Elucidating the Neurobiological Basis for the Locomotor Effects of Ethanol

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Abstract

The effects of alcohol have been studied immensely in humans. It is still unknown what specifically causes a person to develop alcoholism. By looking at the locomotor effects of alcohol on two *Drosophila* mutants one possible cause for alcohol resistance could be established. Previous studies have revealed that flies deficient in the D_1 dopamine receptor DAMB (D_5-like) and the neighboring gene oxoglutarate carrier (OGC) display attenuated locomotor activities compared to the control parental line ry upon exposure to various concentrations of ethanol. The goal of my study is to identify which gene, *damb* or *ogc*, is responsible for the reduced response to ethanol. The results of this study show that the *damb* mutant was less responsive to alcohol than the *ogc* mutant and the DAMB receptor may play a role in mediating the locomotor activating effect of alcohol.

Introduction

Alcoholism is defined as a disease or illness which is characterized by the inability to limit or control alcohol use. More specifically, alcoholics are unable to limit the duration of the drinking episode, the quantity of alcohol consumed, and the behavioral consequences of drinking (www.ncadd.org). The statistics on alcoholism are staggering. The disease has a prevalence rate of approximately 8 to 14%, and affects twice as many men as women. Measuring the true prevalence of alcoholism is difficult, in part because sufferers do a very good job of hiding their illness. It is known that alcoholism is the fourth leading cause of death for people between the ages of 35 and 55, and 100,000 people die from this disease every year (Enoch and Goldman, 2002). The exact etiology of alcoholism is unknown. It is likely that both environmental and genetic factors are involved in the development of the disease. There are currently several different genetic studies being conducted to determine the specific genes involved in alcoholism (Dick and Foroud, 2003).

Drosophila melanogaster is a very popular animal model in studying developmental processes and nervous system function (Heberlein, 2000). The fly makes an excellent model organism for several reasons. It has a relatively small genome; 14,000 genes compared to a human's with about 70,000 genes. *Drosophila* has a short

life cycle of about two weeks. This makes it extremely easy and inexpensive to generate a large number of offspring for experiments in a short amount of time (http://www.ceolas.org/fly/intro.html). Most important to this study, intoxicated flies display many of the same behaviors as intoxicated humans. After exposure to alcohol the flies display hyperlocomotor activity, become disoriented, and then become sedated (Heberlein, 2000). This is how we can gain insights into human drug addiction by investigating fruit flies.

This study was focused on determining the neuromodulator receptors involved in the locomotor activating effect induced by a relatively mild ethanol exposure. Two mutant *Drosophila* genotypes were employed in this study. The first mutant, *damb,* lacks the genes for the DAMB receptor and the genes for the OGC receptor. DAMB has been identified as a dopamine D1 receptor which is highly concentrated in the mushroom bodies (Han et al., 1996). The mushroom bodies in the fly brain are a center for learning and memory (Han et al., 1996). The other mutant used in this study lacks only the genes for OGC. The exact function of OGC is yet to be determined, but it is found in mitochondrion and may be involved in energy metabolism (Fly Base, 2003).

Dopamine is one of the main neurotransmitters involved in the rewarding effects of ethanol. Dopamine is released when we engage in a dopaminergic neurotransmission activity that is found pleasurable. Alcohol increases dopamine release, therefore likely increasing the feeling of pleasure (Julien, 2001). Many studies have been conducted on the exact role and mechanism that dopamine plays in alcohol-induced responses. Bainton et al. tested flies with reduced dopamine levels and found that these flies had a decreased response to ethanol-induced hyperlocomotion (Bainton et al., 2000). This suggests that dopamine plays a role in the locomotor activating affect of ethanol. Cohen et al. conducted a study looking specifically at the dopamine D1 receptor (Cohen et al., 1999). The results of this study showed that the blockade of the dopamine D1 receptor causes decreases in ethanol self-administration in rats. The study also showed that the dopamine D1 receptor plays a significant role in ethanol induced locomotion (Cohen et al., 1999).

Research Questions

1) Does DAMB mediate the locomotor effects of ethanol?
2) Does OGC mediate the locomotor effects of ethanol?

Significance of the Study

If a person were more resistant to the effects of alcohol, due to malfunction or altered function of a certain receptor, they would drink more to achieve the desired effect of intoxication. If it is known which cellular mechanisms in the brain control the behavioral effects of alcohol, and also the rewarding effects, this may lead to a pharmaceutical treatment for alcoholism. Also, it will help identify people who are more at risk for developing an alcohol abuse problem (i.e. those with malfunctioning receptors).

Methods

The purpose of this study is to elucidate which molecules mediate the locomotor effect of ethanol in fruit flies. The flies were drawn from a random population of laboratory mutants. Only male flies were examined because eggs present in female flies might interfere with the results. This study examined 24 flies. The sample includes two different mutants: the *damb* mutant and the *ogc* mutant.

To elucidate the effects of ethanol on behavioral responses of flies, we employed a locomotor assay system. In this system, individual flies were placed in a glass tube with both ends plugged with cotton balls. The cotton ball in one side was soaked with various concentrations of ethanol (15% and 30%). The other cotton ball was soaked with distilled water. In order to account for side preference, the cotton ball soaked in ethanol was placed on different sides of different tubes. The number of times that flies displayed distinct locomotor behaviors during an hour was recorded using a video camera. The behaviors that were monitored include: single turn 1 (turning around in the middle of the tube), single turn 2 (turning around at the end of the tube), multiple turn 1 (walking in circles), and multiple turn 2 (turning side to side without walking). It is important to note that single turn 2 is normal fly walking behavior.

In this study, there are two different sets of independent variables. The first set includes the two different genetic mutants (*damb* and *ogc*). The next set of independent variables is two different concentrations of ethanol: 15% and 30%. The dependent variables in this study are: the number of single turn 1, single turn 2, multiple turn 1, and multiple turn 2.

The data recorded after all runs of the experiment was put into an Excel spreadsheet for analysis. T-tests were run to test for statistical significance.

Results

The ethanol induced in *ogc* mutants more single turning 1 than *damb* mutants with both 15% and 30% ethanol ($p<.05$) (shown in Figures 1 and 2). This indicates that the *damb* mutants are less responsive to ethanol than the *ogc* mutants. There was no difference in the number of single turn 2 (which is normal fly walking behavior) with water and ethanol exposures in both genotypes (both *damb* and *ogc*) ($p>.05$). This was observed in both trials using 15% ethanol and 30% ethanol (shown in Figures 3 and 4). This observation suggests that ethanol does not influence normal fly walking behavior. The ethanol-exposed *ogc* mutants displayed more multiple turning 1 than the ethanol-exposed *damb* mutants ($p<.05$) (shown in Figures 5 and 6). Ethanol at 15% and 30% induced more activity in *ogc* flies compared to *damb* mutants. The ethanol-exposed *ogc* mutants also displayed more multiple turning 2 than the *damb* mutants ($p<.05$) (shown in

Figures 7 and 8). As seen with single turn 1, the *damb* mutants are less responsive to ethanol than the *ogc* mutants when multiple turns were measured.

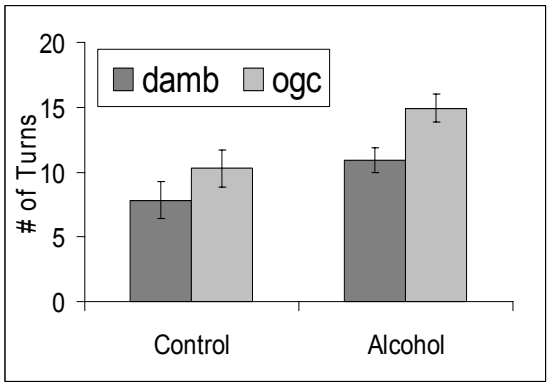

Figure 1: *ogc* and *damb* mutants' number of single turn 1 under 15% ethanol. Each bar represents a mean of 24 and standard error of the mean.

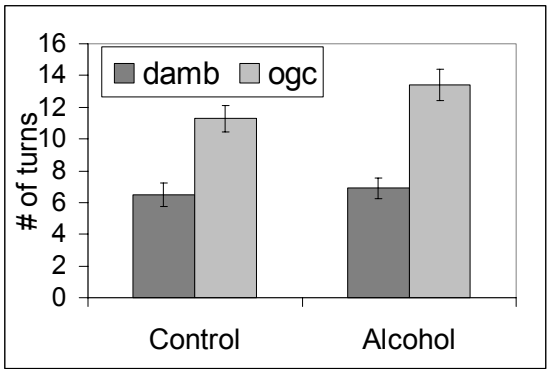

Figure 2: *ogc* and *damb* mutants' number of single turn 1 under 30% ethanol. Each bar represents a mean of 24 and standard error of the mean.

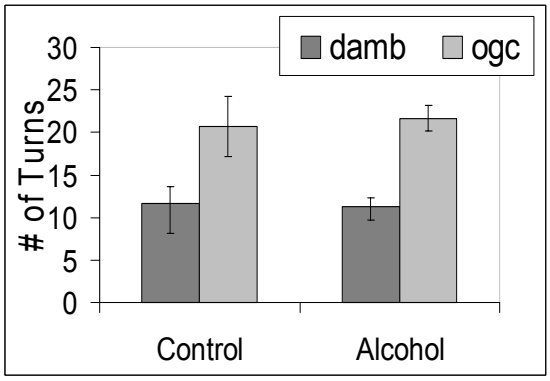

Figure 3: *ogc* and *damb* mutants' number of single turn 2 under 15% ethanol.

Each bar represents a mean of 24 and standard error of the mean.

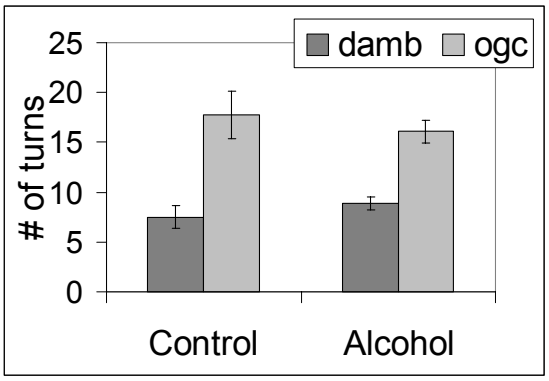

Figure 4: *ogc* and *damb* mutants number of single turn 2 under 30% ethanol
Each bar represents a mean of 24 and standard error of the mean.

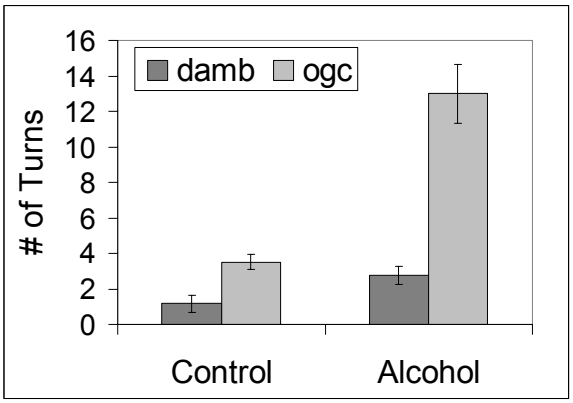

Figure 5: *ogc* and *damb* mutants number of multiple turn 1 under 15% ethanol
Each bar represents a mean of 24 and standard error of the mean.

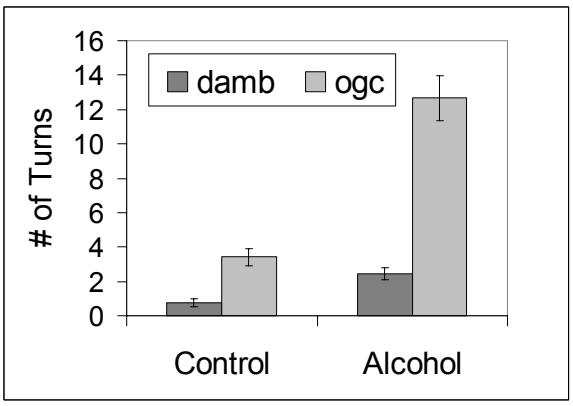

Figure 6: *ogc* and *damb* mutants number of multiple turn 1 under 30% ethanol
Each bar represents a mean of 24 and standard error of the mean

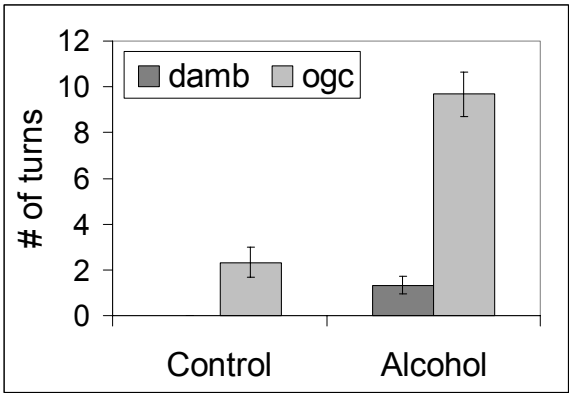

Figure 7: *ogc* and *damb* mutants number of multiple turn 2 under 15% ethanol Each bar represents a mean of 24 and standard error of the mean.

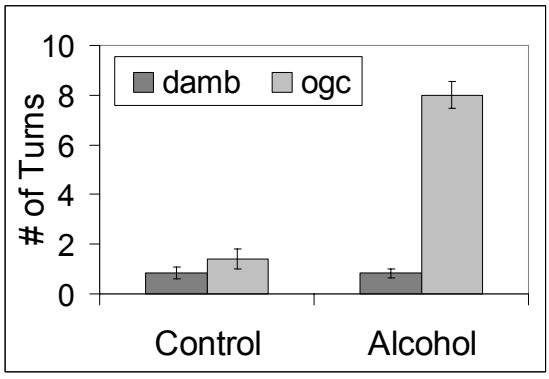

Figure 8: *ogc* and *damb* mutants number of multiple turn 2 under 30% ethanol Each bar represents a mean of 24 and standard error of the mean.

Conclusions

A number of conclusions can be made from these results. Since the *damb* mutants were less responsive to ethanol than the *ogc* mutants, it can be concluded that DAMB plays a role in mediating the locomotor activating effect of alcohol (single turn 1 and multiple turn 1) and also disorientation (multiple turn 2). In broad terms this implies that people, who have fewer numbers of the DAMB receptor, or malfunctioning or less active DAMB receptors, may be more resistant to the effects of alcohol, which may cause them to drink more.

For future work, these experiments need to be repeated with the same variables to test reliability and validity, and with different concentrations of ethanol in order to establish a dose-response relationship.

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