Animal Models of Alcohol Studies: A Literature Review

Katie Livingood

The University of Tulsa

Abstract

The study of the effects of alcohol on humans has been going on for quite some time. However, given moral and ethical standard, it is often prohibited to perform research on humans. Using animal models to conduct research is the solution. Both vertebrates and invertebrates have been used in research, with invertebrates being more advantageous when it comes to size and cost. With the advent of genome mapping, several invertebrates, *Drosophila melanogaster* and *Caenorhabditis elegans*, have been found to have similar genes as humans. With this knowledge, researchers can use these animals to test effects of ethanol and receive similar results.

Introduction to Alcohol Models

Although studies on the effects of alcohol on humans exist, many of those studies focus on the disease of alcoholism, rather than actual alcohol metabolism. Studies that would use human subjects to focus on human alcohol intake, alcohol metabolism, or dependence upon alcohol are hard to come by because of the ethical concerns, and possibly experimental concerns, that would surround the studies. Because of these concerns, scientists have turned to animals. Animal models in alcohol studies have become a staple in the field of study because they allow scientists to experiment in ways that would not be acceptable if human subjects were used. Scientists cannot ethically use humans to research topics such as excessive intake, organ damage, or tolerance. Animal models provide scientists the ability to research these topics more efficiently and in a controlled environment.

Human Subject Research

Humans have been used as experimental subjects as far back as the Middle Ages. Human dissections, drug testing, and experiments concerning physiology occurred during these times. Early in the 1900s, it became clear that there needed to be a code of ethics researchers adhered to when it came to using human subjects in experimental research. In 1964, using the Nuremberg Code as a springboard, and looking at its flaws, the World Medical Association developed what became known as the Declaration of Helsinki (Childress, 2000). The Declaration of Helsinki stressed two important tenets – the human subject would voluntarily take part in the experiment and differentiated between therapeutic research and non-therapeutic clinical research (Childress, 2000). Helsinki was been revised since to include informed consent from the potential human subject.

The Institutional Review Board was created later on because of this declaration. The Institutional Review Board, IRB, acts as an independent ethics committee. This committee is charged with reviewing and monitoring any research that would involve human subjects. These boards are most often used in areas of behavioral science and biomedical science. In the United States, the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS) oversee the IRBs. Institutional Review Boards are used across the US, companies in public and private sectors, as well as universities.

Animal Subject Research

Since it has been found that humans and mice share a relatively similar genome, mice have been used in experimental research in which the end result would benefit humans. Using mice in research has several benefits – mice are easy to come by and do not need informed consent, mice reproduce rather quickly so it is easy to see results across several generations, and mice are inexpensive to maintain (food and housing). Other animals also provide ease for experimental research. Similar to human subject research, animal subject research also has guidelines that must be followed. In the United States, the Department of Agriculture oversees the compliance with these rules. The Animal Welfare Act of 1966 regulates the treatment of animals that are used in research. However, there are some exemptions to the Act. Mice specifically bred for research, cold-blooded amphibians and reptiles, fish, and invertebrates are some of the exemptions.

Ethanol and Its Metabolsim

Ethanol is a clear, flammable liquid that is most often found in alcoholic beverages. Chemically speaking, ethanol is a straight-chain alcohol and has the formula of CH_3CH_2OH. It is commonly abbreviated EtOH. Ethanol can be produced freely in nature by the fermentation of sugar. Excess ethanol can produce drug-like effects on the individual ingesting it. Ethanol is considered a psychoactive drug because it has the ability to change consciousness. Ethanol also acts as an enhancer for the GABA receptors in the nervous system and as a depressant on the central nervous system.

Ethanol is broken down by the enzyme by alcohol dehydrogenase to acetaldehyde. Acetaldehyde can be toxic if it accumulates. Acetaldehyde is broken down further to acetic acid by the enzyme acetaldehyde dehydrogenase. Depending upon the organism., these enzymes may vary.

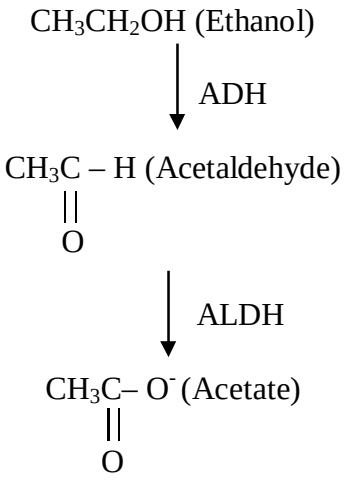
History of Human Alcohol Ingestion

The history of human intake of alcohol, in the form of ethanol, begins with frugivorous primates and their intake of fruit (Dudley, 2004). Primates eat fleshy fruit in the wild and often that fruit has rotted, producing low levels of ethanol. Fruit rot is the fermentation of fructose by yeast (Levey, 2004). The process of fermentation begins when yeast (*Saccharomyces cerevisiae*) begins to metabolize the fruit sugars, fructose. The products of this process are ethanol and carbon dioxide. The chemical reaction is as follows: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$. It is important to know that only certain yeasts will ferment fruit sugars and that is must be done in an anaerobic environment (Levey, 2004). Because of that, the fermentation by the yeast is limited and will only have a low level of ethanol.

Eating these fruits each day exposed primates to low levels of ethanol for long periods of time and might have primates and subsequent humans to associated "ethanol consumption with nutritional reward" (Dudley, 2004). It has been hypothesized that humans might have an affinity for ethanol because of this history of primate frugivory. However, once all the evidence has been looked at, it is difficult to attribute evolutionary frugivory to alcohol-intake of humans (Milton, 2004).

Biochemical Pathway of Ethanol in Humans

Alcohol is metabolized in humans along a certain pathway. The ethanol enters the body and passes from the stomach to the small intestine, where it is absorbed. The liver is responsible for detoxifying the ethanol – about 90% of the ethanol is oxidized here. The enzyme alcohol dehydrogenase (ADH) oxidizes ethanol in to acetaldehyde, which is in turn oxidized into acetic acid or acetate by aldehyde dehydrogenase (ALDH). Acetic acid is a nutrient for humans and a precursor to acetyl CoA, which can be used for energy.



It may be noted that some research indicates alcoholism to be a genetic disease, enhanced by environmental factors. It has been shown that many East Asians have a gene which inhibits the process acetaldehyde dehydrogenase (ALDH). Since ALDH does not oxidize acetaldehyde in this scenario, it builds up and becomes toxic. Because this mutation creates adverse effects, it is found that it decreases the incidence of alcoholism in this population (Ocaranza et al., 2008).

Animal Models

In order for an animal to be considered a model for alcohol studies the following should be met: "(1) voluntary self-administration of alcohol, (2) tolerance after a period of continuous consumption, (3) dependence on alcohol as revealed by withdrawal symptoms, and (4) biomedical complications associated with chronic alcohol consumption that are similar to those observed in humans" (Abramson et al., 2000).

Animal models have been used increasingly in drug research. Animal models allow researchers to bypass complex factors and high costs related to human studies (Wolf & Heberlein, 2003). For drug research, medicinal or drug-induced behaviors, rats have been the animal model of choice. Scientists have been able to study brain, neurotransmitter and molecule behavior that might cause the drug-induced behaviors.

Using animal models for alcohol research give scientists an advantage in their field of study. Scientists can circumvent ethical concerns and possible physiological and psychological consequences (Tabakoff & Hoffman, 2000). Depending on the nature of the research, different animals are chosen by the researchers. Many animals are chosen because of the similarities of their biological system to humans. Animals may be chosen based upon their face validity or predictive validity to human systems. Face validity means the animal might actually mimic the human condition and predictive validity means the model might hold predictive value, such as treatment value (Tabakoff & Hoffman, 2000).

Historically, vertebrate models have been used in alcohol research. Mice, rates and primates have been offered as models by scientists. These animals offered the ability to study tolerance, withdrawal and organ damage. Some behavioral studies have also been completed using vertebrate models. More recently, invertebrates have been selected as models for alcohol

research. Invertebrates such as *Drosophila melanogster*, *Caenorhabditis elegans* and *Apis mellifera* have been used as models. Invertebrate models are used to study the biochemistry behind alcohol intake, alcohol metabolism and sometimes behavioral experiments been performed as well.

Vertebrate Models

Researchers have used primates as models in alcohol research; however, these studies can be costly. Baboons have been used in studies focusing on alcohol-induced liver diseases. These studies started as early as 1973, when a technique to feed baboons a nutritional liquid diet, along with ethanol, was first developed. Charles Lieber and Emanuel Rubin developed this technique in order to form an animal model of liver disease that might closely resemble that seen in humans. Lieber and Rubin worked on this technique and refined it into the 1980's. They had recently performed studies on rats, but felt that the baboon metabolism was closer to human metabolism. They were able to reproduce the histologic features of human liver disease in baboons (Lieber & Rubin, 1973).

Primates have also been the focus of imaging studies to learn about how the brain functions during certain alcohol-induced behaviors. These studies also focus on brain activity and neurotransmitter activity during exposure to alcohol, (Sullivan et al., 2005).

Recently the chicken genome has been found to be more similar to the human genome than that of a mouse. However, considerations have to be made in deciding whether to use the chicken as a model, because of the strong differences between human and avian behavior (Tabakoff & Hoffman, 2000).

Mice models are often used in alcohol research because of their "considerable genetic homology with humans" (Bennett et al., 2006). The mice model offers researchers the ability to manipulate the genes associated with alcoholism. Researchers can study tolerance and withdrawal with these models without doing any harm to human subjects. Genetically modified mice have proved convenient and successful when it comes to manipulating certain genes and producing different, but specific strains of mice that react differently to certain levels of ethanol.

Researchers have the ability to manipulate genes in mice by several different technologies – over-expression, complete elimination, and modification (Bennett et al., 2006). Using these technologies, researchers have been able to produce mice with genetic phenotypes that are useful for alcohol models. Tolerance, withdrawal, motivational effects, and high-dose sensitivity are four such phenotypes that have been used in alcohol studies (Bennett et al., 2006). By using these genetically modified mice strains, researchers are closer to identifying specific genes associated with alcohol-related behaviors, and possibly alcoholism as well. By identifying the genes associated with alcoholism, the biochemical pathways influenced by alcohol will also be identified.

Rats have also been used in alcohol studies. Researchers have recently used rats in neuro-imaging studies to examine brain activities during ethanol exposure (Sullivan et al., 2005). Specifically, rats have been used to study the kinetics of alcohol metabolism (Sullivan et al, 2005). There is much variability among all individuals in the uptake of alcohol in blood and in brain. Rats have also been used for studies of alcohol dependence and tolerance (Matthews et al., 2001). It is difficult to study dependence and tolerance when the subjects are forced to ingest the alcohol. Rats were trained to self-administer alcohol through a "sucrose-fading" technique. This consisted of training rats to respond to available sucrose solutions for a period of time, and slowly adding ethanol to the sucrose solution as time progressed (Matthews et al., 2001).

Recently a study was performed using a genetically modified strain of rat. In this study, researchers hoped to show that gene therapy might be beneficial to alcoholics (Ocaranza et al., 2008). The strain of rat used was one that was predisposed to high levels of ethanol intake; therefore, "alcoholics". The study found that the "alcoholic" rats, when injected with a gene that acts against ALDH, will ingest roughly 50% less alcohol (Ocaranza et al., 2008). This study

shows gene therapy as a possible solution to alcoholism in humans. However, much more research is needed in this area.

Invertebrate Models

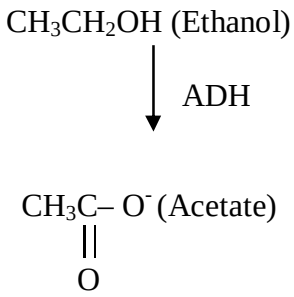
Invertebrates are increasingly being used in alcohol research because of their low cost, the ease of care, and the ability to see the experiment through several generations in a short amount of time. Invertebrates also allow researchers to explore areas of alcohol research at the cellular, molecular and genetic levels that are not readily available to research in vertebrates (Davis, 2008).

Invertebrate models are being chosen as models for alcohol research because of their simplistic nervous system and the ability to rapidly see genetics at work (many generations in a short amount of time). Studies show that vertebrates and invertebrates share molecular similarities in their nervous systems and many of the major neurotransmitters are shared. Sites of ethanol action, such as ligand-gated ion channels, are shared among humans and invertebrates such as *Drosophila melanogaster* and *C. elegans*. Because of these shared sites, these two invertebrates continue to provide proof that invertebrates can be used as models for alcohol studies.

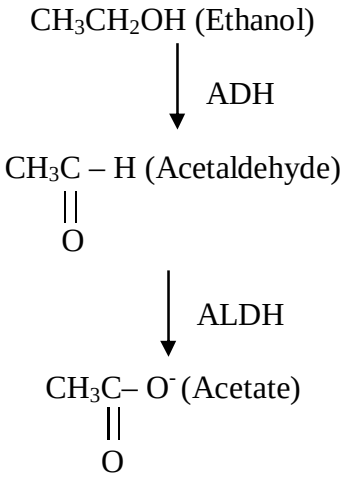
Drosophila Melanogaster

Biochemical Pathway of Ethanol in *Drosophila melanogaster*

In the fruit fly, ethanol is oxidized into something useful by the organism roughly following the same pathway as in humans. *Drosophila melanogaster* contains relatively the same enzymes used in this pathway as humans. However, for the enzyme alcohol dehdydrogenase, there exists four different alleloenzymes. It has also been noted that acetaldehyde dehydrogenase (ADH) in *Drosophila* may have the ability to oxidize acetaldehyde to acetate by itself (Geer, Heinstra, and McKechnie, 1991). This would mean that the acetaldehyde dehydrogenase (ALDH) in *Drosophila* is not as important as it is in the pathway in humans. The following would be an example of the possible pathway in *Drosophila*, if ALDH is unnecessary.



More recent research has noted that ALDH does play in important role in ethanol metabolism in *Drosophila*; however, it was also discovered that ALDH has variable expression among different populations of *Drosophila* (Frye et al., 2004). With this information, it would more probable that ethanol is metabolized by the following pathway, the same as humans:



Drosophila melanogaster as a Potential Model

Drosophila melanogaster has been used in many different kinds of research because of its fully mapped genome and the knowledge that it has both genes and biochemical pathways that are similar to humans. The *Drosophila* genome is easily manipulated and allows researchers the ability to modify it. The ability to identify genes that might contribute alcohol-related behaviors or alcohol-related phenotypes in *Drosophila* opens doors to the same type of research with humans because of the molecular similarities which *Drosophila* and humans share (Heberlein, 2000).

Drosophila melanogaster has a history of ethanol consumption. Many species of *Drosophila* breed in fermented fruit, and therefore can be exposed to concentrations up to 5% ethanol (Fry et al., 2004). Because of this history, *Drosophila* can provide a wide range of tools (genetic, molecular, etc) for scientists to study. The wide range of species, some breeding in fermented fruit and others not, enzymatic activity as it relates to tolerance can be studied (Fry et

al., 2004). Specific enzymatic activity, such as alcohol dehydrogenase and aldehyde dehydrogenase, can vary the tolerance found in *Drosophila* populations (Fry et al., 2004).

The use of *Drosophila melanogaster* has increased in recent years as the impetus for animal models in alcohol research has grown. Behavioral, genetic and molecular studies have been performed. Because of the molecular similarities between *Drosophila* and humans, research alcohol research has increased using the insect. *Drosophila* have many of the same neurotransmitters that humans do. Mutants of *Drosophila* have been made to evaluate different roles of genes in alcohol-related behaviors. When intoxicated, *Drosophila* exhibit some of the same behaviors as humans – "incoordination, loss of postural control, and eventually sedation and immobility" (Heberlein et al., 2004). When exposed to certain concentrations of ethanol, *Drosophila* show alcohol-induced behavioral responses similar to that of vertebrates exposed to the same concentrations (Wolf & Heberlein, 2003).

Recently, *Drosophila* have been used in the study of alcohol tolerance. *Drosophila* exhibit a tolerance to ethanol after a single exposure, which is termed functional tolerance. However, *Drosophila* can also exhibit metabolic tolerance to ethanol. Scientist studying metabolic tolerance in *Drosophila* have found that the molecular mechanisms controlling tolerance is similar between flies and mammals (Wolf & Heberlein, 2003). This provides researchers with another area of alcohol studies. The ability to pinpoint the genes behind alcohol tolerance provides researchers and medical doctors information that could impact the treatment of alcoholism in humans.

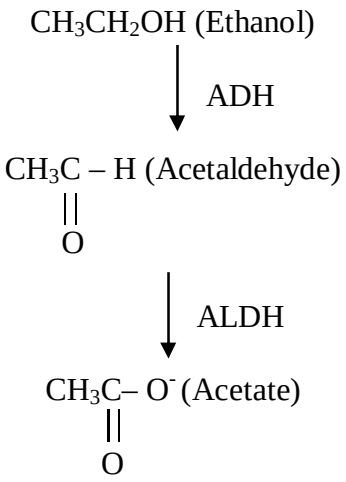
The ability of researchers to produce certain strains of *Drosophila melanogaster* and to use them experimentally has proved successful in research. Similar to the experiments with genetically altered mice, researchers have genetically altered *Drosophila* to give mutants have

altered ethanol sensitivity. The mutants *cheapdate*, *light-weight*, and *tipsy* show increased ethanol sensitivity, while the mutant *barfly* shows reduced ethanol sensitivity (Heberlein et al., 2004). Further research shows that *cheapdate* was able to disrupt the gene mutant *amnesiac*, which had been found to disrupt cAMP signaling (Heberlein et al., 2004). The use of these genetically altered mutants allowed researchers to find genetic evidence that the cAMP signaling pathway is essential in ethanol sensitivity (Moore et al., 1998). With this information, it could be deduced that the same cAMP pathway in human cells also plays a part in ethanol sensitivity, possibly reducing sensitivity in humans, contributing to alcoholism. Furthermore, the use of several different genetically altered strains of *Drosophila* affords the ability of the researcher to pinpoint certain genes that might be responsible for certain aspects of alcohol ingestion. This was exhibited in experiments that used several different strains of fruit fly to pinpoint which genes might affect locomotor control under acute ethanol ingestion (Singh & Heberlein,2000). All this evidence points to *Drosophila melanogaster* being an excellent model for alcohol studies (Moore et al., 1998).

Caenorhabditis elegans

Biochemical Pathway of Ethanol in *Caenorhabditis elegans*

Research suggests that *Caenorhabditis elegans* contains two different types of alcohol dehydrogenase enzymes.



Caenorhabditis elegans as a Potential Model

This invertebrate has also been recently used in alcohol studies. *C. elegans* is a small, soil-dwelling worm found in temperate regions. The complete genome has been mapped, neurons characterized, and embryologic development thoroughly studied (Davis, 2008). In working with this invertebrate, researchers have found that exposure to alcohol effects size, rate of development, and reproductive success of *C. elegans* (Davis, 2008). These findings are similar to those in vertebrate studies. These studies offer evidence that *C. elegans* is would be a useful model for alcohol studies.

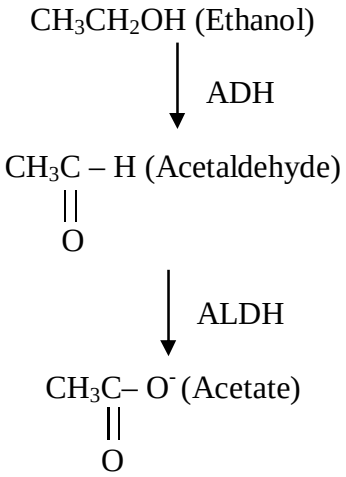
Studies have shown that when *C. elegans* have been exposed to different concentrations of ethanol, the behavior is similar to that of *Drosophila* and of vertebrates. A period is stimulation is followed by a period of sedation and immobility (Wolf & Heberlein, 2003 and Mitchell et al, 2006). *C. elegans* have the ability to intake ethanol in two different ways – either

through actual ingestion or through their cuticle. It has been observed that the concentration of ethanol crossing over the cuticle through immersion is higher than when *C. elegans* ingests the ethanol orally (Mitchell et al, 2006). In order for alcohol research with *C. elegans* to be useful, it would be necessary to clarify whether immersion or ingestion was used. Mitchell et al (2006) found that immersion of *C. elegans* provides a good model for alcohol research because it can provide a controlled way to expose the worm to ethanol. However, it was also found that the alcohol-induced behavior is concentration dependent and should be reported as such in all studies (Mitchell et al, 2006).

Apis Mellifera

Ethanol Metabolism in *Apis mellifera*

While much research has been done concerning the actual ingestion of ethanol of honeybees (which will be covered in the following section), only some research has been completed concerning the physiology of the honey bee and the ingested ethanol. It has been found ethanol ingestion in honey bees can be measured by using the hemolymph (Bozic et al., 2007). Honey bee hemolymph is analogous to human blood, acting as a circulating agent for the insect. While ethanol has been found to follow the same metabolism pathway as in the other organisms mentioned, it is unclear to what extent these enzymes are expressed or are varied (Maze, Wright, and Mustard, 2006).



Apis mellifera as a Potential Model

In 2000, Abramson proposed that *Apis mellifera*, the honeybee, could also serve as a useful model in alcohol research. The honeybee willing consumes ethanol (and not just when mixed with a sucrose solution), ethanol effects the behavior of the honeybee, and the honeybee will self-administer ethanol (Abramson et al., 2000). Honeybees meet the first criterion that was set forth for an animal to be considered as a model. Further research is to be done in order to see

if honeybees will meet the other three criteria, with special attention paid to the last criterion of having the same biomedical complications associated with chronic alcohol consumption as humans do (Abramson et al., 2000).

Honeybees carry a similar molecular structure to that of humans. The use of honeybees provides several advantages over other invertebrates – honeybees are cost effective, their entire genome has been sequenced, they exhibit a wide-range of complex behaviors that can be studied, they provide a means to study early development, and honeybees will consume ethanol, as well as self-administer ethanol (Abramson et al., 2007). It has been found that honeybees will exhibit some of the same type of alcohol-related behaviors as other vertebrate models.

Recent studies have corroborated the thought that ethanol can affect *Apis mellifera* in the same ways it affects humans, *Drosophila melanogaster*, and *Caenorhabditis elegans*. One particular study found that ethanol affects were dose-dependent and acute ingestion caused symptoms to appear earlier and last longer (Maze et al., 2006). It was noted in this study that *Apis mellifera* seemed to stay inebriated longer than *Drosophila melanogaster* (Maze et al., 2006). This was attributed to the fact that honeybees have a "honey stomach", a stomach which is impermeable and allows the insect to store nutrients which will not be used by the individual. This study concluded that while ethanol does have effects on motor function of other invertebrates, *Apis mellifera* is a prime model because of the ability for honey bees to self-administer ethanol and certain characteristics that the honey bees exhibit – social behavior, the ability to learn and the ability to have memory (Maze et al., 2006).

With the information from the above experiment, more research was conducted concerning the learning aspect. It has been observed that alcohol ingestion in humans while learning a task can delay learning abilities. While much research has been completed concerning

Apis mellifera and ethanol ingestion, the majority of the research has focused on the physical effects it has on the organism (Mustard et al., 2008). It was found that acute ethanol ingestion can impair learning in the honey bee, specifically with olfactory cues (Mustard et al., 2008).

Conclusion

The effects of ethanol on humans will continue to be a growing research area. While vertebrates provide researchers the ability to examine motor function and organ function similar to humans, invertebrates also provide researchers the ability to examine motor function and molecular function. The use of invertebrates afford the researcher a faster life cycle, lower costs, and less maintenance. Using either of the three invertebrates mentioned in this paper would provide researchers with a wealth of knowledge, especially as more work is done at the molecular level concerning the effects of ethanol on the nervous system. Of particular interest to the author, is the use of *Apis mellifera* as a potential model for alcohol studies. Literature suggests that it is a prime organism for a model because of the similarity of molecular structure and characteristics such as social behavior, memory, and learning. Further research concerning the honey bee's use of the "honey stomach" in its intake of ethanol will be performed.

References

Abramson, C.I., Stone, S.M., Ortez, R.A., Luccardi, A., Vann, K.L., Hanig, K.D., and Rice, J. (2000). The development of an ethanol model using social insects I: Behavior studies of the honey bee *(Apis mellifera* L.). *Alcoholism: Clinical and Experimental Research, 24*(8), 1153-1166.

Abramson, C.I., Wells, H., and Bozic, J. (2007). A social insect model for the study of ethanol induced behavior: The honey bee. *Trends in Alcohol Abuse and Alcoholism Research,* 197-218.

Bennett, B., Downing, C., Parker, C., and Johnson, T.E. (2006). Mouse genetic models in alcohol research. *TRENDS in Genetics, 22,* 367-374.

Bozic, J., DiCesare, J., Wells, H., Abramson, C.I. (2007). Ethanol levels in honeybee hemolymph resulting alcohol ingestion. *Alcohol, 41,* 281-284.

Childress, J. (2000). Nuremberg's legacy: Some ethical reflections. *Perspectives in Biology and Medicine, 43,* 347-361.

Davis, J.R., Li, Y., and Rankin, C.H. (2008). Effects of developmental exposure to ethanol on *Caenorhaditis elegans*. *Alcoholism: Clinical and Experimental Research, 32*(5), 853-867.

Dudley, R. (2004). Ethanol, fruit ripening, and the historical origins of human alcoholism in primate frugivory. *Integrative and Comparative Biology, 44,* 315-323.

Fry, J.D., Bahnck, C.M., Mickucki, M., Phadnic, N., and Slattery, W.C. (2004). Dietary ethanol mediate selection on aldehyde dehydrogenase activity in *Drosophila melanogaster*. *Integrative and Comparative Biology, 44,* 275-283.

Geer,B.W., Heinstra, P.W.H., and McKechnie, S.W. (1993). The biological basis of ethanol tolerance in Drosophila. *Comparative Biochemistry and Physiology, 105B,* 203-229.

Heberlein, U. (2000). Genetics of alcohol-induced behaviors in Drosophila. *Alcohol Research & Health, 24*(3), 185-188.

Heberlein, U., Wolf, F., Rothenfluh, A., and Guarnieri, D.J. (2004). Molecular genetic analysis of ethanol intoxication in *Drosophila melanogaster*. *Integrative and Comparative Biology, 44*, 269-274.

Levey, D.J. (2004). The evolutionary ecology of ethanol production and alcoholism. *Integrative and Comparative Biology, 44*, 284-289.

Lieber, C and Rubin, E. (1973). Experimental alcohol hepatitis: A new primate model. *Science, 182*, 712-713.

Matthew, D.B., Overstreet, D.H., Rezvani, A.H., Devaud, L.L., and Morrow, A.L. (2001). Effects of sweetened ethanol solutions on ethanol self-administration and blood ethanol levels. *Pharmocology, Biochemistry and Behavior, 68*, 13-21.

Maze, I.S., Wright, G.A., and Mustard, J.A. (2006). Acute ethanol ingestion produces does-dependent effects on motor behavior in the honey bee (*Apis mellifera*). *Journal of Insect Physiology, 52*, 1243-1253.

Milton, K. (2004). Ferment in the family tree: Does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? *Integrative and Comparative Biology, 44*, 304-314.

Mitchell, P.H., Bull, K. Glautier, S., Hopper, N.A., Holden-Dye, L., and O'Connor, V. (2007). The concentration-dependent effects of ethanol on *Caenorhabditis elegans* behavior. *The Pharmacogenomics Journal, 7*, 411-417.

Moore, M.S., DeZazzo, J., Yuk, A.Y., Tully, T., Singh, C.M., and Heberlein, U. (1998). Ethanol intoxication in *Drosophila*: Genetic and pharmacological evidence for regulation by the

cAMP signaling pathway. *Cell, 93,* 997-1007.

Mustard, J.A., Edgar, E.A., Mazade, R.E., Wu, C., Lillvis, J.L., and Wright, G.A. (2008). Acute ethanol ingestion impairs the appetitive olfactory learning and odor discrimination in the honey bee. *Neurobiology of Learning and Memory, 90,* 633-643.

Ocaranza, P., Quintanilla, M.E., Tampier, L., Karahanian, E., Sapag, A., and Israel, Y. (2008). Gene therapy reduces alcohol intake in an animal model of alcohol dependence. *Alcoholism: Clinical and Experimental Research, 32,* 52-57.

Singh, C. and Heberlein U. (2000). Genetic control of acute ethanol-induced behaviors in Drosophila. *Alcoholism: Clinical and Experimental Research, 24,* 1127-1136.

Sullivan, E.V., Sable, H.J.K, Strother, W.N., Friedman, D.P., Davenport, A., Tillman-Smith, H., … Grant, K.A. (2005). Neuroimaging of rodent and primate models of alcoholism: Initial reports from the integrative neuroscience initiative on alcoholism. *Alcoholism: Clinical and Experimental Research, 29,* 287-294.

Tabakoff, B., and Hoffman, P.L. (2000). Animal models in alcohol research. *Alcohol Research & Health, 24,*(3), 77-84.

Wolf, F.W., and Heberlein, U. (2003). Invertebrate models of drug abuse. *Drug Abuse in Invertebrates,* 161-178.