent any way to guarantee success. But to the Ganetzky lab of that time—true believers in the power of genetics—this was a sure thing. There was no doubt, and we were right (Atkinson, Robertson, & Ganetzky, 1991).

I inherited the *slo* project from Tom Elkins, Barry's graduate student, who had just finished his dissertaion on *slo*. It was Tom who performed the original electrophysiology connecting the *slo* gene to a Ca^{2+}-activated K^+ current. Tom proposed that *slo* encoded either the channel itself or a factor that was essential for channel function (Elkins, Ganetzky & Wu, 1986; Elkins & Ganetzky, 1988). Tom also described how temperature shifts could be used to tease out the *slo* mutant behavioral phenotype. I think that I memorized Tom's first two papers and parts of his thesis. Tom was about to leave Madison when I interviewed and we only met again at the Fly Meeting where I brought him up to date concerning my (at the time, lack of) progress on *slo*. I am always a bit sad that I was unable to show Tom what grew out of his work on *slo*. Tom died in a collision with a drunk driver in 1989. Tom was personable and bright and made a lasting impression. When he died it was like someone had poured a clingy sadness onto everything in the fifth floor of the Genetics building. To say that Barry was distraught is a very great understatement.

What have I done after Barry's lab? I wanted *slo* to give up more of its behavioral secrets. I was certain that changes in channel expression would be linked to changes in behavior. Understanding the *slo* transcriptional control region was supposed to be a quick study, but it ended up being much more complicated than imagined—having about a 7 kb transcription control region with at least five tissue-specific promoters (Brenner, Thomas, Becker, & Atkinson, 1996; Chang et al., 2000; Bohm, Wang, Brenner, & Atkinson, 2000). This really became a profitable avenue of research when I discovered drugs and alcohol. Not for me, but for my flies. The first drug that I tried was imidacloprid—an acetylcholine mimic that produced seizures in flies. I thought that in response, BK channel expression would be induced in a homeostatic "effort" to reinstate normal neuronal excitability. Bayer, Inc. sent me an imidacloprid sample dissolved in a solvent. It had interesting effects but was hard to work with because it often killed flies. We wanted to test the solvent as a control, but at the time Bayer decided that the name of the solvent was proprietary. Fortunately, I had in the lab a very creative undergraduate (Yazan Al Hasan) who had friends in a mass spectroscopy and NMR lab. One day he came in and told me that the solvent carrier was benzyl alcohol. That stuff was amazing.

It had almost no toxicity to flies. Flies knocked out with benzyl alcohol fumes recovered rapidly when moved into fresh air, and after recovery they demonstrated tolerance (induced resistance) to its sedative effects. Most important, tolerance was completely dependent on the benzyl alcohol–induced expression of the *slo* gene! For us, this carrier solvent was a

more attractive model drug than imidacloprid was (Ghezzi, Al-Hasan, Larios, Bohm, & Atkinson, 2004).

I thought that this was great; benzyl alcohol was a drug that induced expression of a channel gene, and this induction caused a behavioral change in response to the drug. We immediately started studying how *slo* was induced by benzyl alcohol. My only problem was that as a drug, benzyl alcohol lacked a constituency of individuals who abused or were addicted to it. It turned out that NIH had developed a practical streak that required drug abuse research to actually focus on drugs that people abuse. I had two choices. Switch to a drug that NIH viewed as an important social problem or try to get a bunch of people addicted to benzyl alcohol. I chose the former. Not being organic chemists, we figured alcohol was alcohol and so we just tried a different alcohol—ethanol. We obtained nearly identical results when we sedated flies with ethanol. I instantly became tied to modeling alcoholism in flies.

This has worked surprisingly well. It turns out that flies have many of the same alcohol responses that humans do. They seek ethanol by choice, use it as a pharmaceutical to treat infection, and are intoxicated by it, recover from it, and acquire tolerance to it (Milan, Kacsoh, & Schlenke 2012; Scholz, et al 2000). We began with the study of tolerance, which is defined as a drug-induced resistance to an effect of the drug. By chance, adult flies proved ideal for the study of functional tolerance (reduced response of the nervous system to ethanol) because they do not acquire metabolic tolerance (changed rate of ethanol clearance), whereas in mammals both phenomena occur simultaneously, complicating analyses (Cowmeadow, Krishnan, & Atkinson, 2005; Cowmeadow et al., 2006).

Functional tolerance is probably the first neural adaptation that occurs following ethanol exposure. Understanding this adaptation could help one understand the myriad of changes that occur during the development of alcoholism. We showed that the *slo* BK channel gene was central to this process. Increased BK channel expression acted as a neural excitant by reducing the neuronal refractory period and thereby increasing the capacity for repetitive neuronal firing. While alcohol was on board, this change could help counter alcohol sedation. However, after alcohol clearance, the increased excitability remained and caused the animals to have an overly excitable nervous system and an increased susceptibility for seizures. Alcohol-withdrawal hyperexcitability is also seen after alcohol clearance in people. We showed that alcohol induction of a single gene, *slo*, could produce two alcohol-related responses—tolerance and alcohol-withdrawal excitability (Ghezzi, Krishnan, & Atkinson, 2014; Ghezzi, Pohl, Wang, & Atkinson, 2010).

Barry’s lab was where I first learned to study behavior. Then, it was all new to me. Barry expected that we would learn or develop any assay that we needed. This was a valuable lesson for me, and I can see it echoed in my own laboratory. We assay all kinds of behavior: if it’s a behavior, we assume that we can assay it. I think the farthest afield that this has taken us are experiments on how ethanol dependence can be assayed using a learning assay. Alcoholism is a disease that perverts higher-order thinking in humans, and it is generally recognized that alcoholics become cognitively dependent on ethanol. We rationalized that this could be studied in flies. We showed that when larvae first consume food laced with 5% ethanol, their capacity to perform associative learning drops. With time, adaptation occurs

and the animals learn normally. However, these adapted animals are now functionally dependent on ethanol, and when ethanol is withheld, their ability to learn plummets. Ethanol reinstatement very quickly restores the normal capacity to learn (Robinson, Khurana, Kuperman, & Atkinson, 2012).

We have since moved on to chromatin-immunoprecipitation assays (ChIP) as a way to figure out how *slo* senses ethanol sedation. A common event in gene activation is histone acetylation triggered by activating transcription factors. Using ChIP to measure ethanol-induced histone acetylation, we could determine which regions of *slo*'s large transcriptional control region was being "touched" by transcription factors. We showed that alcohol induction of *slo* required modulation by CREB and that CREB-induced histone acetylation across the *slo* control region, resulting in induction of the *slo* neural transcripts (Wang, Krishnan, Ghezzi, Yin, & Atkinson, 2007; Wang, Ghezzi, Yin, & Atkinson, 2009). We also identified a negative putative regulatory element that when deleted from the endogenous gene caused *slo* to "overreact" to the alcohol. The slight boost in the alcohol-induced expression in animals lacking this 60 n element caused the perdurance of alcohol tolerance to jump from about 10 days to more than 21 days (Krishnan, Li, Ghezzi, & Atkinson, 2016; Li, Ghezzi, Pohl, Bohm, & Atkinson, 2013).

Then we used the alcohol-induced histone acetylation profile in a genomics survey to identify other genes that responded in kind to ethanol sedation. We ended up where many doing genomics find themselves, in the middle of gene network diagrams that by themselves appear as rational as astrological zodiac designs. However, because benzyl alcohol and ethanol are employing the same mechanism of tolerance, we look at only those genomic changes that are common to both drugs. This tack proved a godsend, allowing us to filter out almost all nonspecific alcohol effects and to focus on those effects responsible for the shared behavioral response of tolerance (Ghezzi et al., 2013). In addition, we can fall back on the power of Drosophila genetics to test the validity of the interactions and to separate important elements from statistical artifacts.

During my time, the Ganetzky lab was me, Mike Stern, Rachel Drysdale, Maurice Kernan, Katie Schlimgen, Gail Robinson, Pat Powers, Kate Loughney, Justin Thackeray, Jeff Warmke, and Doris Ursic. All of our work was facilitated by Bob Kreber, Barry's lab manager. Bob made really valuable contributions to the lab. Barry always said that if Bob ever retired that he was going to retire. I wonder if this is what happened. The most intense memories of my scientific life are from this time with Barry and the hand-picked group of people that I was fortunate to be a part of.

Acknowledgments

The Atkinson lab is currently supported by NIH grant number 2R01AA018037-06A1 to NSA.

References

Atkinson NS, Robertson GA, Ganetzky B. A component of calcium-activated potassium channels encoded by the Drosophila slo locus. Science. 1991; 253:551–555. [PubMed: 1857984]

Benzer S. Behavoiral mutants of Drosophila isolated by countercurrent distribution. Proc Natl Acad Sci U S A. 1967; 58(3):1112–1119. [PubMed: 16578662]

Benzer S. Genetic dissection of behavior. Sci Am. 1973; 229(6):24–37. [PubMed: 4202065]

Bohm RA, Wang B, Brenner R, Atkinson N. Transcriptional control of Ca2+-activated K+ channel expression: identification of a second, evolutionarily-conserved, neuronal promoter. J Exp Biol. 2000; 203:693–704. [PubMed: 10648211]

Brenner R, Thomas TO, Becker MN, Atkinson NS. Tissue-specific expression of a Ca2+-activated K+ channel is controlled by multiple upstream regulatory elements. J Neurosci. 1996; 16:1827–1835. [PubMed: 8774450]

Chang W, Bohm RA, Strauss JC, Kwan T, Thomas T, Cowmeadow RB, et al. Muscle-specific transcriptional regulation of the slowpoke Ca2+-activated K+ channel gene. J Biol Chem. 2000; 275:3991–3998. [PubMed: 10660555]

Cowmeadow RB, Krishnan HR, Atkinson NS. The *slowpoke* gene underlies rapid ethanol tolerance in Drosophila. Alcoholism: Clinical and Experimental Research. 2005; 29:1777–1786.

Cowmeadow RB, Krishnan HR, Ghezzi A, Al'hasan Y, Wang YZ, Atkinson NS. Ethanol Tolerance Caused by *slowpoke* Induction in *Drosophila*. Alcohol Clin Exp Res. 2006; 30(5):745–753. [PubMed: 16634842]

Drysdale R, Warmke J, Kreber R, Ganetzky B. Molecular characterization of eag: a gene affecting potassium channels in Drosophila melanogaster. Genetics. 1991; 127:497–505. [PubMed: 1901818]

Elkins T, Ganetzky B. The roles of potassium currents in Drosophila flight muscles. J Neurosci. 1988; 8:428–434. [PubMed: 3339425]

Ganetzky B, Wu CF. Genes and membrane excitability in Drosophila. Trends Neurosci. 1985; 8:322–326.

Ghezzi A, Al-Hasan YM, Larios LE, Bohm RA, Atkinson NS. *slo* K+ channel gene regulation mediates rapid drug tolerance. Proc Natl Acad Sci U S A. 2004; 101:17276–17281. [PubMed: 15569939]

Ghezzi A, Krishnan HR, Atkinson NS. Susceptibility to ethanol withdrawal seizures is produced by BK channel gene expression. Addiction Biology. 2014; 19:332–337. [PubMed: 22734584]

Ghezzi A, Krishnan HR, Lew L, Pardo FJ 3rd, Ong DS, Atkinson NS. Alcohol-induced Histone Acetylation Reveals a Gene Network Involved in Alcohol Tolerance. PLoS Genetics. 2013; 9(12):e1003986. [PubMed: 24348266]

Ghezzi A, Pohl JB, Wang Y, Atkinson NS. BK Channels Play a Counteradaptive Role in Drug Tolerance and Dependence. Proc Natl Acad Sci U S A. 2010; 107(37):16360–16365. [PubMed: 20798347]

Gho M, Ganetzky B. Analysis of repolarization of presynaptic motor terminals in Drosophila larvae using potassium-channel-blocking drugs and mutations. J Exp Biol. 1992; 170:93–111. [PubMed: 1328458]

Kernan MJ, Kuroda MI, Kreber R, Baker BS, Ganetzky B. napts, a mutation affecting sodium channel activity in Drosophila, is an allele of mle, a regulator of X chromosome transcription. Cell. 1991; 66:949–959. [PubMed: 1653649]

Krishnan H, Li X, Ghezzi A, Atkinson NS. A DNA element in the slo gene modulates ethanol tolerance. Alcohol. 2016 In press.

Li X, Ghezzi A, Pohl JB, Bohm AY, Atkinson NS. A DNA element regulates drug tolerance and withdrawal. PLoS One. 2013; 8:e75549. [PubMed: 24086565]

Loughney K, Kreber R, Ganetzky B. Molecular analysis of the para locus, a sodium channel gene in Drosophila. Cell. 1989; 58:1143–1154. [PubMed: 2550145]

Milan NF, Kacsoh BZ, Schlenke TA. Alcohol consumption as self-medication against blood-borne parasites in the fruit fly. Curr Biol. 2012; 22(6):488–493. [PubMed: 22342747]

Robinson BG, Khurana S, Kuperman A, Atkinson NS. Neural Adaptation Leads to Cognitive Ethanol Dependence. Current Biology. 2012; 22:2338–2341. [PubMed: 23200990]

Wang Y, Ghezzi A, Yin JC, Atkinson NS. CREB regulation of BK channel gene expression underlies rapid drug tolerance. Genes Brain Behav. 2009; 8:369–376. [PubMed: 19243452]

Scholz H, Ramond J, Singh CM, Heberlein U. Functional ethanol tolerance in Drosophila. Neuron. 2000; 28:261–271. [PubMed: 11086999]

Stern M, Ganetzky B. Altered synaptic transmission in Drosophila Hyperkinetic mutants. J Neurogenet. 1989; 5:215–228. [PubMed: 2553904]

Wang Y, Krishnan HR, Ghezzi A, Yin JC, Atkinson NS. Drug-Induced Epigenetic Changes Produce Drug Tolerance. PLoS Biol. 2007; 5(10):2342–2353.

Warmke J, Drysdale R, Ganetzky B. A distinct potassium channel polypeptide encoded by the Drosophila eag locus. Science. 1991; 252:1560–1562. [PubMed: 1840699]