THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

THE EFFECTS OF DEVELOPMENTAL ALCOHOL EXPOSURE ON ADULT BEHAVIORS IN *DROSOPHILA MELANOGASTER*

SANDEEP SANDIRASEGARANE

Fall 2009

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Biology

Reviewed and approved* by the following:

Kyung-An Han
Associate Professor of Biology
Thesis Supervisor

Richard J. Cyr
Professor of Biology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Fetal alcohol syndrome (FAS) is caused by maternal alcohol consumption during pregnancy. Children affected with FAS exhibit growth deficiencies, distorted facial features, and central nervous system (CNS) dysfunction. Due to the relatively low tolerance threshold of the neuronal precursors for alcohol, the CNS experiences greater damage than any other part of the developing embryo. The neurological deficits associated with the syndrome lead to the impairment of cognitive and executive functions, learning and memory, and motor functions. In this study, I explored the effects of developmental ethanol exposure on adult behaviors in *Drosophila melanogaster*. The behaviors studied in my research include ethanol sensitivity, eclosion time, locomotor activity, and cocaine sensitivity. Ethanol administration on *Drosophila* pupae, the critical developmental period for brain development, did not affect eclosion clock or cocaine sensitivity, but had the significant effects on sensitivity to the sedative effect of ethanol in adults, and on mechanical stimulus-induced locomotor behavior in a sex-specific manner. While pupal ethanol exposure did not affect the locomotor activity of adult male flies, the fine motor control of adult female flies was compromised. The mechanistic studies of ethanol's effects on CNS development in *Drosophila* should enhance our understanding of the physiological and cellular mechanisms underlying FAS.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

1.1. Fetal alcohol syndrome and central nervous system dysfunction

Fetal alcohol syndrome (FAS) is the most common preventable birth defect in the United States since it is caused by maternal alcohol consumption during pregnancy [1]. Children affected with FAS exhibit growth deficiencies, distorted facial features, and central nervous system (CNS) dysfunction [2-3]. Due to the relatively low tolerance threshold of the neuronal precursors for ethanol (the active ingredient of alcohol beverages), the CNS experiences greater damage than any other part of the developing embryo [4]. Consistently, structural brain imaging has revealed the reduced sizes of the basal ganglia, the corpus callosum, the hippocampus, and the cerebellum in children with FAS compared to control subjects [5-6]. The structural deficits associated with the syndrome lead to the impairments in cognitive functioning, executive functioning, learning and memory, and motor function [2, 7].

1.2. Drosophila as a model to study the effects of developmental ethanol exposure

While studies of human subjects have the advantage of investigating physiologically more relevant processes, experiments are more difficult to control than those involving animal models [8]. Rats are the most extensively used organisms in FAS research and have been helpful to identify the changes in the specific nervous system functions [9]. The fruit fly *Drosophila melanogaster*, however, has not been used to investigate the effects of developmental alcohol

exposure. *Drosophila* is an excellent model to elucidate the genetic and molecular basis of FAS since its developmental period (about 12 days) is shorter than that in higher order species. Long developmental time is one of the major difficulties of using mammalian model systems [8]. *Drosophila* has a relatively simple nervous system with approximately 200,000 neurons [10], but many of its functions are similar to those in humans. For example, ethanol associated behaviors and related molecules are conserved between flies and humans [11]. In addition, the key molecules for various cellular and developmental processes are shared between *Drosophila* and humans [10]. Fruit flies are also inexpensive and easy to rear in a lab.

1.3. Behavioral changes associated with ethanol and cocaine exposure

The *Drosophila* genome can easily be manipulated to generate mutant flies for various experimentations including drug addiction [12]. Flies exposed to cocaine at varying doses show an array of abnormal behaviors [11, 13]. For instance, flies display excessive grooming at low doses, walk in circles (a type of motor stereotypy) at intermediate doses, and lose motor control at high doses. In addition, cocaine-exposed flies exhibit impaired positive phototaxis and negative geotaxis [14]. Of importance, *Drosophila* has also been used for alcohol research since their behavioral responses to ethanol are similar to those in humans [15]. Flies exhibit increased hyperactivity at low ethanol doses and sedation at high doses [16]. They also develop tolerance to the sedative effect of ethanol upon repeated exposures [17]. Furthermore, male flies treated with chronic ethanol display loss of courtship inhibition toward other males, which represents a type of behavioral disinhibition cognitive impulsivity [18]. Ethanol-induced impulsivity is typically observed in inebriated humans. Circadian rhythm has been used to study the

detrimental effects of ethanol on CNS dysfunction in rats and humans [19]. However, the effect of ethanol exposure on circadian rhythm is unknown in *Drosophila*. A measurable characteristic of fruit fly circadian rhythm is eclosion time [20]. Maximum eclosion from pupae to adult flies occurs during the dark-light transition when examined in a 12 hour light/12 hour dark illumination condition. Eclosion time is easy to monitor and thus used in my study to identify the effect of ethanol exposure on circadian rhythm.

1.4. Summary of Thesis

In this study, I explored the effects of developmental ethanol exposure on adult behaviors in *Drosophila melanogaster*. The behaviors studied in my research include ethanol sensitivity, eclosion time, locomotor activity, and cocaine sensitivity. Ethanol administration on *Drosophila* pupae, the critical developmental period for brain development, did not affect eclosion clock or cocaine sensitivity, but had the significant effects on sensitivity to the sedative effect of ethanol in adults, and on mechanical stimulus-induced locomotor behavior in a sex-specific manner. While pupal ethanol exposure did not affect the locomotor activity of adult male flies, the fine motor control of adult female flies was compromised. The mechanistic studies of ethanol's effects on CNS development in *Drosophila* should enhance our understanding of the physiological and cellular mechanisms underlying FAS.

CHAPTER 2. MATERIALS AND METHODS

2.1. *Drosophila* strains and culture

All flies were reared on standard cornmeal medium in food bottles (Figure 1) at 25^{o}C with 50% relative humidity under the 12h light/12h dark illumination condition [18]. The adult flies were collected within two days after eclosion, and groups of 33 flies (either mixed gender or males and females alone) were used for behavioral assays [18]. The wild-type *Canton-S* *Drosophila* strain was used in all experiments.

Figure 1. Food bottle used to house and rear flies

2.2. Preparation of ethanol solutions

The 0% ethanol solution was composed of distilled water. 95 mL of 5% ethanol solution was prepared by mixing 90 mL of distilled water with 5 mL of 95% ethyl alcohol (Pharmco-

AAPER Product Inc., Brookfield, CT, USA). 95 mL of 10% ethanol solution was made by mixing 85 mL of distilled water with 10 mL of 95% ethanol solution.

2.3. Ethanol administration during the pupal stage of development

At 23^{o}C, a group of approximately 100 *Canton-S* flies was transferred into a plastic bottle containing cornmeal medium. The bottle was then plugged with a cotton ball and kept in an incubator maintained at 25^{o}C with 50% relative humidity under a 12h light/12h dark cycle. Approximately seven days later, a relatively dense population of wandering third-instar larvae was observed on the bottle's wall. Upon this observation, the adult flies were discarded, and a box cutter was used to remove the food-containing bottom from the bottle (Figures 2 and 3). The bottle was then inverted, and the newly created opening was covered with plastic wrap, which was secured with clear tape (Figure 4). The bottle was returned to the incubator and was kept (plastic wrap side up) for additional two days at which time third instar larvae became pupae.

Figure 2. Cutting off bottom of the bottle

Figure 3. Food-containing bottom separated from bottle

Figure 4. Plastic wrap covering opening

The cotton ball was then removed from the bottle opening and a Petri dish (35 mm diameter) containing a cotton pad applied with 1mL of 0%, 5% or 10% ethanol was prepared (Figures 5 and 6). The Petri dish was covered with a Kimwipe and the bottle was placed on the Petri dish (Figures 7 and 8). The whole unit was kept in an incubator and the ethanol pad was replaced daily until adult flies eclosed from pupal cases, which took two to three days. Eclosed flies were reared in the incubator without ethanol for four days before testing.

Figure 5. Petri dish with cotton pad used to administer ethanol

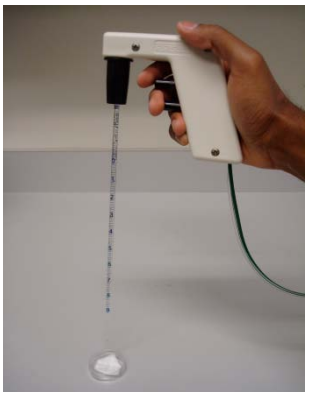


Figure 6. Ethanol added to cotton pad

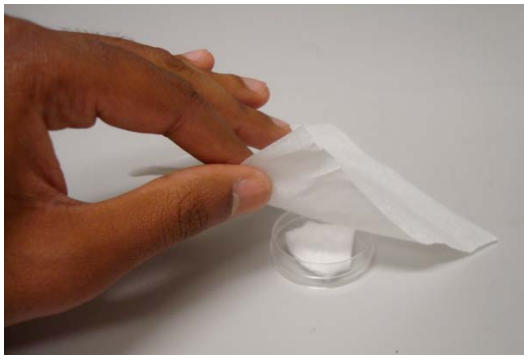


Figure 7. Kimwipe coving Petri dish

Figure 8. Bottle placed over Petri Dish with Kimwipe

2.4. Measurement of ethanol sensitivity

Ethanol exposures were carried out in Flypub at room temperature (23^{o}C). Flypub is a plastic chamber with a clear ceiling for viewing and an open bottom for ethanol administration [18]. Three 0%, three 5% and three 10% ethanol groups were tested together. Each group of 4 to 6 day old flies was transferred to Flypub. The flies were given 10 minutes to adjust to the environment before the unit was gently placed on a Petri dish containing a cotton pad applied with 95% ethanol and covered with a Kimwipe (Figure 9).

In order to determine the sedative effect of ethanol, the number of flies that fell to the bottom and remained immobile was recorded every two minutes until all flies were sedated. The percentage of total flies that were unconscious for each of the recorded times was calculated. In addition, the mean sedation time (MST) of the control and ethanol groups were calculated. To calculate MST, individual sedation times were multiplied with the number of flies sedated at the respective sedation times, which were then added for the total sedation time. The total sedation time was then divided by the total number of flies to generate MST of the group [18].

Figure 9. Fly Pub used to sedate flies

2.5. Measurement of eclosion clock

The control (0% ethanol) and ethanol treated pupae (six groups for each ethanol concentration) were reared at 25^{o}C with 50% relative humidity under the 12 hour light/12 hour dark illumination condition. Two days after the initial ethanol administration, the number of eclosed flies was recorded every two hours from 8am to 8pm (the light period of the light/dark cycle). After eclosed flies were counted, they were discarded. The percentage of total flies that eclosed in each time interval was then calculated.

2.6. Measurement of locomotor activity induced by mechanical stimulus

The experiments were carried out at room temperature. Within two days after eclosion, flies were collected while sedated under carbon dioxide to allow for gender separation (Figure 10). Groups consisting of 33 flies of the same gender were placed in food vials and reared in the incubator. Four days after eclosed flies were collected, male groups and female groups were transferred from food vials to empty glass vials. A black line had been drawn in advance around each glass vial 0.5 inch from the bottom (Figure 11). Two hours after the transfer, the flies were vortexed for 60 seconds using a VWR Mini Vortexer (model VM-3000; VWR, West Chester, PA, USA) set to speed 8 (Figure 12). After vortexing, the flies that fell down to the base of the vial attempted to climb up the glass wall. The number of flies that were unable to coordinate their movements and climb upward past the black line was recorded for each group at 10 sec, 30 sec, and 60 sec after vortexing. The percentages of the flies that were disoriented and unable to

climb upward were used to compare the control and ethanol-treated groups for mechanical stimulus-induced locomotor activity.

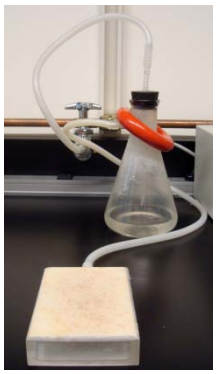


Figure 10. CO_2 pad used to sedate flies for counting

Figure 11. Glass vial in which flies were vortexed

Figure 12. Vortexer used to disorient flies

2.7. Measurement of cocaine sensitivity

The experiments were conducted at room temperature (25^{o}C). Within two days after eclosion, flies were collected while sedated under carbon dioxide to allow for gender separation. Female flies were discarded, and groups of 33 male flies were placed in food vials. Four days after the collection, flies were transferred from food vials to glass vials with a line drawn 0.5 inch from the base. Two hours after transfer, the flies were subjected to cocaine exposure. 0 µl, 5 µl, or 10 µl of 15 mg/ml of cocaine solution in ethanol was applied to the 28-gauge Nichrome wire on the stopper plug (Figures 13 and 14). After evaporating ethanol vapor for 10 minutes, the stopper was placed on the glass vial with the wire exposed to the flies inside (Figure 15). The stopper was then applied with 2.2 V electric power, under which condition cocaine was

volatized (Figure 16) [14]. Four minutes after volatization, the glass vial was transferred to a drop apparatus and dropped three consecutive times over a distance of 10.7 cm (Figure 17).

Figure 13. Stopper plug with Nichrome wire

Figure 14. Cocaine being added to plug

Figure 15. Glass vial with specialized plug

Figure 16. Power supply used to volatilize cocaine

A **B**

Figure 17. Drop Apparatus used to disorient flies (A) before drop and (B) after drop

The number of flies that were unable to coordinate their movements to climb upward and thus remained under the line was recorded for each group at 10 sec, 30 sec, and 60 sec after the final drop. The percentages of flies that remained under the black line were used to compare the control and ethanol-treated groups to measure the effect of pupal ethanol exposure on sensitivity to cocaine's effects on motor behavior.

2.8. Statistical analyses

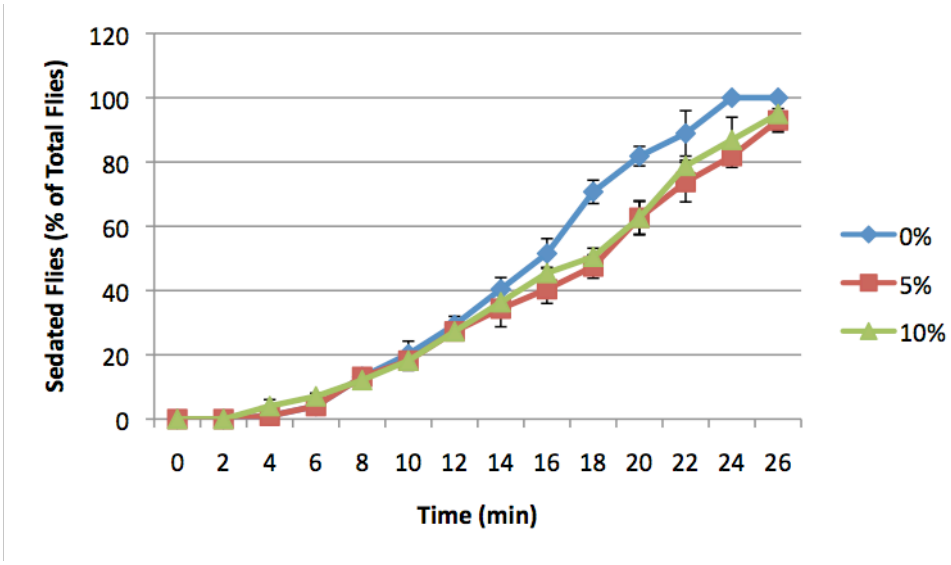
Statistical analyses were performed using Microsoft Excel 2007. With more than two groups, analysis of variance (ANOVA) was conducted. To compare the means of two groups, a two-tailed student t-test was performed.

CHAPTER 3. RESULTS

3.1. Effect of developmental ethanol exposure on ethanol sensitivity

The 4 to 6 day-old *Canton-S (CS)* flies that had been exposed to 0%, 5% or 10% ethanol during the pupal stage were exposed to ethanol vapor in Flypub containing 95% ethanol as described in materials and methods. As the flies were exposed to ethanol, they exhibited hyperactivity (fast movements), loss of motor control (falls), and then sedation (motionless rest). All three groups exhibited a positive correlation between percentage of total flies that were sedated and time. Interestingly, the flies with the developmental ethanol exposure showed slower sedation profiles than the control flies (Figure 18). MST of the control group (15.98 ± 0.29 min) was significantly lower than that of the 5% (18.14 ± 0.43 min) and 10% pupal ethanol group (17.56 ± 0.53 min) (p = 0.014, N = 3). There was no difference in MST between the 5% and 10% pupal-ethanol groups (p>0.05). This result suggests that ethanol exposure during the pupal stage made the flies more resistant to the sedative effect of ethanol in adults.

A



B

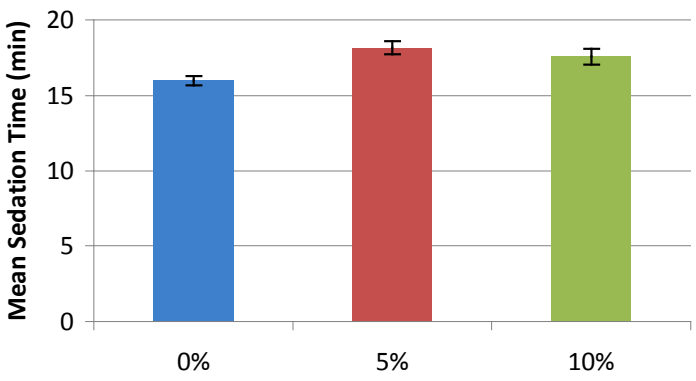


Figure 18. Effect of developmental ethanol exposure on ethanol sensitivity in adults. Flies were exposed to 95% ethanol vapor in Flypub and the numbers of sedated flies were counted every two minutes until all flies were sedated. (**A**) Sedation profiles of the control and the 5% and 10% pupal ethanol treated groups. (**B**) MST analysis. There was a significant difference in MST between the control group and the 5% ethanol group (one way ANOVA followed by *post hoc* two-tailed Student *t*-test, $p = 0.014$, $N = 3$ per each group). No significant differences in MST were observed between two ethanol groups ($p > 0.05$).

3.2. Effect of developmental ethanol exposure on eclosion clock

To investigate the effect of pupal ethanol exposure on eclosion, the adult flies eclosed from 0%, 5% or 10% ethanol-exposed pupae were counted every two hours. In all three groups, maximum eclosion occurred between 8 AM and 10 AM, a two-hour period right after the light

was on. The control group exhibited the largest eclosion percentage during this period followed closely by the 5% pupal-ethanol group. The 10% pupal-ethanol group showed the reduced percentage at this peak eclosion time, but it is not significantly different ($p > 0.05$) from those of the control and 5% pupal-ethanol groups (Figure 19). Thus, ethanol exposure during the pupal stage had no effect on eclosion clock.

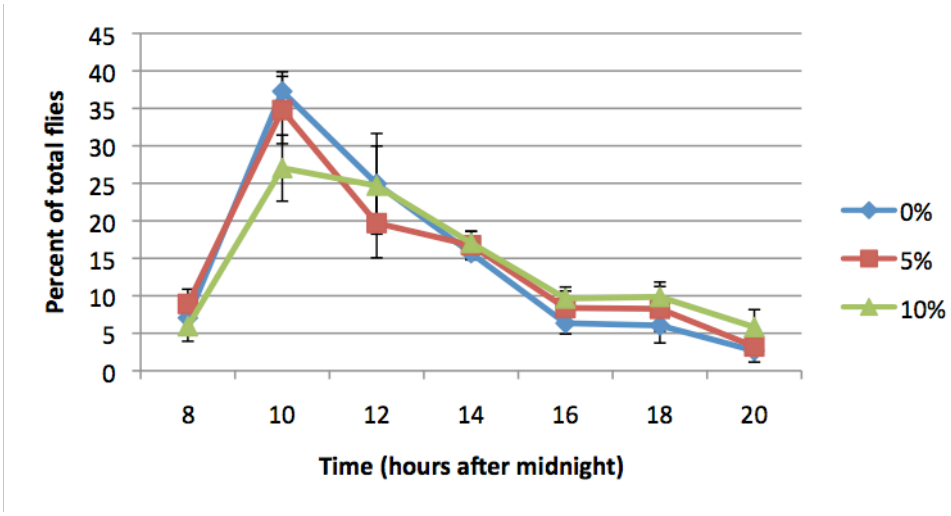
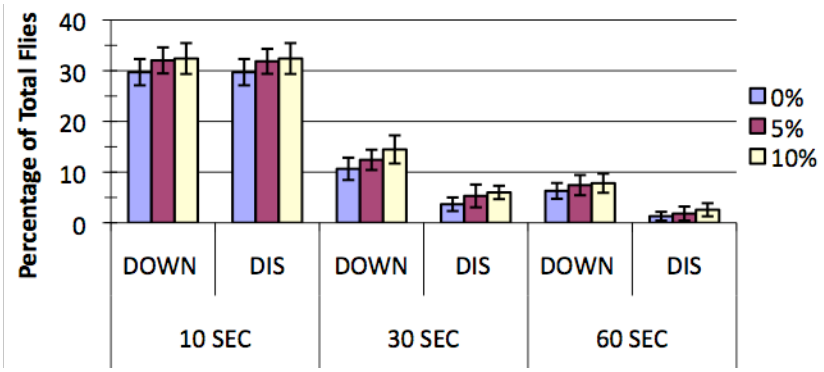


Figure 19. Effect of developmental ethanol exposure on eclosion time. The pupae under the influence of 0%, 5% or 10% ethanol were placed in a 12 hr light/dark cycle. The majority of flies in all groups eclosed between 8 AM and 10 AM. The 10% ethanol group displayed a smaller percentage of the flies eclosed during the peak eclosion time compared to the control and 5% pupal-ethanol groups but the difference is not significant (one way ANOVA followed by *post hoc* two-tailed Student *t*-test, $p > 0.05$, $N = 6$).

3.3. Effect of developmental ethanol exposure on locomotor activity

To explore whether developmental ethanol exposure affects locomotor behavior in adults, the mature (4 to 6 day-old) control and pupal-ethanol treated flies were tested for their capacity to recover from vortex-induced disturbance in motor behavior. Upon vortexing, the flies fell down to the base of the vial and, at the end of vortexing, the flies attempted to climb up the glass wall. The flies that were unable to coordinate their movements stayed at the bottom longer and showed disorientation (i.e. they walked unsteadily at the bottom of the vial and fell quickly during their climbing attempts). At ten seconds after vortexing, the percentages of male flies that were unable to climb up the walls were comparable among the 0%, 5%, and 10% pupal-ethanol groups (Figure 20A). On the other hand, the percentage of the female flies unable to scale the walls at ten seconds after vortexing was significantly greater for the 10% pupal-ethanol group than that of the 0% control group (two-tailed Student *t*-test, $p = 0.00125$) and that of the 5% ethanol group (two-tailed *t*-test, $p = 0.00528$). As shown in Figure 20B, no significant difference was observed between the 5% pupal-ethanol female group and the 0% control female group (two-tailed *t*-test, $p = 0.066$). For both males and females, no significant differences were observed among the groups either at 30 or 60 seconds after vortexing ($p > 0.05$). This observation indicates that ethanol exposure during the pupal development negatively affects the motor system in a sex-specific manner. Ethanol exposure seems to influence the development of the female, but not male, motor system in that the affected females have delayed recovery in motor behavior after mechanical disturbance.

A



B

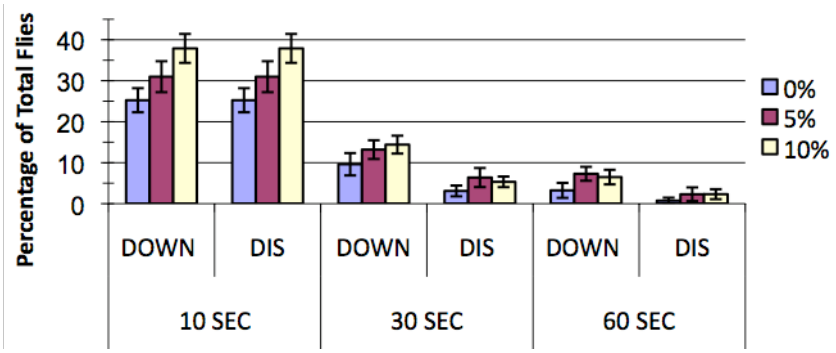


Figure 20. Effect of developmental ethanol on locomotor activity. The "DOWN" bars represent the percentages of flies that remained below the line drawn at 0.5 inch above the base of each vial at 10, 30, and 60 seconds after vortexing. The "DIS" bars represent the percentages of flies that displayed disorientation below the line. (**A**) Males vortexed for 60 seconds (N = 16). The males in the control and ethanol groups showed no difference in their anti-geotatic activities at 10, 30 and 60 seconds after vortexing. (**B**) Females vortexed for 60 seconds (N = 15). At 10 seconds after vortexing,

females in the 10% pupal-ethanol group showed significant difference in the anti-geotatic activity compared to the 0% and 5% pupal-ethanol groups (one way ANOVA followed by *post hoc* two-tailed Student *t*-test, $p = 0.00528$ with 0% group, $p = 0.00125$ with 5% group). No differences were observed at 30 and 60 seconds among all three groups ($p > 0.05$).

3.4. Effect of developmental ethanol exposure on cocaine sensitivity

The mature (4 to 6 day-old) control and pupal-ethanol exposed male flies were transferred to a glass vial with a line drawn at 0.5 inch above the base. A specialized stopper plug containing 0, 75, or 150 µg of cocaine on a nichrome wire was used to cap the glass vials. The plug was then connected to a power source in order to heat the nichrome and volatize cocaine, which appeared as a cloud of white smoke and filled the vial. The flies administered with 75 µg and 150 µg of cocaine, but not the control flies (0 µg cocaine), displayed fast and erratic movements. Some flies lied on their backs and spun rapidly in circles. The flies were then dropped three consecutive times over a distance of 10.7 cm. While displaying quick and uncontrolled movements, the flies that were forced to fall to the bottom of the vials climbed up the vial walls when dropping was stopped. No significant differences were observed in the percentages of flies that were unable to climb the walls among the control and pupal-ethanol exposed groups for all cocaine concentrations tested and at all time points measured after dropping. In both control and pupal-ethanol treated groups, the flies exposed to 0 µg cocaine exhibited a significantly lower percentage of flies at the bottom than those exposed to 75 and 150

µg of cocaine. Likewise, the flies exposed to 75 µg cocaine showed a significantly lower percentage of flies at the bottom than those that were given 150 µg cocaine (Figure 21). Therefore, cocaine had the negative effect on motor control in a dose dependent manner in all groups.

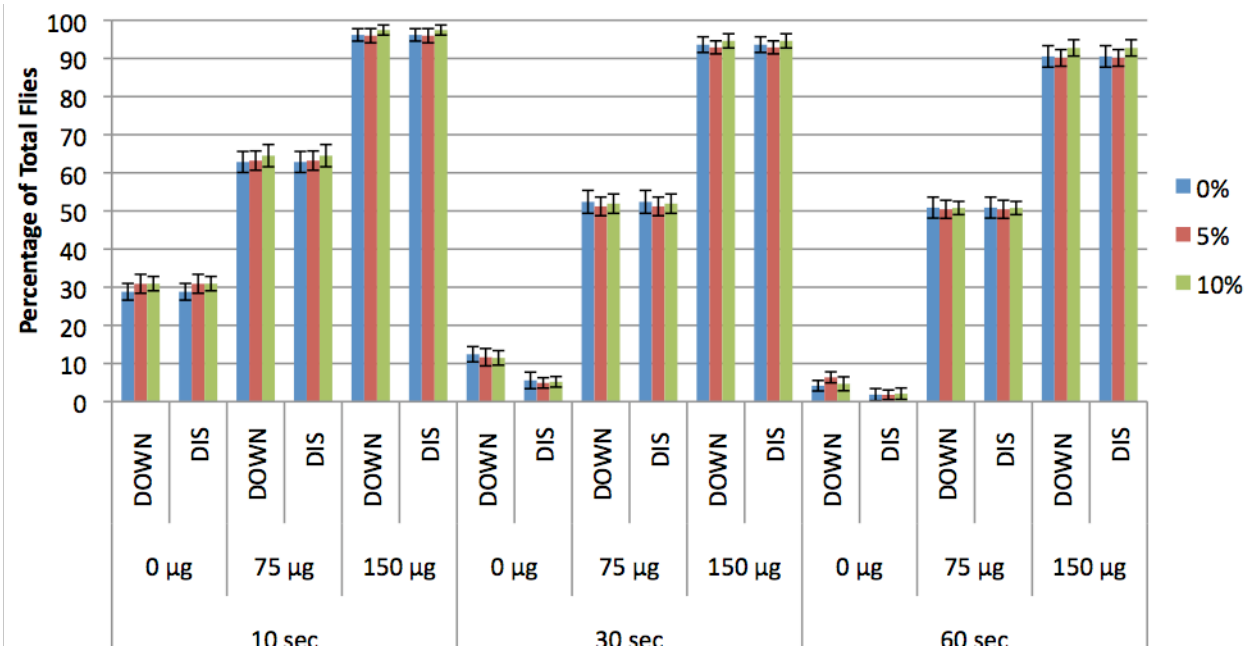


Figure 21. Effect of developmental ethanol on cocaine sensitivity. The "DOWN" bars represent the percentage of flies that remained below the line drawn at 0.5 inch above the base of each vial at 10, 30, and 60 seconds after dropping. The "DIS" bars represent the percentage of flies that displayed disorientation below the line. The male flies that were exposed to 0%, 5% and 10% of ethanol during the pupal stage were dropped three times over a distance of 10.7 cm and the ability of the flies to recover motor control and climb upward was tested. There was no significant difference in the

percentages of flies unable to climb the vial walls (DOWN) or disoriented flies at the bottom (DIS) among all three groups treated with no or two cocaine doses and tested at all time points after dropping (one way ANOVA, $p > 0.05$, $N = 12$ per group).

CHAPTER 4. DISCUSSION

4.1. Overview of experimental results

It is widely accepted that normal development of the fetus in humans is adversely affected by ethanol intake, and both physical deformities and abnormal brain activities are seen in children with prenatal ethanol exposure [21-22]. In the present study, I have shown that administration of ethanol to *Drosophila* pupae had no effects on eclosion time and sensitivity to cocaine's effects on motor behavior. However, the significant effect of pupal ethanol exposure was noted on sensitivity of the adult flies to the sedative effect of ethanol. There was also the significant effect of developmental ethanol exposure on the recovery from loss of motor control caused by strong mechanical disturbance in the female, but not male, flies that were exposed to 10% ethanol during the pupal stage. This research has focused on behavioral changes caused by developmental ethanol exposure. The findings described here could be used to further elucidate the underlying mechanisms, which would in turn enhance our understanding of the physiological and cellular mechanisms underlying FAS by guiding the direction of future research.

4.2. Ethanol sensitivity

Previous studies have shown that the male and female rats with early developmental ethanol exposure exhibit decreased sensitivity to the sedative effects of ethanol [23-24]. In one study, rats were administered with ethanol throughout gestation and during the lactation period, which corresponds to the third trimester of human gestational development [24]. The adult rats

with no or early developmental ethanol exposure were injected with ethanol and placed on their back. The time needed for the rat to reorient itself and stand on all four paws was used to determine ethanol sensitivity. The rats with developmental ethanol exposure showed faster reorientation time than the control rats, indicating reduced sensitivity to alcohol. In another study that involves longitudinal follow-up of human subjects, the ethanol sensitivities of 227 men with alcoholic fathers (i.e. family history of alcoholism) and 227 men with nonalcoholic fathers were measured at the age of 20 [25]. Approximately 10 years later, 124 of the men with family history of alcoholism and 98 of the control subjects were tested for ethanol abuse or dependence based on the DSM-III-R criteria. Regardless of family history, the 20% of the men in both groups that exhibited the smallest response to ethanol at the age of 20 have shown four times more likely to become alcoholics than the 20% of the men with the greatest response to alcohol. These observations demonstrate that reduced sensitivity to ethanol is associated with enhanced propensity to alcoholism in humans.

In the present study, the control flies exhibited ethanol-induced sedation more rapidly than those developmentally exposed to ethanol. This indicates that the flies with developmental ethanol exposure have reduced sensitivity to ethanol. This change in ethanol sensitivity could be due to changes either in the physiological system for ethanol metabolism or in the brain functions for motor control. Future studies of the molecules crucial for ethanol sensitivity will help clarify this issue.

4.3. Eclosion clock

Previous studies have shown that rats with developmental ethanol exposure exhibit alterations in circadian rhythms with shortened sleep-wake cycles [26]. In addition, ethanol exposure during the neonatal stage, which corresponds to third trimester brain development in humans, compromises circadian rhythm in rat suprachiasmatic nucleus, thereby affecting physiological processes and behavior [27]. Eclosion clock was one aspect of circadian rhythm investigated in the present study. Although the control flies and the flies with developmental ethanol exposure had the same peak eclosion time, the flies exposed to 10% ethanol as pupae showed a visible yet statistically insignificant decrease in the percentage of flies eclosed at the peak time. The flies exposed to 5% ethanol as pupae showed a small decrease in the percentage of flies eclosed at the peak time as well. This inverse relationship between ethanol doses administered to pupae and the eclosion percentages at the peak time suggests a potential effect of developmental ethanol exposure on delaying eclosion possibly due to subnormal development. Future studies investigating eclosion clock at shorter measurement intervals and developmental or cellular markers crucial for eclosion should clarify this notion.

4.4. Locomotor activity

In the present study, the significant effect of developmental ethanol exposure on the locomotor activity of adult female *Drosophila* was observed. Compared to the control flies and the flies exposed to 5% pupal-ethanol, the female, but not male, flies treated with 10% ethanol as pupae displayed a significantly reduced ability to regain motor control 10 seconds after severe

mechanical disturbances (vortexing). This finding is consistent with the rat study, in which female rats, but not male rats, exposed developmentally to ethanol exhibit increased, hyperactive locomotor responses to ethanol as adults [24].

Previous studies on both rats and human subjects have shown reduced balance and motor function associated with developmental ethanol exposure [22, 28-30]. In one study, human subjects whose mothers abused alcohol exhibited increased cerebral palsy, ataxia, and kinetic tremors [22]. In another study, 11 native American children with FAS, 11 who had been exposed prenatally to ethanol but without FAS, and another 11 who were not prenatally exposed to ethanol were compared on general, gross and fine motor controls [29]. The results show that the general motor skills of the children with FAS are significantly lower than those of children that were not prenatally exposed to alcohol, while the children without FAS that were prenatally exposed to alcohol exhibit intermediate scores. The fine motor skills of the children affected with FAS are significantly lower than that of both the exposed and non-exposed groups. On the other hand, no significant difference has been noted in the gross motor skill scores of all groups. These studies, however, did not distinguish gender differences.

These observations together suggest that prenatal ethanol exposure induces behavioral deficits associated with motor functions crucial for fine and general motor skills. Reduced motor function in the human subjects with prenatal ethanol exposure is similar to the diminished ability of the female flies with developmental ethanol exposure to control motor behavior in a timely manner. This phenotype is likely due to ethanol's negative effect of on motor system

development. Future studies identifying the underlying mechanisms in *Drosophila* may help enhance our understanding of detrimental effects of developmental ethanol exposure in humans.

4.5. Cocaine sensitivity

Previous studies have shown that FAS increases the risk of future drug usage in humans [5]. One study reports that early alcohol exposure increases the sensitivity of adult rats to the locomotor effects of cocaine [24]. While control rats show a decreased response to cocaine upon repeated exposures, the rats developmentally exposed to ethanol show prolonged hyperactive activity to repetitive cocaine treatment. In the study reported here, no significant effect of pupal alcohol exposure was observed on cocaine sensitivity in adult male flies. Nonetheless, the assay used in my study did not examine the effects of repeated cocaine exposure. It is possible that developmental ethanol exposure may only affect the behaviors induced by repeated cocaine exposure such as behavioral sensitization or cocaine sensitivity only in females, which have not been investigated in this study.

4.6. Future Studies

The study described here has offered significant insights into behavioral deficits associated with developmental ethanol exposure in *Drosophila.* Future studies investigating various mutants and experimental modifications should help elucidate the underlying cellular and physiological mechanisms. The observation described in the ethanol sensitivity section shows a significant decrease in ethanol sensitivity in the flies exposed to ethanol as pupae. Since

this study was conducted on a small sample size (N = 3), additional trials should be conducted in future studies to offer additional support to the finding. The effect of pupal ethanol exposure on eclosion clock should also be investigated further, because a greater sample size may uncover significant differences between control and experimental groups. Furthermore, the locomotor recovery assays described above identified deficits in motor control and loss of coordination associated with developmental ethanol exposure. Future studies using various locomotor assays should help elucidate additional effects of developmental ethanol exposure. For example, fly locomotor activity may be measured in a chamber in which flies walking speed with or without ethanol exposure could be examined to measure the locomotor activating effect of ethanol [31]. Lastly, the cocaine sensitivity study investigated only male flies. The similar study should be repeated using female flies since the locomotor activity assay revealed an effect of developmental ethanol exposure in females but not males.

4.7. Conclusions

In summary, the findings described here have shown that developmental ethanol exposure led to reduced sensitivity of the adult flies to the sedative effect of ethanol. Reduced ethanol sensitivity is linked to increased propensity to alcoholism in humans. Thus this finding should help understand whether and how developmental ethanol intake affects alcoholism. Furthermore, pupal ethanol exposure had the significant effect on motor control and coordination in the affected adult females. These behavioral effects of developmental ethanol exposure in *Drosophila* will ultimately help identify the underlying cellular and physiological mechanisms of FAS.

CHAPTER 5. REFERENCES

1. Niccols A: Fetal alcohol syndrome and the developing socio-emotional brain. *Brain Cogn* 2007, 65(1):135-142.
2. Paley B, O'Connor MJ: Intervention for individuals with fetal alcohol spectrum disorders: treatment approaches and case management. *Dev Disabil Res Rev* 2009, 15(3):258-267.
3. Kelly SJ, Day N, Streissguth AP: Effects of prenatal alcohol exposure on social behavior in humans and other species. *Neurotoxicol Teratol* 2000, 22(2):143-149.
4. Welch-Carre E: The neurodevelopmental consequences of prenatal alcohol exposure. *Adv Neonatal Care* 2005, 5(4):217-229.
5. Mattson SN, Schoenfeld AM, Riley EP: Teratogenic effects of alcohol on brain and behavior. *Alcohol Res Health* 2001, 25(3):185-191.
6. Archibald SL, Fennema-Notestine C, Gamst A, Riley EP, Mattson SN, Jernigan TL: Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev Med Child Neurol* 2001, 43(3):148-154.
7. Nash K, Sheard E, Rovet J, Koren G: Understanding fetal alcohol spectrum disorders (FASDs): toward identification of a behavioral phenotype. *ScientificWorldJournal* 2008, 8:873-882.
8. Cudd TA: Animal model systems for the study of alcohol teratology. *Exp Biol Med (Maywood)* 2005, 230(6):389-393.
9. Weinberg J, Sliwowska JH, Lan N, Hellemans KG: Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *J Neuroendocrinol* 2008, 20(4):470-488.

10. Reichert H, Boyan G: Building a brain: developmental insights in insects. *Trends Neurosci* 1997, 20(6):258-264.

11. Heberlein U, Tsai LT, Kapfhamer D, Lasek AW: Drosophila, a genetic model system to study cocaine-related behaviors: a review with focus on LIM-only proteins. *Neuropharmacology* 2009, 56 Suppl 1:97-106.

12. Wolf FW, Heberlein U: Invertebrate models of drug abuse. *J Neurobiol* 2003, 54(1):161-178.

13. George R, Lease K, Burnette J, Hirsh J: A "bottom-counting" video system for measuring cocaine-induced behaviors in Drosophila. *Methods Enzymol* 2005, 393:841-851.

14. McClung C, Hirsh J: Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in Drosophila. *Curr Biol* 1998, 8(2):109-112.

15. Lovinger DM, Crabbe JC: Laboratory models of alcoholism: treatment target identification and insight into mechanisms. *Nat Neurosci* 2005, 8(11):1471-1480.

16. Scholz H, Ramond J, Singh CM, Heberlein U: Functional ethanol tolerance in Drosophila. *Neuron* 2000, 28(1):261-271.

17. Berger KH, Heberlein U, Moore MS: Rapid and chronic: two distinct forms of ethanol tolerance in Drosophila. *Alcohol Clin Exp Res* 2004, 28(10):1469-1480.

18. Lee HG, Kim YC, Dunning JS, Han KA: Recurring ethanol exposure induces disinhibited courtship in Drosophila. *PLoS One* 2008, 3(1):e1391.

19. Spanagel R, Rosenwasser AM, Schumann G, Sarkar DK: Alcohol consumption and the body's biological clock. *Alcohol Clin Exp Res* 2005, 29(8):1550-1557.

20. Hall JC: Tripping along the trail to the molecular mechanisms of biological clocks. *Trends Neurosci* 1995, 18(5):230-240.

21. Calhoun F, Warren K: Fetal alcohol syndrome: historical perspectives. *Neurosci Biobehav Rev* 2007, 31(2):168-171.

22. Mattson SN, Riley EP: A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. *Alcohol Clin Exp Res* 1998, 22(2):279-294.

23. Becker HC, Hale RL, Boggan WO, Randall CL: Effects of prenatal ethanol exposure on later sensitivity to the low-dose stimulant actions of ethanol in mouse offspring: possible role of catecholamines. *Alcohol Clin Exp Res* 1993, 17(6):1325-1336.

24. Barbier E, Houchi H, Warnault V, Pierrefiche O, Daoust M, Naassila M: Effects of prenatal and postnatal maternal ethanol on offspring response to alcohol and psychostimulants in long evans rats. *Neuroscience* 2009, 161(2):427-440.

25. Schuckit MA: Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry* 1994, 151(2):184-189.

26. Earnest DJ, Chen WJ, West JR: Developmental alcohol and circadian clock function. *Alcohol Res Health* 2001, 25(2):136-140.

27. Allen GC, West JR, Chen WJ, Earnest DJ: Developmental alcohol exposure disrupts circadian regulation of BDNF in the rat suprachiasmatic nucleus. *Neurotoxicol Teratol* 2004, 26(3):353-358.

28. Gallo PV, Weinberg J: Neuromotor development and response inhibition following prenatal ethanol exposure. *Neurobehav Toxicol Teratol* 1982, 4(5):505-513.

29. Kalberg WO, Provost B, Tollison SJ, Tabachnick BG, Robinson LK, Eugene Hoyme H, Trujillo PM, Buckley D, Aragon AS, May PA: Comparison of motor delays in young

children with fetal alcohol syndrome to those with prenatal alcohol exposure and with no prenatal alcohol exposure. *Alcohol Clin Exp Res* 2006, 30(12):2037-2045.

30. Roebuck TM, Simmons RW, Richardson C, Mattson SN, Riley EP: Neuromuscular responses to disturbance of balance in children with prenatal exposure to alcohol. *Alcohol Clin Exp Res* 1998, 22(9):1992-1997.

31. Rothenfluh A, Heberlein U: Drugs, flies, and videotape: the effects of ethanol and cocaine on Drosophila locomotion. *Curr Opin Neurobiol* 2002, 12(6):639-645.

SCHREYER HONORS COLLEGE

ACADEMIC VITA OF SANDEEP SANDIRASEGARANE

Name: Sandeep Sandirasegarane

Address: 305 E. Prospect Ave.
State College, PA 16801

Alternate Address: 59 Carousel Circle
Hershey, PA 17033

E-Mail Id: sos5027@psu.edu

Education

Major: Biology

Minor: Classical Ancient Mediterranean Studies

Honors: Biology

Thesis Title: "The Effects of Developmental Alcohol Exposure on Adult Behaviors in Drosophila melanogaster."

Thesis Supervisor: Dr. Kyung-An Han, Associate Professor of Biology

Work Experience

Date: Sept. 2009 – Present

Title: Private instructor

Description: I tutor Penn State undergraduate students

Institution/company: PSUKnowHow (State College, PA)

Supervisor's name: Jeremy Robinson

Date: Aug. 2009 – Present

Title: Biology 110 (Concepts and Biodiversity) Teaching Assistant

Description: I teach a 24-person lab section of the course

Institution/company: Penn State University (State College, PA)

Supervisor's name: Dianne Burpee

Date: June 2009 – Aug. 2009

Title: Research Assistant

Description: I assisted a graduate investigator with his research projects

Institution/company: Penn State University (State College, PA)

Supervisor's name: Dr. Kyung-An Han

Date: Jan. 2009 – May 2009

Title: Biology 437 (Histology) Teaching Assistant

Description: I taught a 12-person laboratory section of the course

Institution/company: Penn State University (State College, PA)

Supervisor's name: Dr. James Strauss

Date: Aug. 2009 – Dec. 2008

Title: Biology 110 (Concepts and Biodiversity) Teaching Assistant

Description: I taught a 24-person lab section of the course

Institution/company: Penn State University (State College, PA)

Supervisor's name: Dianne Burpee

Grants Received:

N/A

Awards:

2008 Theta Delta Chi Education Foundation Scholarship

2009 Penn State Edward C. Hammond, Jr. Memorial Scholarship in Biology

Professional Memberships:

Alpha Epsilon Delta, Premedical Honor Society *Fall 2007-Present*

Publications:

N/A

Presentations:

Undergraduate Exhibition at Pennsylvania State University *April 9, 2008*

- Presented poster displaying undergraduate thesis research achievements

Community Service Involvement:

Penn State Interfraternity Council – Philanthropy Chairman *Spring 2009- Fall 2009*

- Assisted IFC fraternities with the organization of their philanthropies
- Marketed IFC philanthropy events

Penn State Dance Marathon – Dancer *Feb 20, 2009- Feb 22, 2009*

- Danced for 46 straight hours to support pediatric cancer patients and their families

Theta Delta Chi Fraternity – 2009 Dance Marathon Chairman *Fall 2008- Spring 2009*

- Organized fundraising initiatives to raise over $28,000 as a fraternity

Mount Nittany Medical Center Cardiac Catheterization Lab *Jan 2008- Apr 2008*

- Shadowed cardiologists and viewed interventional procedures

Mount Nittany Medical Center Surgical Unit *Aug 2007- Dec 2007*

- Assisted nurses and shadowed general surgeons

Mount Nittany Medical Center Patient Floors *Jan 2007- Apr 2007*

- Delivered newspapers, flowers, and snacks to patients

Theta Delta Chi Fraternity – Philanthropy/Service Chairman *Fall 2006-Spring 2009*

• Organized philanthropies and motivated fraternity members to perform community service

Penn State Dance Marathon – Morale Committee Member *Fall 2007- Spring 2008*

• Motivated dancers to stay awake and on their feet for 46 hours

International Education:

Tropical Field Ecology (BIOL 499A) in Costa Rica *Dec 2007- Jan 2008*

Language Proficiency:

English