SELF-ADMINISTRATION OF ALCOHOL IN HONEY BEES

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ALCOHOL IN HONEY BEES

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PREFACE

Cicero (1979) asserted that any animal model of alcoholism should include 1) animal must voluntarily self-administer the alcohol, 2) tolerance to alcohol should be demonstrated following a period of continuous consumption, 3) dependence on alcohol as demonstrated by withdrawal symptoms, and 4) the biomedical complications associated with chronic alcohol consumption seen in humans also develop in animals.

Many animal models of alcoholism have been developed using, for instance, primates (Mello, 1976), mice (Rijk, Crabbe, & Rigter, 1982), and goldfish (Marcucella & Abramson, 1978). Several techniques of alcohol-induction have been tried to increase alcohol consumption including intracerebral injections (Cicero & Myers, 1969), sweetening of the alcohol solution (Gilbert, 1974; Siegel & Brodie, 1984), sucrose-fading (Tolliver, Sadeghi & Samson, 1988), food deprivation (Macenski & Meisch, 1992; Pakarinen, Williams & Woods, 1999), direct stomach tube implantations (Deutsch & Eisner, 1977), and inhalation (Rijk, Crabbe & Rigter, 1982). Honey bees are an attractive model for an alcohol model.

First, honey bees are inexpensive to procure and maintain as compared to other animals. Second, much is known about their history, physiology, genetics, and behavior. Third, automated and non-automated techniques exist to study various honey bee behaviors. Fourth, honey bees, like humans, are social animals and allow examination of social behaviors within a colony. Fifth, honey bee eggs are laid in cells which allow

observation and video recording of larvae development. Finally, honey bees meet the self-administration requirement of Cicero's (1979) development of animal models.

The results of the preliminary and current experiments indicate that 1) under harnessed conditions, honey bees will readily consume 1%, 5%, 10%, and 20% alcohol solutions, 2) sucrose stimulation, as well as sensory bypass, elicits excitation and consumption of 95% alcohol, 3) alcohol consumption decreases locomotion, 4) honey bees readily consume fruit juice and fruit flavored wine, and 5) honey bees will self-administer alcohol at an artificial feeder, a behavior similar to that observed by Hassan (1992). His field studies found that honey bees will consume fermented nectar containing up to 10% ethanol. However, further research is needed to determine the development of alcohol tolerance and dependence in honey bees. Additionally, the biomedical complications of self-administration of alcohol in honey bees needs to be examined. The results of these studies may indicate the use of honey bees in bioassay procedures. That is, if it is found that honey bees meet all of Cicero's (1979) requirements, honey bees may provide answers regarding the use of alcohol inhibiting drugs in humans (e.g., Antabuse and Naltrexone).

The completion of this project would not have been possible without the assistance of Dr. Charles I. Abramson. His unwavering patience and guidance was invaluable to the furtherance of my education. Thank you. Additionally, I would like to express my appreciation to my committee members Dr. Doug Hershey and Dr. Marc Pratarelli. Their input and support was very helpful to this project.

Last, but definitely not least, I would like to thank Marty. You have always supported my endeavor to broaden my horizons. Without your companionship and love, I would not have continued to attain my goals. Thanks also goes to my family for providing an incentive to persevere during this past year.

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Comparative Analysis of Alcohol Consumption: Review of the Literature

Researchers have used animals in various ways in an attempt to understand the effects of alcohol on humans. The use of animals offers advantages over using humans for such research. For instance, Mello (1973) emphasized the ethical advantages of using animals because they could be used in studies that prohibit human use. These ethical advantages are clearly outlined in the ethical guidelines established by the American Psychological Association (APA). The APA does not allow examination of some neurophysiological, endocrinological, biochemical, and behavioral aspects of alcohol addiction in humans. Animals, in contrast, are not covered by the stringent guidelines established for the human population and are more easily used in such studies.

Another advantage of using animals for alcohol research involves the ability to selectively breed animals. Alcohol-preferring strains of rats and mice have been produced and studied in an attempt to develop an animal model of alcoholism (Eriksson 1968; Waller, McBride, Gatto, Lumeng, & Li, 1984; Sandbak, Murison, Sarviharju, & Hyytiae, 1998). Finally, animal models provide experimental manipulation techniques not available in human models. These techniques allow the researcher to study the factors influencing human alcohol consumption (McGregor, Saharov, Hunt, & Topple, 1999). Thus, animal models are attractive to researchers as they endeavor to understand mechanisms involved with self-administration of alcohol.

Cicero (1979) noted that any animal model of alcoholism should include the following 1) the animal must voluntarily self-administer. Voluntary consumption refers to

the preferential consumption of alcohol when the animal is faced with a choice between alcoholic and non-alcoholic solutions. The alcohol should be consumed orally and exceed the metabolic capacity of the animal to produce pharmacologically significant blood alcohol levels, 2) tolerance to alcohol should be demonstrated following a period of continuous consumption, 3) dependence on alcohol, as demonstrated by withdrawal symptoms, should be demonstrated following a period of continuous consumption, and 4) the biomedical complications associated with chronic alcohol consumption seen in humans should also develop in animals.

In an effort to develop an animal model of alcoholism, various alcohol-induction techniques have been used to increase alcohol consumption. These include intracerebral injections (Cicero & Myers, 1969), sweetening of the alcohol solution (Gilbert, 1974; Siegel & Brodie, 1984), sucrose-fading (Tolliver, Sadeghi, & Samson, 1988), food deprivation (Macenski & Meisch, 1992; Pakarinen, Williams, & Woods, 1999), direct stomach tube implantations (Deutsch & Eisner, 1977), and inhalation (Rijk, Crabbe, & Rigter, 1982).

Historical Attempts to Explore Alcohol Consumption in Vertebrates

Previous attempts have been made to develop vertebrate models of alcohol consumption using, for example, rodents (Ludvig, Fox, Kubie, Altura, & Altura, 1998) and goldfish (Marcucella & Abramson, 1978). The goldfish models were particularly attractive because the alcohol could be poured directly into the tank and produced intoxication in minutes. Many other vertebrate models either inject the animal with alcohol or expose it to alcohol vapors.

Although it is possible to produce physical dependence on alcohol in animals, much of the addictive process of alcohol remains unknown (Mello, 1976). However, because of the ability to produce alcohol dependence in animals, several specie have been studied to examine the various consequences resulting from alcohol consumption.

Primates

Reproductive consequences of alcohol consumption have been studied by Mello and her associates. In one study, Mello, (1983) found that female macaque monkeys demonstrated disrupted reproductive functions such as decreased ovarian mass, uterus atrophy, and lower hormone levels following high doses of self-administered alcohol. These disrupted functions paralleled clinical results of human alcoholic woman. Additionally, female macaque monkeys demonstrated varying self-administration patterns of alcohol during the menstrual cycle phase. Alcohol self-administration was significantly lower during menstruation than during the mid-cycle or late luteal phase (Mello, Bree, Skupny, & Mendelson, 1984).

Mello, Bree, Mendelson, and Ellingboe (1986) examined the pattern of alcohol consumption in rhesus monkeys as a function of menstrual cycle phase. They found that during chronic self-administration of alcohol, the rhesus monkeys, similar to human alcoholic women, developed increased amenorrhea, anovulatory cycles, and inadequate luteal phases. However, it could not be determined if the chronic self-administration was attributed to learning or physical discomfort of the premenstrual cycle tension symptoms such as increased anxiety, depression, irritability, and headaches.

Finally, Mello, Bree, Mendelson, and Ellingboe, (1984) reported that alcohol consumption produced immediate and sustained disruption of menstrual cycle regularity

in female macaque monkeys. The monkeys who self-administered high doses of alcohol (2.95 to 4.41 kg per day) developed pathological changes in their ovaries and uterus, one monkey died of alcohol overdose, and one died of alcohol-related pulmonary disease. In contrast, monkeys who self-administered low doses of alcohol (1.35 to 1.66 kg per day) continued to have stable menstrual cycles. Mello, Bree, and Mendelson (1986) compared the self-administration of alcohol to the self-administration of food in relationship to the menstrual cycle. They found that female rhesus monkeys self-administered alcohol and food significantly less during their menstruation cycles than during their midcycle or late luetal phases. Similarly, monkeys who self-administered high doses of alcohol (3 to 5.5 kg per day) also showed stable patterns of food self-administration whereas the low-to-moderate doses of alcohol self-administration (0.3 kg per day) showed a decrease in self-administration of food during the midcycle. These findings supported the hypothesis that estrogen plays a pivotal role in the self-administered consumption of food and alcohol across the menstruation cycle.

Food restriction in rhesus monkeys was shown to increase the preference for ethanol in both males and females, although less in the females (Pakarin, Williams, & Woods, 1999). Ritz, George, deFiebre, and Meisch (1986) found that in ethanol-preferring rats, ethanol easily served as a reinforcer during food deprivation. Finally, results have shown that alcohol possesses appetitive properties in addition to the aversive properties. For instance, alcohol was shown to suppress responses which were previously established and maintained by food reinforcement (Denoble & Begleiter, 1978).

Rodents

Animals and humans have been used to study the effects of alcohol on psychological attributes. For instance, rats specifically bred to prefer alcohol have demonstrated less fear responses to aversive stimuli such as shock prod and water-immersion stress tests than rats specifically bred to avoid alcohol (Sandbak, Murison, Sarviharju, & Hyytiae, 1998). This study also found that the alcohol-avoiding rats developed more stomach gastric ulcerations than did the alcohol-preference rats. Based on these results, Sandbak and his associates suggested that the fear and gastric sensitivity responses were caused by a common biochemical mechanism and hypothesized it was the dopaminergic system. In humans, this system is involved in both the motivational effects of alcohol (Wise & Rompre, 1989) and the psychological effect of decreased anxiety (Cowan, 1983).

Other areas of research have examined the effects of food deprivation on alcohol self-administration behavior. The results of various studies have indicated a relationship between the two. For instance, rats who were provided with free access to 2.7% beer and 8% sucrose preferred the beer when deprived of food (McGregor, Saharov, Hunt, & Topple, 1999).

Hangover effects have been defined using numerous behavioral disturbances such as reduced ambulatory and physical activity, and physiological measures such as increased nausea and decreased temperature. Although researchers define hangover effects differently, all agree that they are aversive to the subject. Sinclair and Gustafsson (1987) injected rats with ethanol and compared their running wheel activity, body temperature as measured by rectal probe, and number of vocalizations during intoxication

and post intoxication (20-24 hours after injection of ethanol). A reduction in running wheel activity occurred during intoxication but increased significantly 24 hours after ethanol injection. In addition, rats who were provided access to a running wheel 24 hours after ethanol injection exhibited decreased temperatures and vocalizations and increased ambulatory behavior when compared to rats barred from running wheel activity.

The hangover behaviors and physiological changes of the rats supported previous findings from human studies. Humans display a similar time course of alcohol-induced hangover effects. McGregor, Saharov, Hunt, & Topple (1999) reported that rats preferred 2.7% beer to 8% sucrose during food deprivation. They suggested that hangover effects, defined as conditioned tastes aversions, served to alter subsequent high-strength (5%) beer intake but not low-strength (2.7%) beer intake.

Gauvin, Goulden, and Holloway (1993) defined a hangover effect by the ethanol's delayed versus normal basal homeostasis as measured in discrimination accuracy tests. Their results showed a time-dependent (48 hours) cycle return from the hangover state to the normal state. Finally, Briscoe and Gauvin (1999) found that male rats, with experimentally induced ethanol hangovers, self-administered ethanol significantly less than cocaine induced rats.

Self-administration studies, sometimes referred to as free-feeding or open field feeding, have provided evidence that animals will continue to self-administer alcohol when they are reinforced with food or sucrose during training. For example, Grant and Samson (1985) examined whether ethanol self-administration could be maintained with food access in a free-feeding condition. They found that rats reinforced with sucrose

during the initial ethanol consumption training period would continue to self-administer ethanol.

Results of forced alcohol consumption studies have shown that conditioned aversions to the taste and smell of alcohol develop quickly and that tolerance also develops very rapidly (Briscoe & Gauvan, 1999). However, Deutsch and Eisner (1977) found that implanting a tube into the stomach of rats, thus bypassing the sensory organs, produced voluntary consumption of alcohol that initially developed into a conditioned aversion.

Frogs

The spinal cords of frogs have been used to study the effects of alcohol on neuronal plasticity (Glanzman & Epperlein, 1981). Their results indicated that increased alcohol concentration simultaneously increased motorneuron habituation in the nervous system. Glanzman and Schmidt (1981), based on the finding of the spinal cord section of the frog, attempted to replicate the motorneuron pathway habituation results in intact frogs. When whole-body immersion in alcohol solutions was administered to the frogs, they found that the initial nictitating membrane response was reduced providing further evidence that alcohol inhibits motorneuron responses.

Elephants

Siegal and Brodie (1984) examined self-administration behaviors of unflavored 7% alcohol solutions and fruit flavored 10% alcohol solutions in Asian and African elephants. The results of their field study showed that, when water deprived for 12 hours, 7% alcohol solution was the highest concentration that the elephants would consume

when water was also available. However, when the alcohol solutions were flavored with fruit extracts, the elephants readily self-administered 10% concentrations.

Consumption of either 7% or 10% alcohol solutions resulted in exhibition of inappropriate behaviors. For instance, although elephants are social animals and tend to stay with the herd, the intoxicated elephants separated from the herd, decreasing their feeding, drinking, bathing, and exploratory behaviors, and demonstrated increased lethargy and ataxia. The researchers suggested that consumption of fermented fruit in the natural habitat may be related to the elephants' shrinking environment.

Humans

The majority of human studies of alcohol consumption have focused on the detrimental physiological and behavioral attributes. Brookhuis (1998) used various car driving behaviors to measure the detrimental effects of alcohol. Behaviors such as steering wheel handling, speed control, and use of pedals were measured after alcohol consumption. The results indicated that alcohol consumption increased heart rates, decreased reaction times, and impaired car driving skills. These impairments were similar to those caused by hypnotic drugs such as sedatives.

Pharmacological benefits of alcohol consumption in premenstrual woman has been examined by several researchers. For instance, Belfer, Shader, Carroll, and Hermatz (1971) reported that many clinical studies of alcoholic women indicated that alcohol consumption increased during premenstruum. However, Ruble (1977) argued that increased alcohol consumption is attributable to learned responses. That is, women are expected to feel discomfort such as bloating, increased anxiety, and backaches during the premenstrual phase but these discomfort symptoms diminish with the self medicating

properties of alcohol. Yet, women may induce hangover effects as a result of excessive alcohol consumption as a means of self-medication.

In contrast to the detrimental effects of alcohol, a few researchers have suggested that the benefits of alcohol must be considered. Chick (1998), for instance, suggested that moderate alcohol use would benefit patients with uncontrollable risk factors for coronary heart disease. Alcohol-induced escape from depression, frustration, and anxiety have been suggested as a short-term benefit of self-administered alcohol (Martin, Hewett, Baker, & Haertzen, 1977). Cowan (1983) suggested that decreased memory for unpleasant emotional stimuli may also be a beneficial effect eliciting the consumption of alcohol.

Nature-vs-Nurture of Alcoholism

Genetic influences have been hypothesized to underlie the divergent ethanol drinking behaviors in alcohol preferring rats. Additionally, results have shown that these behaviors are present as early as 3-4 weeks (McKinzie, Nowak, Murphy, Li, Lumeng, & McBride, 1998) in rats and provide support for a genetic basis of alcoholism. In contrast, other researchers report that the environment plays a more causative role in alcohol consumption behavior. Johnson and Johnson (1998), for example, reported that human males responded more favorably than females on attitude scales designed to measure drinking behavior. They also revealed that the male attitudes significantly related to the presence of adult intoxication in the home.

Finally, social interaction studies conducted with human alcoholics showed that alcohol consumption decreased with isolation and increased with social interactions (Griffiths, Bigelow, & Liebson, 1973). Animal studies have also shown environmental effects of alcohol consumption. For instance, isolated environments were shown to

increase self-administration of alcohol in several rat strains, however, alcohol consumption significantly decreased the anxiety produced by the isolation. Under low light Fawn Hooded rats were less anxious in mazes while bright light conditions resulted in less anxious behaviors in Wistan rats (Hall, Huang, Fong, Pert, & Linnoila, 1998). Behavioral genetics researchers, however, stress the importance of both genetic and environmental influences of alcohol consumption. They also suggest that animal and human models must be used jointly to fully understand the complexities of alcoholism (George, 1987; Witt, Cunningham, Dudek, Finn, Henderson, Plomin, & Samson, 1998).

Historical Attempts to Explore Alcohol Consumption in Invertebrates

Although invertebrates may seem unlikely subjects, several attempts have been made to explore the effects of alcohol consumption and intoxication in these animals. The naturalist John Lubbock anecdotally described one of the first observed and documented attempts in 1888. He fed "spirits" to ants and observed how intoxicated individuals interacted with nest mates. As Lubbock noted, "the sober ants were puzzled; but after examining the intoxicated individuals, they picked up the strangers and threw them into the ditch, while they carried their own friends into the nest, where no doubt they slept off the effects of the spirits (pgs. 233-234)."

Other researchers have also attempted to explore alcohol consumption and its effects in invertebrates. For instance, Traynor, Schlapfer, Woodson, and Barondes (1979) used the sea hare (*Aplysia californica*) to examine both tolerance and neurophysiological effects of ethanol exposure at the cellular level. Most recently, Moore, DeZazzo, Luk, Tully, Singh, and Heberlein (1998) described ethanol intoxication in the fruitfly

(*Drosophila melanogaster*) and isolated a mutation sensitive to alcohol-induced postural control.

In an effort to understand the neurological basis of alcohol, various invertebrates have been examined. Barker (1975) examined the excitatory and inhibitory effects of neuromuscular cells in lobsters, crayfish, sea hare, and snails when exposed to a depressant drug. He studied the effects of the central nervous system depressant, pentobarbital, on membrane and synaptic activity of crustacean neuromuscular junctions and the neurons of mollusks. In the crustaceans, he found that the drug depressed the excitatory postsynaptic potentials (EPSP) of the neuromuscular junctions but did not change the inhibitory postsynaptic potentials (IPSP) or membrane properties. In the molluscan neurons, the drug depressed the EPSP but only depressed the depolarizing phase of the IPSP.

Honey bees

Self-administration situations address issues not inherent in experimentally controlled studies. For example, the author has observed self-administration of alcohol in natural environments such as recycling centers that collect beer cans and wine bottles. Natural environments such as orchards and areas with high humidity similar to the tropics pose risks of fermented food consumption (Hassan, 1992). The sugar contained in the nectar of blossoms of flowering plants ferment in high temperatures and humidity. Therefore, consumption of fermented foods may result in behavioral effects similar to those produced by alcohol consumption. For instance, in laboratory experiments, Hassan (1992) found that fermented nectar may contain as high as 10 percent alcohol

concentration and that European honey bees (*Apis mellifera* L.) are attracted to and consume the fermented nectar.

In field studies, Hassan observed that honey bees intoxicated from fermented nectar have difficulty finding their hive when returning from foraging expeditions. He suggested that if the intoxicated bees did make it back to their hives, they may be rejected by the bees guarding the hive. Despite being marked with recognition pheromones prior to their departure from the hive, guard bees, according to Hassan, may deem the intoxicated foragers as outsiders.

Individuals who become ill after consumption of fermented food may develop strong taste aversions to the food. These food aversions are long lasting and extremely resistant to change (Abramson, 1994). In addition to the behavioral consequences of fermented food consumption, the physiological and biological effects of fermented food consumption may not differ from the negative consequences of prolonged alcohol consumption. Results from the current preliminary studies have demonstrated similar results in honey bees. The antennae of honey bees, analogous to the human nose, contain their sensory organs. When the antennae stimulation is bypassed, the honey bees consumed aversive stimuli as strong as 95% alcohol solutions.

Development of An Alcohol Model Using Honey Bees

Honey bee models are as attractive as other invertebrates or vertebrates for development of an alcohol model and have several unique advantages. First, honey bees are inexpensive to procure and maintain. For example, vertebrates and mollusks cost, on average, between $3.00 and $15.00 each, respectively, while honey bee colonies containing approximately 60,000 animals can be purchased for about $50.00. Except for

routine spraying for mite infestation, the colonies require very little maintenance. Second, much is known about their history, physiology, genetics, and behavior. Third, automated and non-automated techniques are available to study a wide range of honey bee behavior including habituation, sensitization, Pavlovian, and Operant conditioning. Fourth, because honey bees are social animals, the effects of alcohol consumption on such advanced behaviors as the "dance communication" and social caste interactions can be studied. These behaviors are a unique feature among some invertebrate and vertebrate species. Fifth, because honey bee eggs are laid in cells, the cell environment is an ideal environment to explore the effects of alcohol consumption on the larvae development. For example, alcohol can be injected directly into the cell and the effect on the developing larvae may be examined. Sixth, in contrast to existing invertebrate models and vertebrate models, honey bees will readily consume alcohol and will self-administer alcohol by flying to an artificial feeder containing a 5% alcohol solution.

Our laboratory research suggests that the European honey bee (*Apis mellifera* L.) could be a suitable animal model and may provide insights into the human behavior of alcohol consumption, tolerance, dependence, and biomedical consequences of addiction and alcoholism.

Chapter III

Summary of Relevant Work Conducted in Our Laboratory

The proboscis extension reflex (PER) has been used extensively to study a wide array of behavioral, genetic, and neurobiological perspectives of behavior. The honey bees are first harnessed in small metal tubes then they are presented with stimuli. The response elicited, or the PER, is used to measure both associative and non-associative learning (Smith, Abramson, & Tobin, 1991). Additionally, the PER has been used to measure learning in the Africanized honey bee (Abramson, Aquino, Silva, & Price, 1997), the effects of insecticides on learning in European (*Apis mellifer*) honey bees (Stone, Abramson, & Price, 1997) and Africanized (*Apis mellifera* L.) honey bees (Abramson, Aquino, Ramalho, & Price, 1999), and as a rapid bioassay to measure detection of beeswax (Aquino, Abramson, & Payton, 1999). Therefore, the proboscis extension response is used to measure alcohol consumption in honey bees in this series of experiments.

A necessary first step in the development of an alcohol model was to ascertain if the honey bees would drink alcohol. We believed that honey bees would readily consume alcohol because anecdotal evidence suggested that honey bees forage on discarded beer and wine bottles at recycling centers and trash dumpsites. The purpose of the first series of experiments was to determine how much alcohol and in what concentration level a honey bee would consume. All of the treatment solutions, unless otherwise noted, were prepared by diluting 95% ethanol with filtered water from our laboratory.

EXPERIMENT 1: ALCOHOL CONSUMPTION IN HONEY BEES AS MEASURED BY AMOUNT CONSUMED

Subjects. European honey bees (*Apis mellifera* L.) were collected from the hive one day (24 hours) before training in order to allow them time to habituate to the laboratory environment. They were transported to the laboratory in individual glass vials. The lids of the vials had four air holes to allow ventilation. After being brought to the laboratory, the honey bees were cooled briefly by placing the vials in ice to render unconsciousness and reduce movement. The animals were then harnessed in small metal tubes. For details see Smith, Abramson, and Tobin (1991). After being restrained and adequate time to recover from the unconscious state, the honey bees were fed to satiation from a drop of 1.8 Molar sucrose solution. The honey bees then remained in the apparatus overnight. This was done to ensure all the honey bees had the same level of motivation to feed prior to their assignment to subsequent treatment groups during training. The honey bees were randomly assigned to treatment groups the following day.

The alcohol solutions differed only according to their respective alcohol concentration level, (0%, 1%, 5%, 10%, 20%, and 95%). The honey bees were stimulated to feed by touching their antenna to a one micro liter droplet of their respective group's alcohol solution. When the proboscis extended the honey bee was allowed to drink until it stopped.

Apparatus. Small metal tubes restrained the honey bees by placing a thin piece of duct tape between the head and thorax to keep them secure during testing. The heads, including the antennae and proboscis, were able to move freely.

Procedure. One hundred and fifty honey bees were randomly assigned to six treatment groups consisting of 25 animals each. The independent variable was the percentage of alcohol concentration of the alcohol solution (0%, 1%, 5%, 10%, 20%, and 95%). All of the honey bees were stimulated to feed by touching their antenna to a one micro liter droplet of their respective group's alcohol solution. The dependent variable was the proboscis extension response (PER) after stimulation by the alcohol solution. When the proboscis extended the honey bee was allowed to drink until it stopped.

Results. Figure 1 depicts the mean number of micro liter drops of alcohol solution consumed by the honey bees. Means and standard deviations were computed for the groups. Group 0% (M = 15.80, SD = 4.72), Group 1% (M = 17.00, SD = 7.64), Group 5% (M = 15.60, SD = 4.86), Group 10% (M = 15.60, SD = 5.59), Group 20% (M = 13.60, SD = 3.96), and Group 95% (M = .20, SD = 1.00). As indicated, the honey bees readily consumed all except the 95% alcohol solutions. There was no significant difference between groups when the 95% alcohol solution group was excluded from the Analysis of Variance (ANOVA).

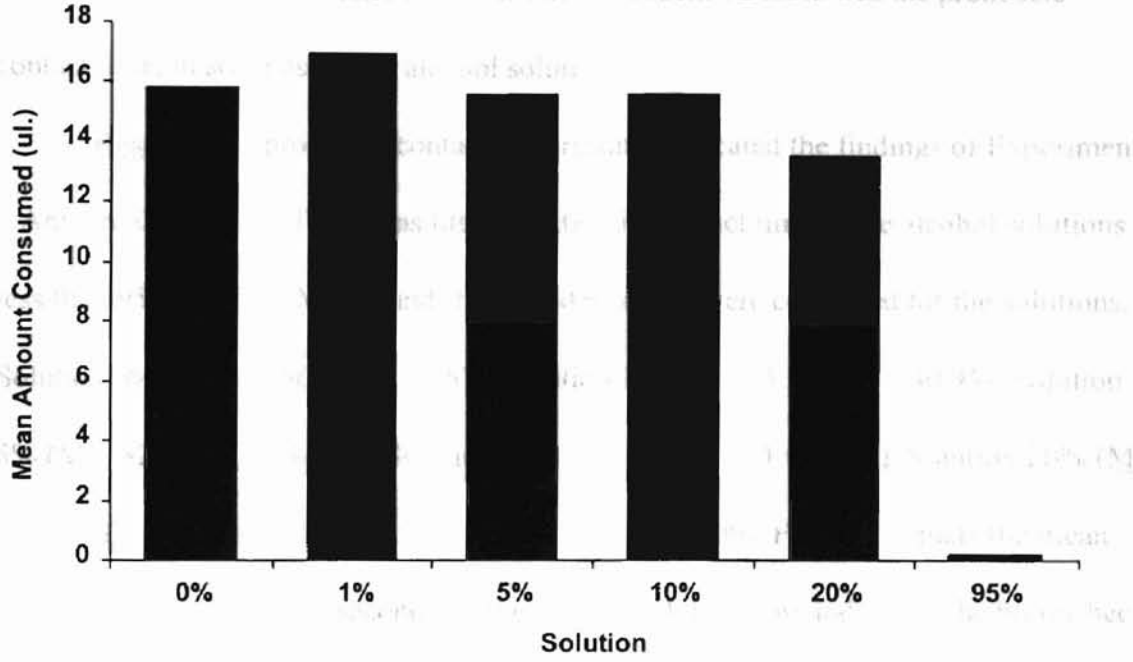

Figure 1. Micro liter drops of consumed solutions.

EXPERIMENT 2: ALCOHOL CONSUMPTION IN HONEY BEES AS MEASURED BY PROBOSCIS CONTACT TIME WHEN MIXED WITH SUCROSE

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. One hundred and fifty honey bees were randomly assigned to six treatment solutions consisting of 25 animals each. The independent variable was the percentage of alcohol solutions (0%, 1%, 5%, 10%, 20%, and 95%). All of the honey bees were stimulated to feed by touching their antenna to a 1 micro liter droplet of their respective group's alcohol solution. The honey bee was measured for proboscis extension

after stimulation to the alcohol solution. The dependent variable was the proboscis contact time, in seconds, to the alcohol solutions.

Results. The proboscis contact time results replicated the findings of Experiment 1 Amount Consumed. There was little variation in contact time to the alcohol solutions less than 95% alcohol. Means and standard deviations were computed for the solutions. Solution 0% (M = 89.56, SD = 38.57), Solution 1% (M = 83.32, SD = 40.95), Solution 5% (M = 82.52, SD = 44.35), Solution 10% (M = 75.92, SD = 46.02), Solution 20% (M = 63.88, SD = 33.22), and Solution 95% (M = .00, SD = .00). Figure 2 depicts the mean proboscis contact time, in seconds, to the alcohol solutions. As indicated, the honey bees responded to all except the 95% alcohol. There was no significant difference between solutions when the 95% alcohol was excluded from the ANOVA.

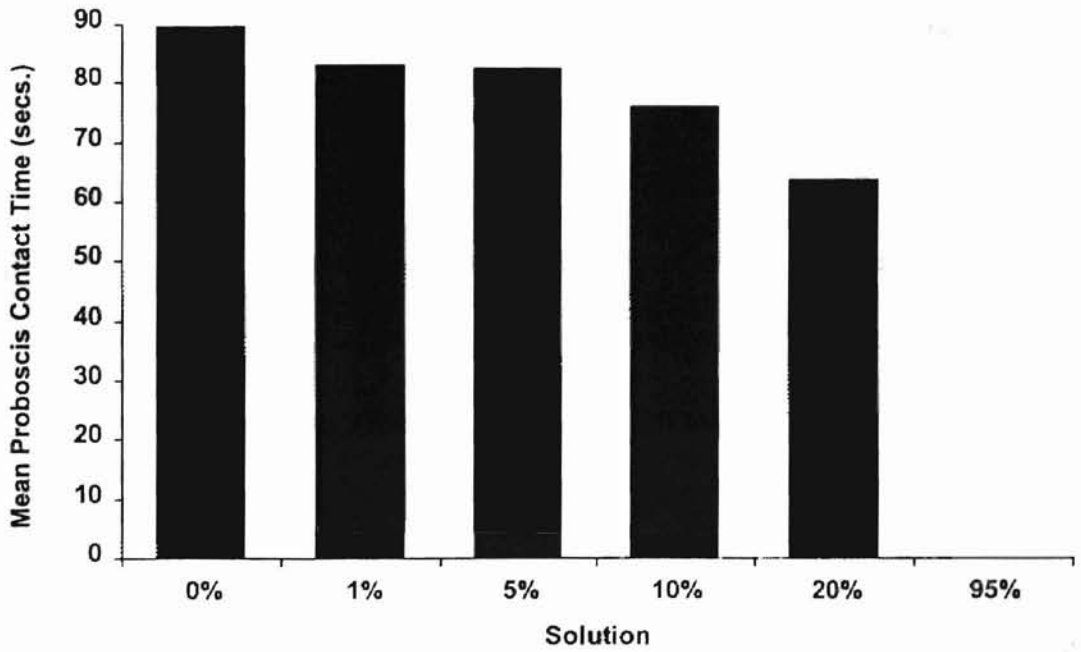

Figure 2. Amount of proboscis contact time, in seconds, to the alcohol solutions.

EXPERIMENT 3: CONSUMPTION OF 95% ALCOHOL AFTER LESSER CONCENTRATION

The lack of differences in drinking and proboscis contact time to the 1%, 5%, 10%, and 20% alcohol solutions suggested that once honey bees began to drink, they continue to do so regardless of the concentration used in these experiments. Experiment 3 was conducted to test for disruption of drinking caused by contrast effects of the alcohol solutions. The results of Experiment 2 indicate that the honey bees did not consume the 95% alcohol solution. This suggested that contrast effects were detected and that the 95% alcohol solution was perceived as an aversive stimulus.

Experiment 3 used a lesser concentration of alcohol solution to determine if it served to excite the honey bees' consummatory responses and elicited consumption of alcohol solutions with higher concentrations. If the honey bees detected a concentration differences in the alcohol solutions then they would disrupt their drinking because the aversiveness of the higher concentrations.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience. This experiment used a single subject design to test the contrast effects of the alcohol solutions because it is a more sensitive test of preference.

Apparatus. Same as Experiment 1.

Procedure. One hundred honey bees were randomly assigned to four treatment groups consisting of 25 animals each. The dependent variable was the proboscis

extension response (PER) after stimulation by the alcohol solution. The independent variables were Solution (10%, 20%, 95%) and Group (order of Solution presentation).

Group 1 was antennae stimulated by 10% alcohol, moved to 20% alcohol and antennae stimulated, then moved to 95 % alcohol and antennae stimulated. Group 2 was antennae stimulated by 20% alcohol, moved to 10% alcohol and antennae stimulated, then moved to 95% alcohol and antennae stimulated. Group 3 was antennae stimulated to 20% alcohol, moved to 20% alcohol and antennae stimulated, then moved to 95% alcohol and antennae stimulated. Group 4 was antennae stimulated by 10% alcohol, moved to 10% alcohol and antennae stimulated, and finally moved to 95% alcohol and antennae stimulated.

Results. As presented in Figure 3, stimulation by the alcohol solutions containing less than 95% alcohol increased the probability of proboscis extension response to higher concentrated alcohol solutions excluding the 95% alcohol solution. Analysis of variance yielded a significant Group effect $F(2, 72) = 1933.75$, $p = .00$, a significant Solution effect $F(2, 144) = 212.91$, $p = .00$, and a significant Group x Solution interaction $F(4, 144) = 13.208$, $p = .00$.

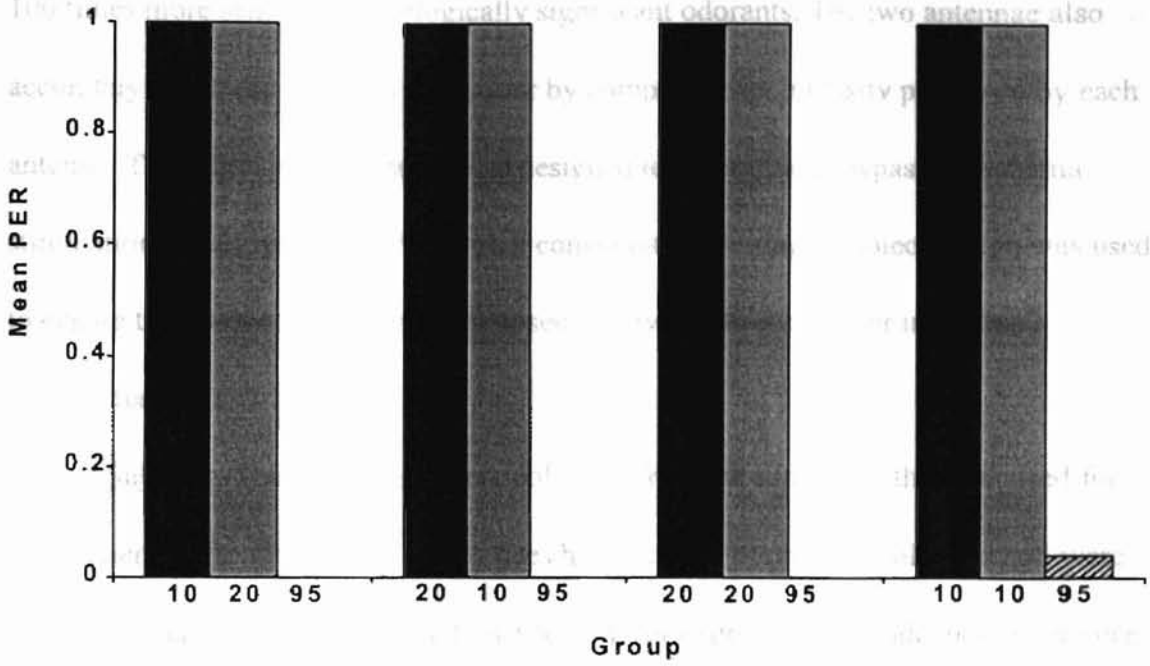

Figure 3. Proboscis extension response to 95% alcohol solution following a lesser concentration. PER = Proboscis Extension Response.

EXPERIMENT 4: CONSUMPTION OF 95% ALCOHOL SOLUTION WITH AND WITHOUT ANTENNAE TOUCH

Previous findings by Deutsch and Eisner (1977) indicated that implanting a tube directly into the stomach of rats bypassed the sensory organs and resulted in voluntary consumption of alcohol that was initially perceived as aversive. Based upon these findings and the results of Experiments 1, 2, and 3 which indicated that the honey bees perceived the 95% alcohol as an aversive stimulus, Experiment 4 used a sensory bypass procedure. Antennae, as first demonstrated by Von Frisch (1914) is analogous to the human nose. He showed that honey bee workers could be trained to visit dishes containing odors of natural flowers or essential oils. When the antennae were surgically removed, olfactory discrimination ability was eliminated. Subsequent experiments showed that the olfactory acuity of worker honey bees, as compared to humans, is 10 to

100 times more sensitive to biologically significant odorants. The two antennae also accurately detect the direction of an odor by comparing the intensity perceived by each antenna. Therefore, an experiment was designed to determine if bypassing antenna stimulation would result in 95% alcohol consumption. A single subject design was used to ensure the greatest level of statistical sensitivity and to control for individual differences.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. Twenty-five honey bees were tested using a single subject design. The independent variable was the alcohol concentration of the solution. Also, each level of solution consisted of antennae stimulation (touch) and no antennae stimulation (no touch). Each animal received all levels of the independent variable and the dependent variable was the proboscis extension response (PER).

First, each honey bee was stimulated by antennae touch to a 10% alcohol solution. Once the proboscis extended the animal was allowed to drink for 10 seconds, moved to the 20% alcohol solution and stimulated, then allowed to drink for 10 seconds. After 10 seconds the animal was moved to the 95% alcohol and stimulated. If the proboscis did not extend or the animal consumed the 95% alcohol solution for 10 seconds, it was moved back to the 20% alcohol solution, stimulated and allowed to drink for 10 seconds. Finally, after drinking for 10 seconds on the 20% alcohol solution, the animal was moved to the 95% alcohol solution before the proboscis retracted.

Results. Although the previous experiments suggested that the 95% alcohol was an aversive stimulus, the results shown in Figure 4 indicate that honey bees will consume 95% alcohol as long as the proboscis remained extended and the antennae did not contact it. This suggests that the sensory mechanism is located in the antennae. Therefore, the antennae act as the on-off switch to consummatory behavior. Analysis of variance yielded a significant Solution effect $F(4, 96) = 576.00$, $p = .00$.

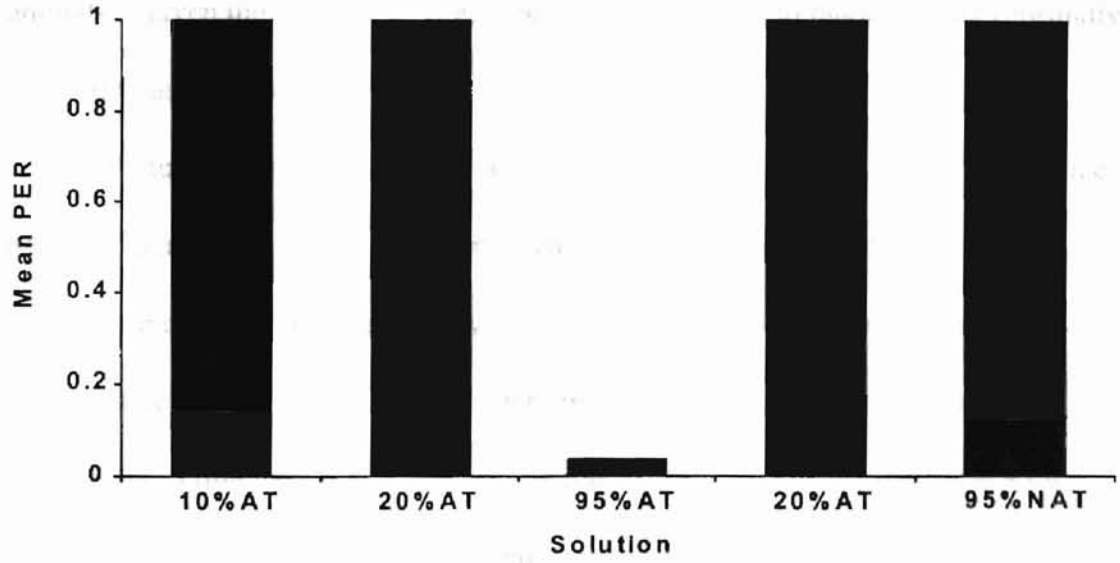

Figure 4. Consumption of 95% alcohol with (AT) and without (NAT) antennae touch. PER = Proboscis Extension Response.

EXPERIMENT 5: CENTRAL EXCITATORY STATE AND ALCOHOL CONSUMPTION

A hungry animal will become excited upon presentation of food and thus will consume any subsequently presented food even if it is normally unattractive to the animal. This behavior is referred to as Central Excitatory State (CES). Responses elicited during CES, however, may be a result of pseudo conditioning.

Pavlovian learning experiments test for acquisition of a behavior by pairing the conditioned stimulus (CS) with the unconditioned stimulus (US). If, after several CS-US presentations, the individual responds to the CS, it is assumed that an association between the stimuli has occurred and, thus, learning is inferred. However, if the animal responds to the CS without prior exposure to the CS-US paired presentations then pseudo conditioning has occurred. To test for the presence of pseudo conditioning a group of animals is given the CS and US presentations but in a pseudo random order (normally referred to as the ABBA sequence).

Water is not normally an attractive stimulus for honey bees. Given the choice between sucrose and water, honey bees will choose sucrose. However, if sucrose is not available and the temperature is very hot, the honey bees will consume water. Because CES may be the mechanism that produces pseudo responses, water was used in this experiment to rule out CES and determine if the honey bees would drink the 95% alcohol. Experiment 5 was also designed to replicate the results of Experiment 4 that indicated bypassing antenna touch increased the probability of consumption of aversive stimuli and stimuli not normally consumed.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. Seventy-five honey bees were randomly assigned to three treatment groups, 25 animals each. Group 1 was exposed to 95% alcohol and antenna stimulated, moved to sucrose and antenna stimulated, then moved back to the 95% alcohol without

antenna stimulation. Group 2 was exposed to a 95% alcohol and antenna stimulated, moved to sucrose and antenna stimulated, then moved to water and antenna stimulated. Group 3 was exposed to water and antenna stimulated, moved to sucrose and antenna stimulated, then moved back to water and antenna stimulated.

Results. The consumption of solutions not normally attractive after excitation is referred to as Central Excitatory State and was supported by the results of this experiment. Sucrose stimulation, as presented in Figure 5, increased consumption of water and 95% ethanol as long as no antennae touch occurred. Analysis of variance yielded a significant Group (antenna touch vs. no antenna touch) effect $F(2, 72) = 1933.75$, $p = .00$, a significant Solution effect $F(2, 144) = 212.91$, $p = .00$, and a significant Group x Solution interaction $F(4, 144) = 13.208$, $p = .00$.

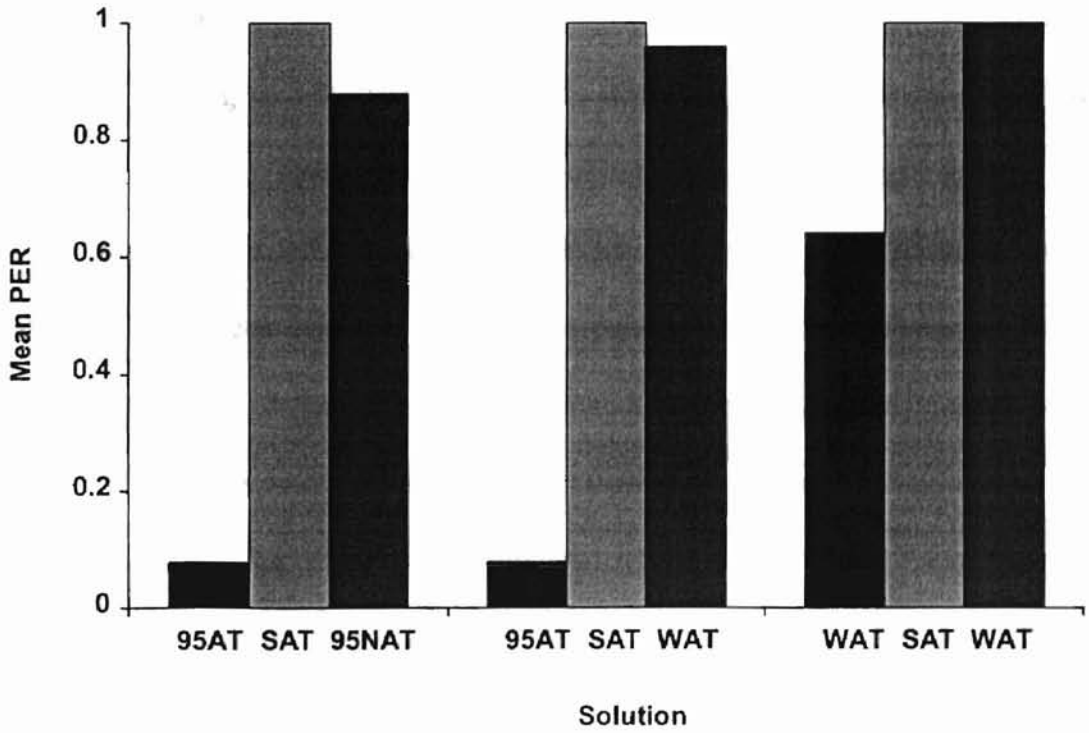

Figure 5. Consumption of water (W) and 95% ethanol due to sucrose (S) excitation. AT = Antennae Touch, NAT = No Antennae Touch. PER = Proboscis Extension Response.

EXPERIMENT 6: RECOVERY TIME AFTER ALCOHOL CONSUMPTION

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. One hundred and twenty five honey bees were randomly assigned to five treatment groups consisting of 25 animals each. The independent variable was the alcohol solution (0%, 1%, 5%, 10%, and 20%). Five minutes after consumption of assigned alcohol solution, the honey bees were tested for proboscis extension response (PER) by touching their antennae to a sucrose solution (0% alcohol) every minute. Recovery time was measured by the occurrence of proboscis extension responses for 5 consecutive trials.

Results. Figure 6 illustrates that greater alcohol concentration requires more trials for recovery to occur. That is, the more alcohol in the solution, the longer the time it required to reach five consecutive proboscis extension responses. The mean number of trials required for recovery, as measured by responses in five consecutive trials, was calculated. Solution 0% animals recovered immediately and responded on trials 1-5, Solution 1% animals recovered after trial 15, responding on trials 16-20, Solution 5% animals recovered after trial 40, responding on trials 41-45, Solution 10% animals recovered after trial 165, responding on trials 166-170, and Solution 20% animals recovered after trial 210, responding on trials 211-215. The results of the Analysis of variance yielded a significant Solution effect $F(4, 124) = 31.32$, $p = .00$.

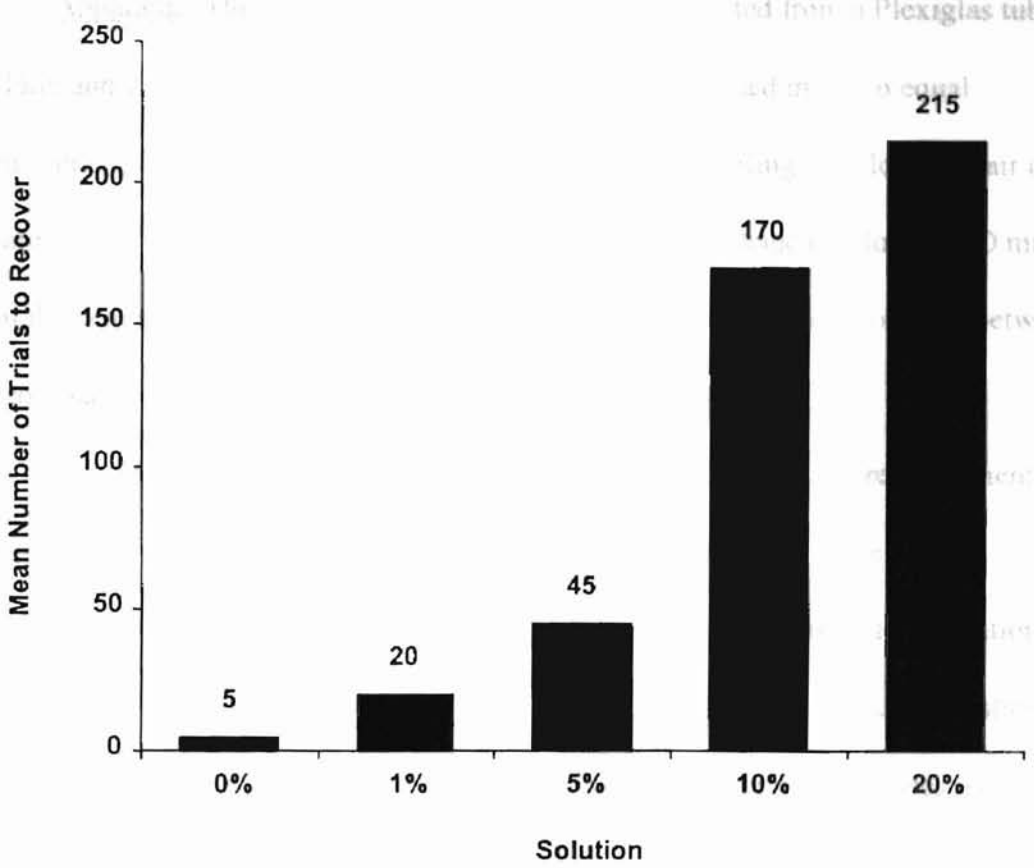

Figure 6. Number of trials required to respond 5 consecutive times after alcohol consumption.

EXPERIMENT 7: EFFECT OF ALCOHOL ON LOCOMOTOR BEHAVIOR: SHUTTLE BOX

Informal evidence from the laboratory suggested that the honey bees became intoxicated after alcohol consumption. When set free from the apparatus they staggered and had difficulty flying compared to the honey bees who had not consumed alcohol. Therefore, shuttle box behavior was used to measure the effect of alcohol on locomotion.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. The apparatus was a shuttle box constructed from a Plexiglas tube (7.5 cm long and 2.5 cm in internal diameter). The tube was divided into two equal compartments by a hurdle 5-mm crawl space between the ceiling and floor. A pair of infrared photoemitters monitored the honey bee's position. Detectors, located 10 mm from the center on each side, automatically registered the number of crossings between the compartments.

Procedure. Seventy-five subjects were randomly assigned to three treatment groups consisting of 25 animals each. The independent variable was the alcohol concentration of the treatment solution (0%, 10%, and 20%). A 5-minute adaptation period elapsed before placing the honey bee in the shuttle box. Once inside the shuttle box the session began. Each session lasted 10 minutes with shuttle responses recorded in one-minute intervals. The dependent variable was the number of shuttle crossings made by the animal.

Results. The mean number of shuttle crossings, as depicted in Figure 7, indicated a difference between the 0% alcohol solution and alcohol solution groups. The 0% alcohol solution group of honey bees responded, on average, 3 times per minute while the alcohol groups of honey bees responded about once per minute. Analysis of variance yielded a significant Group effect $F(2, 72) = 17.31$, $p = .00$ and a significant Trial (minute) effect $F(9, 648) = 11.16$, $p = .00$. Post hoc Tukey analyses revealed no significant differences in shuttle box responses between those given 10% and 20% alcohol solutions. Tukey analyses revealed a significant difference between the 0% and 10% alcohol solutions groups at every Trial (minute), except Trial 6, and total Trials (minutes) (HSD = 14.08, $p = .00$). Finally, Tukey analyses revealed a significant

difference between the 0% and 20% alcohol solutions groups at every Trial (minute) and total Trials (minutes) (HSD = 18.32, p = .00).

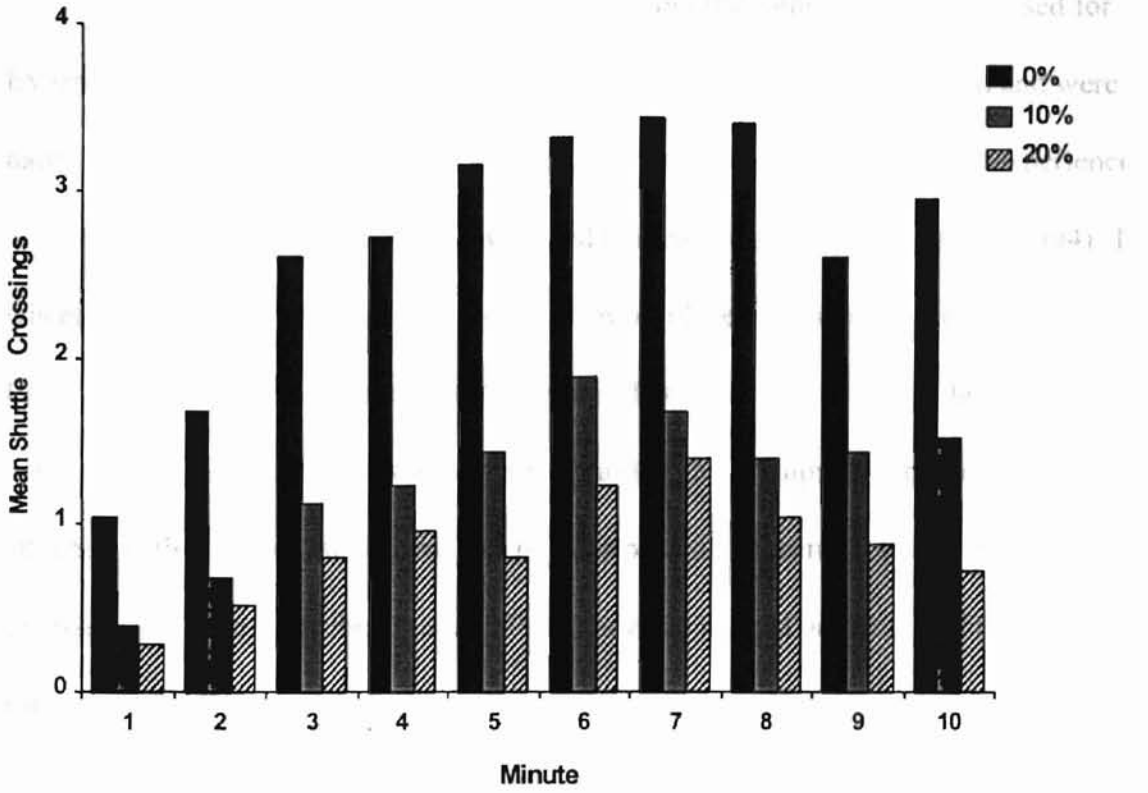

Figure 7. Shuttle Box locomotion following consumption of group solution.

EXPERIMENT 8: EFFECT OF ALCOHOL ON LOCOMOTOR BEHAVIOR: RUNNING WHEEL

The effect of alcohol consumption on locomotor behavior was also measured through running wheel rotations. The purpose of using a running wheel experiment was to confirm the shuttle box results using a more sensitive measure of locomotion. That is, each movement of the honey bee was recorded. The shuttle box could not record all movements because the honey bee was required to cross the hurdle to trip the photocell. If the honey bee moved while on one side or the other of the shuttle box the movements

were not recorded. Therefore, the running wheel was used to follow up and confirm the results of the shuttle box test.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. A rotating wheel was used to measure activity (Abramson, 1994). The wheel was attached to a board and photocells were placed on either side of the wheel. Each rotation of the wheel was detected by the photocells and increased the frequency count by one. The honey bee was tethered by a straight pin dipped in melted wax and attached to the back of the animal. The pin was placed between the wings and held over the wheel so the legs touched the rim. The honey bee walked on the rim of the wheel to rotate it.

Procedure. Seventy-five subjects were randomly assigned to three treatment groups, 25 animals each, and fed (0%, 10%, and 20%) alcohol. A 5-minute adaptation period elapsed before placing the honey bee on the running wheel. Once tethered to the wheel the session began. Each session lasted 10 minutes with running wheel rotations recorded in one-minute intervals.

Results. The mean number of running wheel rotations, as depicted in Figure 8, indicated a difference between the sucrose and alcohol groups. Sucrose honey bees responded, on average, 20 times per minute while alcohol honey bees responded about five times per minute. Analysis of variance yielded a significant Group effect $F(2, 72) = 14.87$, $p = .00$. Post hoc Tukey analyses revealed no significant differences in running wheel rotations between those given 10% and 20% alcohol solutions. Post hoc Tukey

analyses revealed a significant difference between the 0% and 10% alcohol solutions groups at every Trial (minute), and total Trials (minutes) (HSD = 134.08, p = .00). Finally, post hoc Tukey analyses revealed a significant difference between the 0% and 20% alcohol solutions groups at every Trial (minute) and total Trials (minutes) (HSD = 163.52, p = .00).

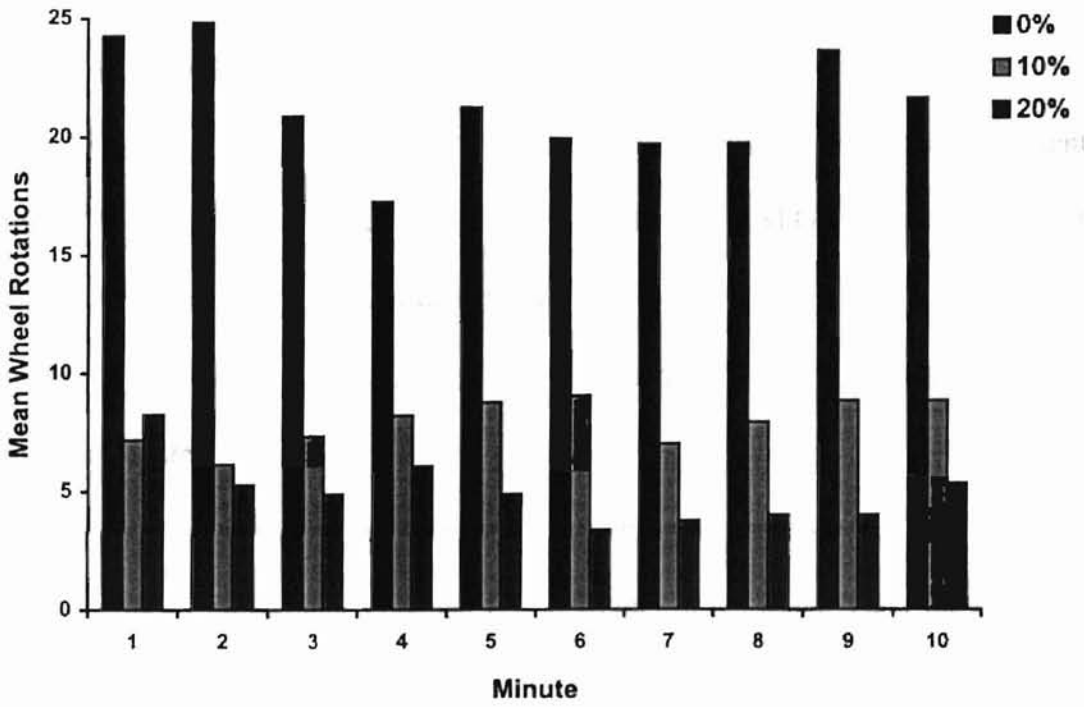

Figure 8. Running Wheel locomotion responses following consumption of treatment solutions.

EXPERIMENT 9: CONSUMPTION OF FRUIT JUICE AND FRUIT FLAVORED WINE

Consumption of Fruit Juice. Aquino and Abramson (1995) observed that honey bees would consume soft drinks around recycling centers and trash dumpsites. Thus, it was hypothesized that fruit nectar in naturalistic environments would be as equally

attractive to honey bees as flower nectar in naturalistic environments. This experiment was conducted to test this hypothesis by measuring proboscis extension to fruit juice.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience. The apparatus was the same as in Experiment 1.

Apparatus. Same as Experiment 1.

Procedure. One hundred and fifty subjects randomly assigned to six treatment groups, 25 animals each, Sucrose, Apple, Peach, Banana, Red Grape, and Nectarine fruit juices. The honey bees were stimulated to feed by touching their antenna to a 1 micro liter droplet of fruit juice. When the proboscis extended the honey bee was allowed to drink until it stopped.

The fruit juice solutions were made by cutting fruit into small pieces and placing them in a plastic cup which contained 1.5 micro liter of tap water. The fruit was allowed to sit in refrigeration for 24 hours to stimulate the metabolism of sugars and to simulate natural situations of fruit fermenting on the ground. To ensure none of the fruits began the fermentation process during the course of the experiment, new fruit juice solutions were prepared every day. Thus, the fruit juice stimuli for each day's testing was prepared 24 hours prior to experimentation.

Results. Figure 9 depicts the mean proboscis extension response (PER) for each fruit juice solution. As indicated, and in contrast to experiments using varying concentrations of alcohol, the honey bees readily consumed all of the fruit juice solutions. Means and standard deviations of the PER for each group was computed, Sucrose (M =

.85, SD = .37), Apple (M = .80, SD = .41), Peach (M = 1.00, SD = .00), Banana (M = .80, SD = .41), Red Grape (M = .80, SD = .41), and Nectarine (M = .75, SD = .44). The Peach fruit juice solution was consumed by all of the honey bees assigned to that group whereas the other fruit juice solutions produced more variability within the group of honey bees. Analysis of variance results indicated that no significant group differences existed.

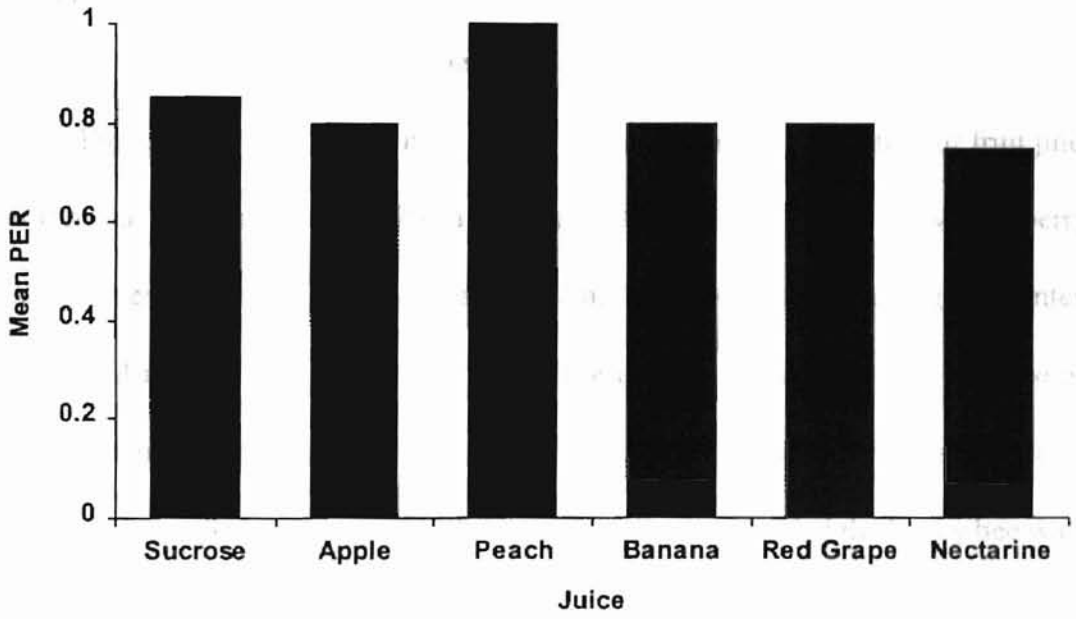

Figure 9. Proboscis Extension Response to fruit juice solutions. PER = Proboscis Extension Response.

Consumption of Fruit Flavored Wine. The consumption of the fruit juice solutions was expected, therefore, it was hypothesized the honey bees would also be attracted to fermented fruit juice solutions. This experiment was conducted to test this hypothesis. Rather than fermenting the fruit juices in our laboratory, we chose to substitute fruit flavored wine. This provided control of the alcohol concentration and fermentation of the fruit flavored solutions. It also more precisely simulates recycling center products. Only

Apple, Peach, Blackberry/Raspberry, and Strawberry/Kiwi wines could be found, thus a two phase experiment was designed to test these flavors.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience. The apparatus was the same as in Experiment 1.

Apparatus. Same as Experiment 1.

Procedure. One hundred honey bees were randomly assigned to four fruit juice and fruit flavored wine flavors, 25 animals each, Apple, Peach, Blackberry/Raspberry, and Strawberry/Kiwi. The honey bees were stimulated to feed by touching their antenna to either a 1 micro liter droplet of fruit juice or a 1 micro liter droplet fruit flavored wine rather than to a sucrose solution as done in previous experiments. This was done to simulate a naturalistic environment. When the proboscis extended the honey bee was allowed to drink until it stopped.

Phase One consisted of purchasing an apple, peach, blackberries, raspberries, strawberries, and kiwis and replicating the previous fruit juice results. The combined fruit juice flavors, blackberry/raspberry and strawberry/kiwi, were made by combining equal parts of each fruit before placing in the tap water.

Phase Two consisted of measuring proboscis extension response to the fermented counterpart of the fruit juice solutions, that is, the fruit flavored wines. The wines used were manufactured by the Boones Farm distillery, California. The Apple, Peach, and Blackberry/Raspberry wines had a 5% alcohol content and the Strawberry/Kiwi wine had an 8% alcohol content. Hassan (1992) previously reported that honey bees would

consume the fermented nectar of plants that contained up to 10% alcohol. However, it was unknown if the honey bees would consume fermented fruit nectar or if the acid of the fruit would alter the honey bees' alcohol consumption behavior. Therefore, this experiment tested a lower alcohol concentration of fermented fruit to determine if honey bees would consume fermented fruit nectar.

Results. Figure 10 depicts the mean response of proboscis extension to the fruit juice and fruit flavored wine. As indicated, the honey bees were equally responsive to the fruit juice solutions and the fruit flavored wine. Means and standard deviations of the PER were computed for each group, Apple (M = .80, SD = .41), Peach (M = 1.00, SD = .00), Blackberry/Raspberry (M = .95, SD = .22), and Strawberry/Kiwi (M = .90, SD = .31). Analysis of variance results indicated no statistical significance existed between groups.

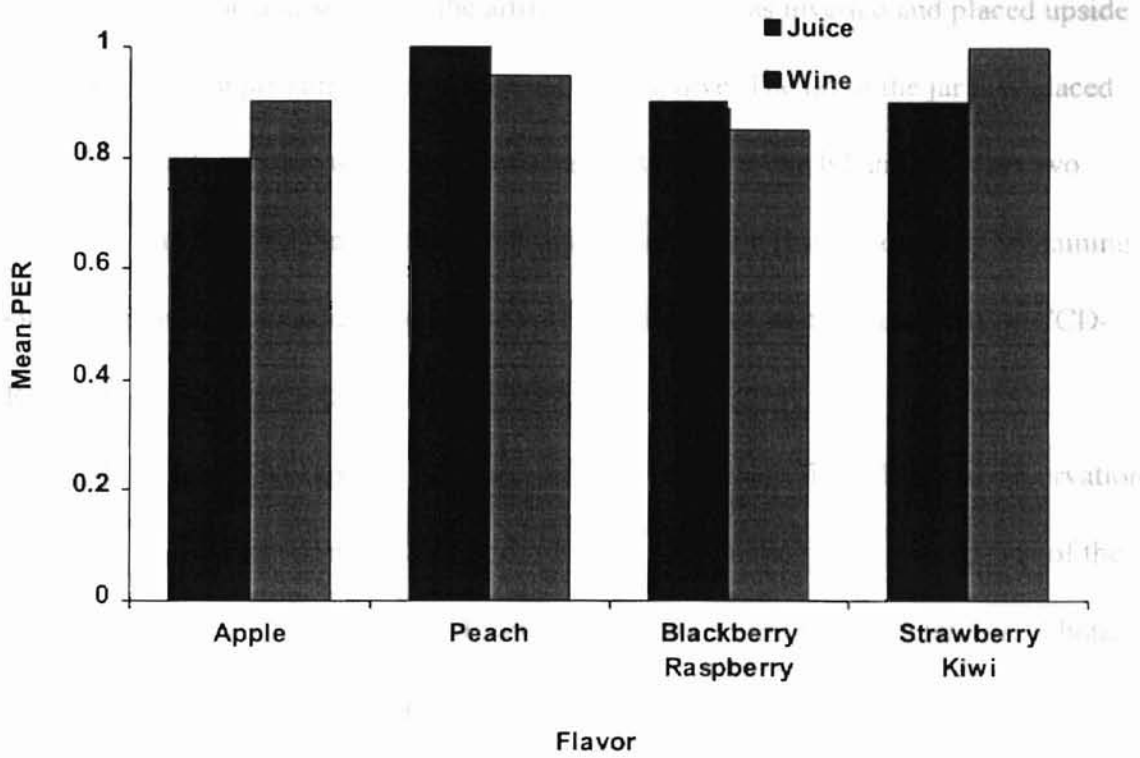

Figure 10. Proboscis extension response to fruit juice and fruit flavored wine. PER = Proboscis Extension Response.

EXPERIMENT 10: FREE-FLYING SELF-ADMINISTRATION OF ALCOHOL

Experiment 10 was conducted to determine if, under naturalistic situations, honey bees would self-administer solutions by visiting an artificial feeder containing alcohol. The feeder represented a "flower." It was predicted that the number of honey bees at the feeder would increase over time.

Subjects. The honey bee colony came from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. The honey bees were observed at their hive rather than captured and brought into the laboratory. A .95 liter glass jar containing 5% alcohol solution was used

to hold the solution and served as the artificial feeder. It was inverted and placed upside down on a stool approximately five meters from the hive. The lid of the jar was placed under the mouth opening with a toothpick inserted between the lid and jar. This two millimeter gap allowed the treatment solution to flow into a plastic container containing both the jar and the jar's lid. The activity of the honey bees was recorded with a CCD-IRIS color video camera (Sony[R] model No. SSC-C374).

Procedure. The experiment was conducted in the open field. The first observation consisted of counting the number of honey bees present at the feeder during each of the one-minute intervals for a total of 30 minutes. This was conducted to determine if honey bees would self-administer alcohol solutions in the field rather than harnessed in a laboratory setting.

Results. Figure 11 presents the results of the 30 one-minute free-flying observation period collapsed into 10 three-minute intervals. As indicated, the number of honey bees at the feeder steadily increased during each observation period. The largest increase in visiting honey bees occurred on the first observation, that is, during the first three minutes. The total number of honey bees for each observation period was calculated by summing the number of honey bees present at the feeder on every one-minute time interval. The mean number of honey bees was calculated by dividing the total number of honey bees in each observation period by three, the number of minutes per period. The total and mean number of honey bees for each observation period (OP) was, 26 honey bees in OP1 ($\underline{M}$ = 8.67), 61 honey bees in OP2 ($\underline{M}$ = 20.33), 68 honey bees in OP3 ($\underline{M}$ = 22.67), 80 honey bees in OP4 ($\underline{M}$ = 26.67), 89 honey bees in OP5 ($\underline{M}$ = 29.67), 94 honey bees in OP6 ($\underline{M}$ = 31.33), 107 honey bees in OP7 ($\underline{M}$ = 35.67), 119 honey bees in OP8

(M = 39.67), 116 honey bees in OP9 (M = 38.66), and 125 honey bees in OP10 (M = 41.67).

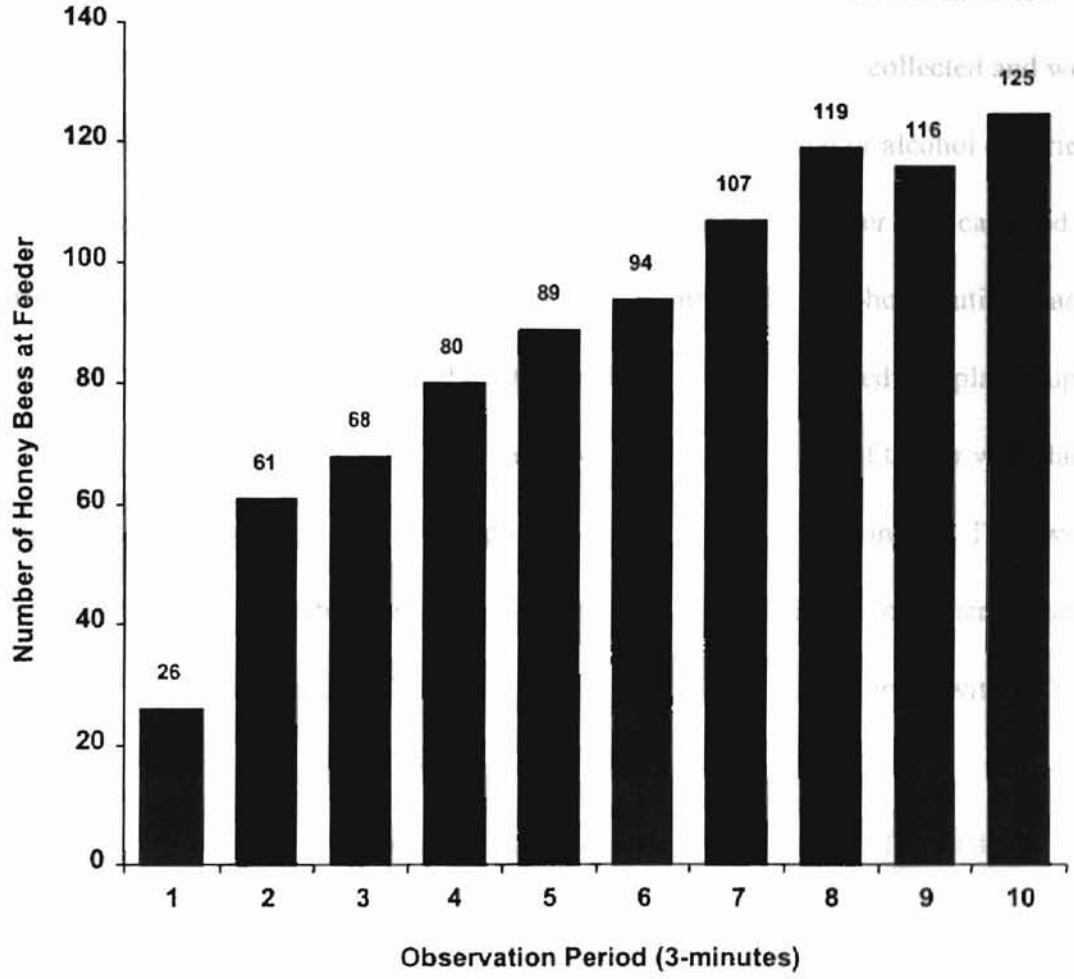

Figure 11. Number of honey bees at the feeder during each of the observation periods (3-minutes each) of the free-flying experiment.

EXPERIMENT 11: RETURN VISITS TO ARTIFICIAL FEEDER BY MARKED HONEY BEES

The results of Experiment 10 indicated that the number of honey bees at the feeder increased over time but there was no way of determining if the same bees were returning more than once. Therefore, Experiment 11 was conducted to determine if, under

naturalistic situations, the same honey bees would return to an artificial feeder containing alcohol.

Subjects. The honey bee colony was from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. The honey bees were observed at their hive rather than captured and brought into the laboratory. A .95 liter glass jar containing 5% alcohol solution was used to hold the solution and served as the artificial feeder. It was inverted and placed upside down on a stool approximately five meters from the hive. The lid of the jar was placed under the mouth opening with a toothpick inserted between the lid and jar. This two millimeter gap allowed the treatment solution to flow into a plastic container containing both the jar and the jar's lid. The activity of the honey bees was recorded with a CCD-IRIS color video camera (Sony^{R} model No. SSC-C374).

Procedure. The experiment was conducted in the open field. The first task was to capture and mark the 10 of the honey bees at the feeder. They were marked with a dot of fingernail polish between their wings. The marking did not interfere with any aspect of the honey bees' behavior. The marked honey bees were observed for 30 minutes or 10 visits, whichever occurred first. The number of visits of each marked honey bee served as the dependent measure. This measure was used to provide support for the prediction that honey bees would self-administer alcohol solutions, a requirement stipulated by Cicero (1979) in the development of an animal alcohol model.

Results. Figure 12 indicates that all of the marked honey bees returned to the feeder at least twice during the free-flying observation period. Of the 10 marked and

monitored honey bees six returned every observation period, one returned six times, one returned four times, one returned three times, and one returned two times. It is unknown why four of the honey bees failed to return every time. It may be due to several factors, including, age of the honey bee, predator attack, or the effects of the alcohol on the foraging honey bee's ability to find the feeder.

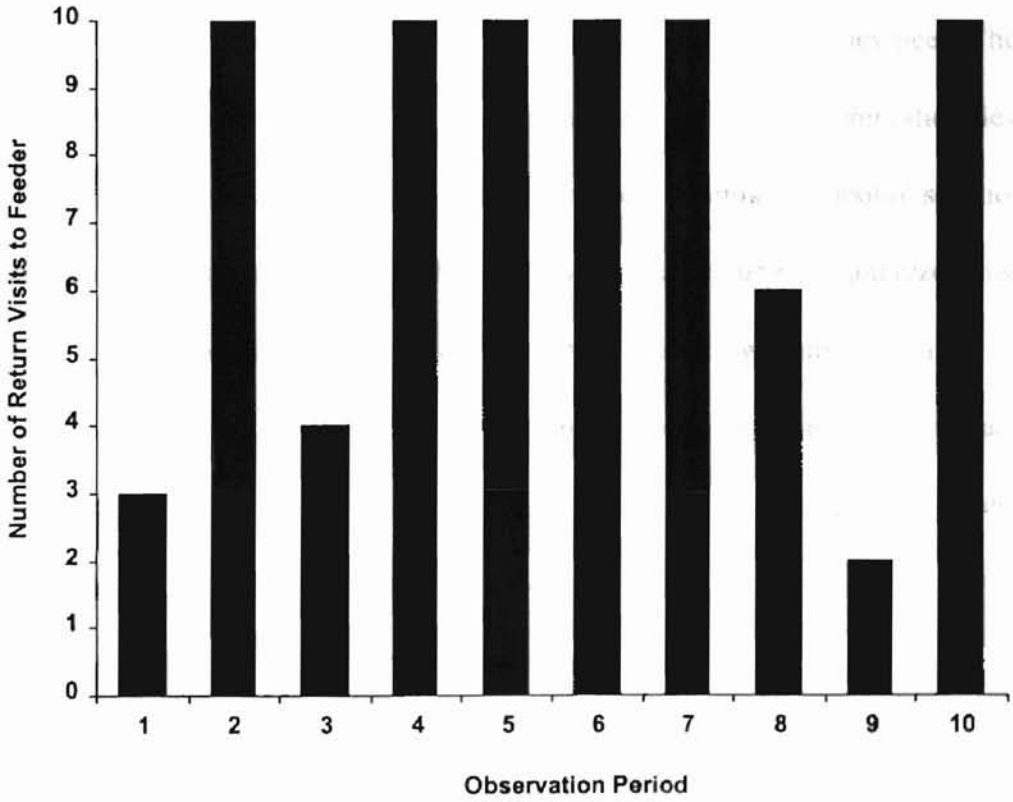

Figure 12. Number of return visits to the feeder by the marked honey bees during the 30 minute free-flying observation period.

Chapter IV

Overview of Multi-Experimental Design

The purpose of the multi-experimental design was to circumvent the event of failure of the first experiment. That is, Experiment 1 was the design, development, construction, and analysis of an alcohol self-administration apparatus because no apparatus existed to monitor the self-administration of alcohol in honey bees. The intent of the apparatus construction was to develop and test a functional observation device.

To determine whether the honey bees were consuming the alcohol solution for caloric or other observable benefits, Experiment 2 was conducted to analyze consumption of alcohol when mixed with water rather than when alcohol was mixed in a sucrose solution. The results, as measured by the amount of proboscis contact time to each solution, were compared to the results of Experiment 1 (Amount consumed when mixed with sucrose) in the preliminary studies

Experiment 3 was conducted to provide additional experimental data of the effects of alcohol on learning when the alcohol was consumed prior to the commencement of the learning trials. In contrast, Experiment 4 evaluated the effects of alcohol on learning when the alcohol was consumed during the learning trials and served as the unconditioned stimulus.

Finally, Experiment 5 was a statistical analysis and comparison of the results obtained in Experiment 3 (Alcohol consumed prior to learning trials) and Experiment 4 (Alcohol consumed during the learning trials). This was done to determine if there was a significant difference in learning as a function of time of consumption and intoxication.

EXPERIMENT 1: DEVELOPMENT OF AN APPARATUS FOR SELF-ADMINISTRATION OF ALCOHOL

The preliminary studies provided evidence of alcohol consumption in honey bees both in harnessed and free-flying conditions. Consequently, the next necessary step in development of a honey bee alcohol model was to examine self-administration of alcohol at the colony level. However, risk factors such as killing colony members or the entire colony did not allow the use of the laboratory's existing hive. Therefore, a separate colony of honey bees was required. This was only possible by obtaining a new colony. In addition, an apparatus was required to house the new colony separately from the existing laboratory colony. No apparatus existed so one was built for this purpose.

A separate observation hive offered several advantages over the harnessed laboratory and preliminary free-flying experiments. First, the observation hive provided a colony of honey bees dedicated solely to the study of the effects of alcohol consumption. Second, the observation hive provided an apparatus to observe and analyze free flying, self-administration of alcohol. Third, the observation hive would allow video taping of the social interactions among the colony members. Finally, analysis of language and communication behaviors, larvae development, queen egg-laying behavior, and queen-colony member interactions would be possible.

Subjects. Due to the mite infestation problems plaguing several suppliers of honey bees, no single frame colony could be procured to test the constructed apparatus. The bee keepers contacted were not willing to part with any of their existing colonies until the honey bee population increased or the mite infestation problem was reduced.

Apparatus. Figure 13 presents a photograph of the observation hive and Figure 14 provides a schematic drawing of the observation hive. A single frame observation hive was procured from Drake's Super Bee Supply Company, Nebraska. It was attached to a 1.727 meter clear vinyl tube. The other end of the tube forked into three separate tubes measuring 25.4 millimeters diameter by .305 meters long. These three tubes led to separate petri dishes. These petri dishes represented a "flower" and were enclosed by their respective covers to keep the honey bees from escaping and also allow observation of the honey bees' feeding behavior. A CCD-IRIS color video camera (Sony[R] model No. SSC-C374) was available to video record all observations.

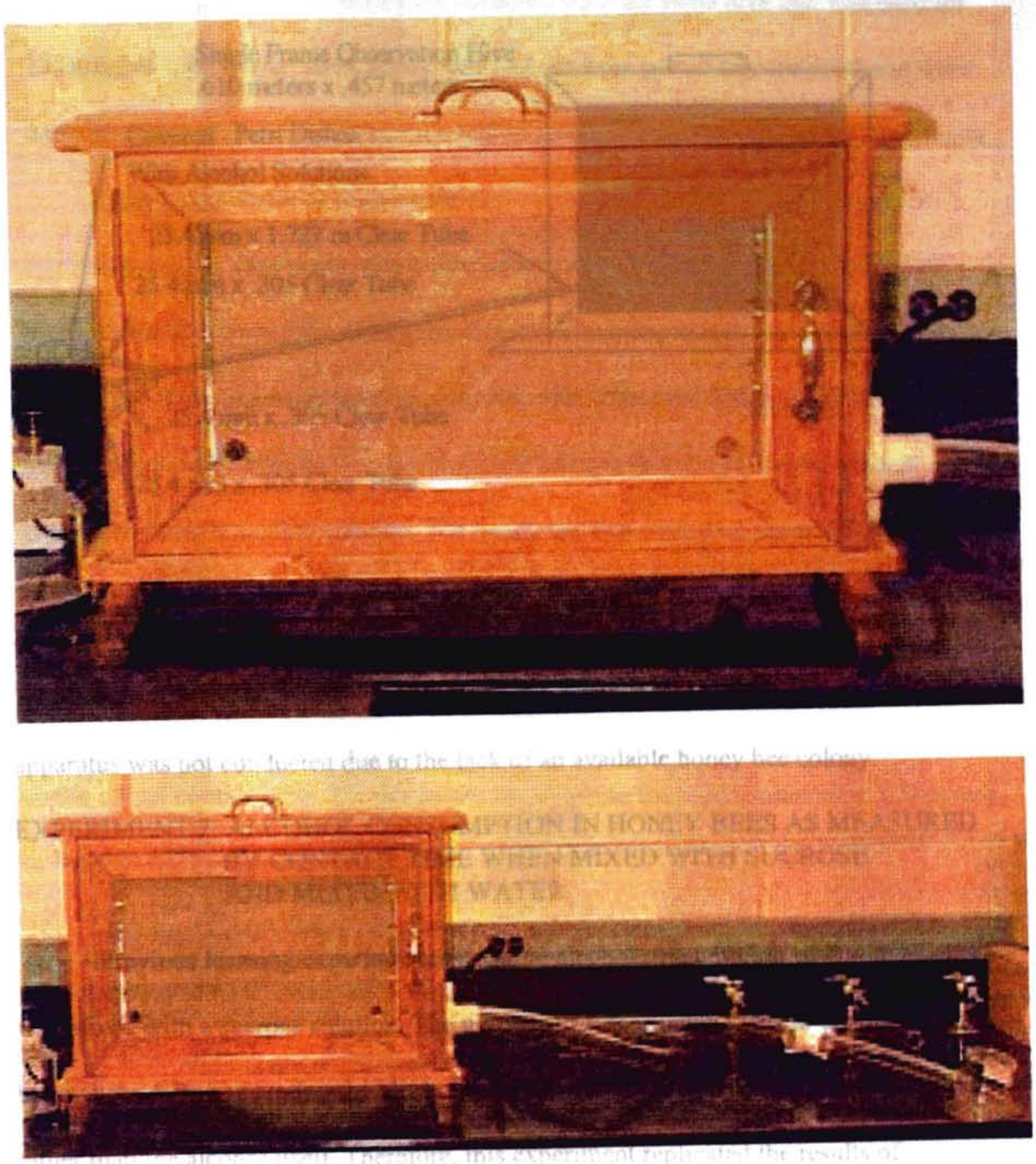

Figure 13. Photograph of single frame observation hive.

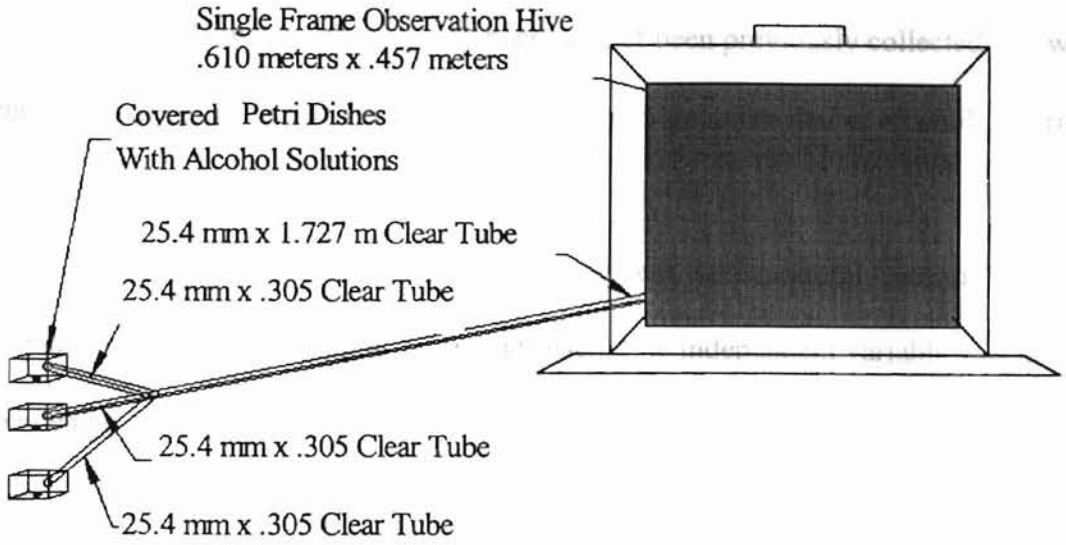

Figure 14. Schematic drawing of single frame observation hive

Results. The apparatus construction was completed. However, testing of the apparatus was not conducted due to the lack of an available honey bee colony.

EXPERIMENT 2: ALCOHOL CONSUMPTION IN HONEY BEES AS MEASURED BY CONTACT TIME WHEN MIXED WITH SUCROSE AND MIXED WITH WATER

Previous learning experiments measured alcohol consumption when the alcohol was mixed with a sucrose solution. The purpose of Experiment 2 was to determine if the honey bees were consuming the alcohol solutions for the caloric benefits of the sucrose rather than the alcohol itself. Therefore, this experiment replicated the results of Experiment 2 (Proboscis contact time) in the preliminary experiments. However, the alcohol in the current experiment was mixed with water rather than sucrose to determine if the honey bees would consume alcohol without sucrose as the reward.

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. One hundred and fifty honey bees were randomly assigned to six alcohol solutions consisting of 25 animals each. The independent variable was the percentage of alcohol solutions (0%, 1%, 5%, 10%, 20%, and 95%). All of the honey bees were stimulated to feed by touching their antenna to a 1.8 M droplet of their respective group's alcohol solution. The honey bee was measured for proboscis extension response after stimulation to the alcohol solution. When the honey bee retracted its proboscis it was immediately stimulated again to test if it would continue to consume the alcohol solution. The initial contact time and the additional contact time were summed to obtain a total contact time. The dependent variable was the total contact time, in seconds, of the proboscis to the alcohol solution.

Results. The proboscis extension response test replicated the findings of the preliminary studies (Experiment 1-Amount Consumed, Experiment 2-Contact Time). Both of these preliminary experiments used sucrose as the mixing agent. This experiment was conducted to analyze the honey bees proboscis extension response to alcohol solutions mixed with water rather than with a sucrose solution. The results indicated that there was little variation in contact time to solutions less than 95% alcohol. As done previously in the preliminary experiments, the 95% solution results were not included in the analyses. Means and standard deviations were computed for each solution. Solution 0% ($M = 21.60$, $SD = 62.03$), Solution 1% ($M = 49.24$, $SD = 42.61$), Solution 5% ($M =$

47.36, SD = 43.77), Solution 10% (M = 44.68, SD = 62.16), and Solution 20% (M = 43.32, SD = 36.86). Figure 15 depicts the mean proboscis contact time to the alcohol mixed with sucrose and alcohol mixed with water.

The Analysis of variance (ANOVA) for the Water mixture data revealed no significant difference between solutions when the 95% alcohol solution was excluded. A comparison of the data from the Sucrose mixture experiment and the Water mixture experiment revealed a significant difference for the Type of mixing agent (sucrose or water) F(1, 300) = 50.11, p = .00 when the 95% alcohol solution was excluded.

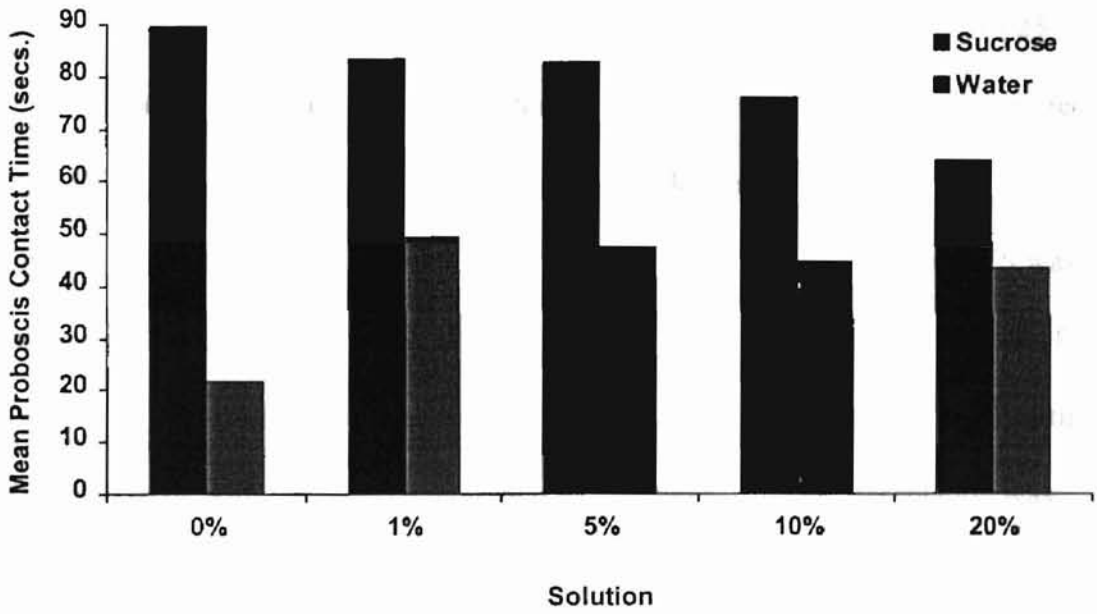

Figure 15. Amount of proboscis contact time (secs.) to the alcohol mixed with sucrose and to alcohol mixed with water.

EXPERIMENT 3: EFFECTS OF ALCOHOL ON LEARNING WHEN CONSUMED PRIOR TO THE LEARNING TRIALS

Experiment 3 examined the effects of alcohol consumption on learning when honey bees were fed alcohol solutions prior to the learning trials (Pre-fed group).

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. One hundred and twenty-five subjects were randomly assigned to five treatment groups (0%, 1%, 5%, 10%, and 20% alcohol solutions) consisting of 25 animals each. These solutions served as the unconditioned stimuli (US) and were fed to the honey bees five minutes prior to the onset of the learning trials. The conditioned stimuli (CS) was the liquid chemical geraniol (a flower odor). A drop of the CS was placed on a piece of filter paper and the filter paper was thumb tacked to the end of a syringe plunger. Each honey bee received 12 acquisition (CS-US) trials and 12 extinction (CS-only) trials with an intertrial interval of six minutes. The CS presentation was immediately followed by presentation of the US and the dependent variable was the proboscis extension response. The honey bee was stimulated to respond to the US by antennae touch.

A Repeated Measures Analysis of Variance (ANOVA) was conducted to analyze Group and Trial main effect differences and Group x Trial interaction differences.

Results. As presented in Figure 16, there was rapid acquisition of the proboscis extension response in all except the 20% alcohol solution group. The results of the

ANOVA revealed a significant Group effect $F(4, 95) = 7.72$, $p = .00$, a significant Trial effect $F(23, 2185) = 19.88$, $p = .00$, and a significant Group x Trial interaction $F(92, 2185) = 2.02$, $p = .00$.

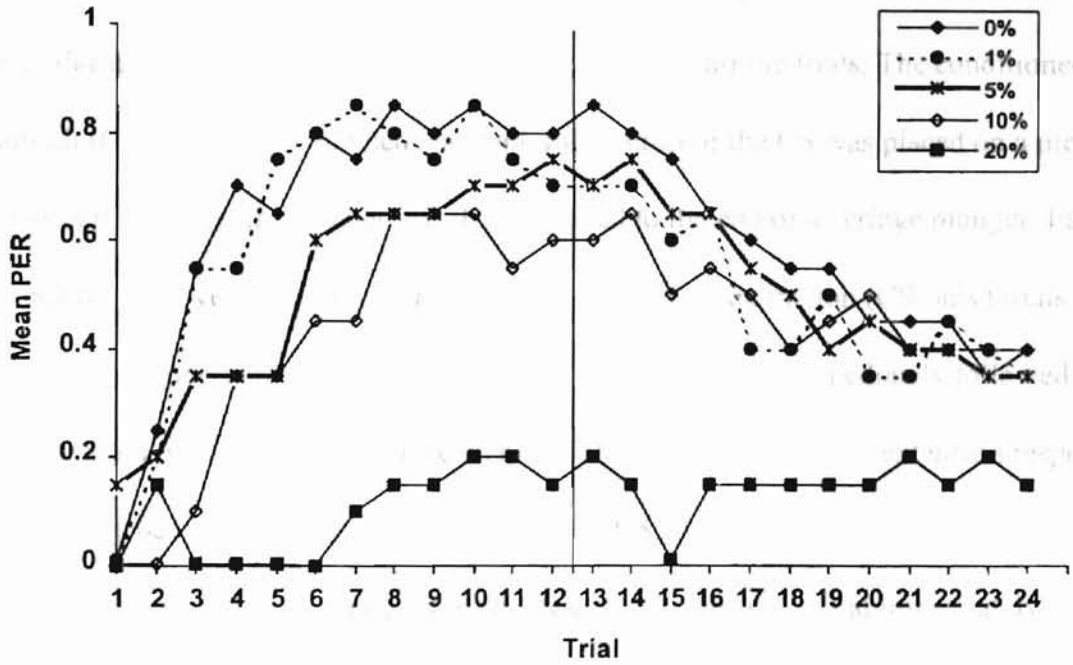

Figure 16. Mean proportion of honey bees responding to alcohol solutions over the 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13.

EXPERIMENT 4: EFFECTS OF ALCOHOL ON LEARNING WHEN CONSUMED DURING THE LEARNING TRIALS

Experiment 4 examined the effects of alcohol consumption on learning in honey bees who consumed alcohol during the learning trials (During-learning group).

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. One hundred and twenty-five subjects were randomly assigned to five treatment groups (0%, 1%, 5%, 10%, and 20% alcohol solutions), 25 animals each. These alcohol solutions served as the unconditioned stimuli (US) and were fed to the honey bees during the learning trials rather than prior to the learning trials. The conditioned stimuli (CS) was the liquid chemical geraniol. A drop of the CS was placed on a piece of filter paper and the filter paper was thumb tacked to the end of a syringe plunger. Each honey bee received 12 acquisition (CS-US) trials and 12 extinction (CS-only) trials with an intertrial interval of six minutes. The CS presentation was immediately followed by presentation of the US and the dependent variable was the proboscis extension response. The honey bee was stimulated to respond to the US by antennae touch. Each bee was presented an air puff of geraniol, the conditioned stimulus (CS), and immediately antennae stimulated to the US. Learning was measured by proboscis extension across 12 acquisition trials. These trials were followed by 12 extinction trials consisting of CS-only presentations.

A Repeated Measures Analysis of Variance (ANOVA) was conducted to analyze Group and Trial main effect differences and Group x Trial interaction differences.

Results. As indicated in Figure 17, when honey bees consumed the alcohol solution during the learning trials there was rapid acquisition of the proboscis extension response in the 0% and 1% alcohol solution groups. In contrast, bees stimulated with 5%, 10%, and 20% alcohol solutions never acquired proboscis extension response The results of the ANOVA revealed a significant Group effect $F(4, 95) = 60.48$, $p = .00$, a significant

Trial effect F(23, 2185) = 14.80, p = .00, and a significant Group x Trial interaction F(92, 2185) = 5.90, p = .00.

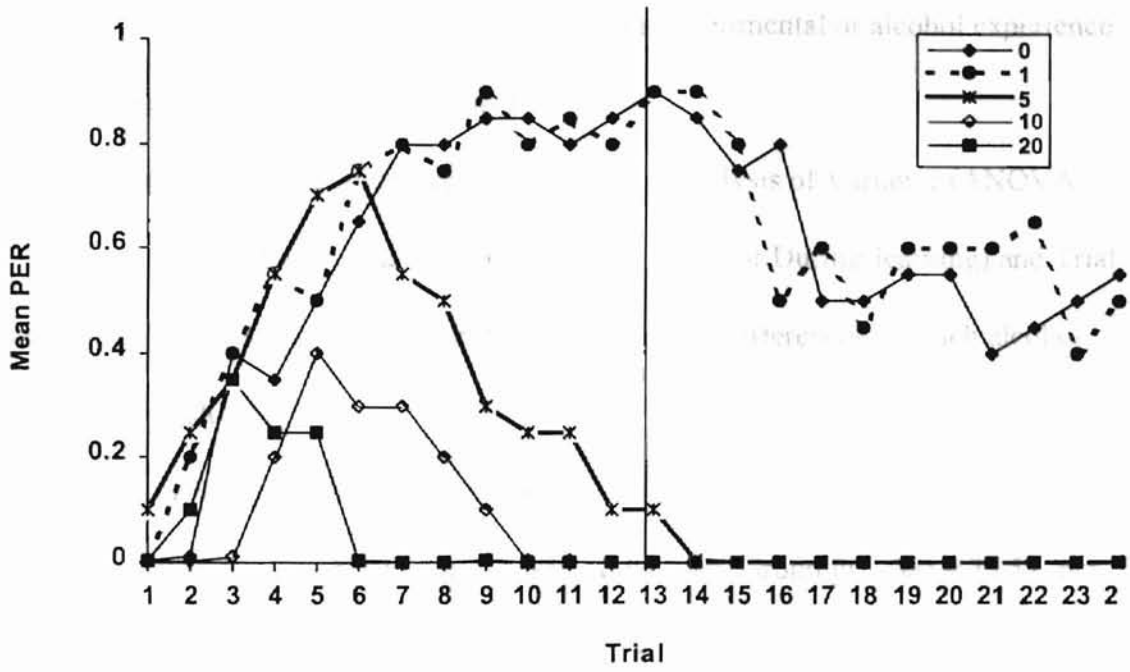

Figure 17. Mean proportion of honey bees responding to the alcohol solutions over the 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13. PER = Proboscis Extension Response.

EXPERIMENT 5: STATISTICAL COMPARISON OF THE EFFECTS OF ALCOHOL CONSUMED PRIOR TO LEARNING TRIALS AND DURING LEARNING TRIALS

Experiment 5 was a statistical comparison of data collected from the honey bees who consumed alcohol solutions prior to the learning trials (Pre-fed) and the data collected from the honey bees who consumed alcohol solutions during the learning trials (During-learning group).

Subjects. The honey bees were collected from the same hive that was used for Experiment 1. However, these honey bees had not been previously collected and were naïve to experimentation. That is, they had no prior experimental or alcohol experience.

Apparatus. Same as Experiment 1.

Procedure. Five separate Repeated Measures Analysis of Variance (ANOVA) procedures were conducted to analyze Condition (Pre-fed or During-learning) and Trial main effect differences and Condition x Trial interaction differences for each alcohol concentration solution.

Results. The Repeated Measures ANOVA revealed significant Condition effects for the 5% Group $F(1, 38) = 12.70$, $p = .000$, and the 10% Group $F(1, 38) = 35.57$, $p = .00$. Trial effects were found for all except the 20% group; 0% Trial effect $F(23, 874) = 15.10$, $p = .00$, 1% Trial effect $F(23, 874) = 11.63$, $p = .00$, 5% Trial effect $F(23, 874) = 9.67$, $p = .00$, and 10% Trial effect $F(23, 874) = 3.77$, $p = .00$. A Condition x Trial interaction effect was found for the 5% group $F(23, 874) = 9.05$, $p = .00$, the 10% group $F(23, 874) = 4.56$, $p = .00$, and the 20% group $F(23, 874) = 4.10$, $p = .00$.

Figure 18 presents the results of the statistical comparison from the Pre-fed and During-learning trial procedures for the 5% alcohol solution.

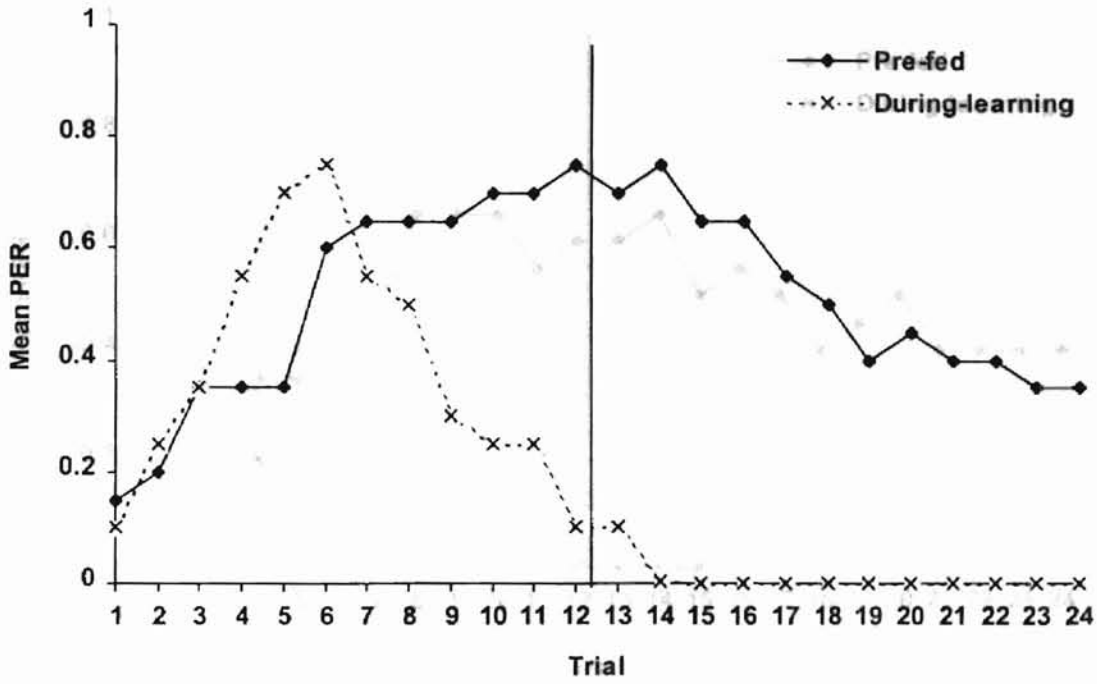

Figure 18. Mean proportion of honey bees responding to the 5% alcohol solutions over the 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13. PER = Proboscis Extension Response.

Figure 19 presents the results of the statistical comparison from the Pre-fed and During-learning trial procedures for the 10% alcohol solution.

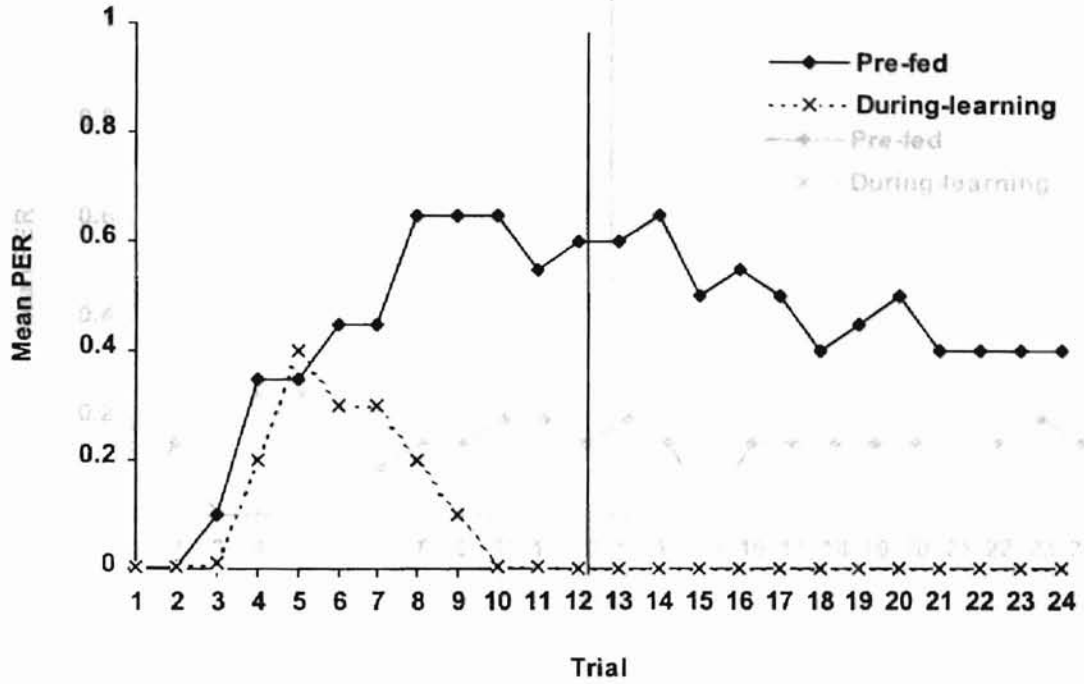

Figure 19. Mean proportion of honey bees responding to the 10% alcohol solutions over the 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13. PER = Proboscis Extension Response.

Figure 20 presents the results of the statistical comparison from the Pre-fed and During-learning trial procedures for the 20% alcohol solution.

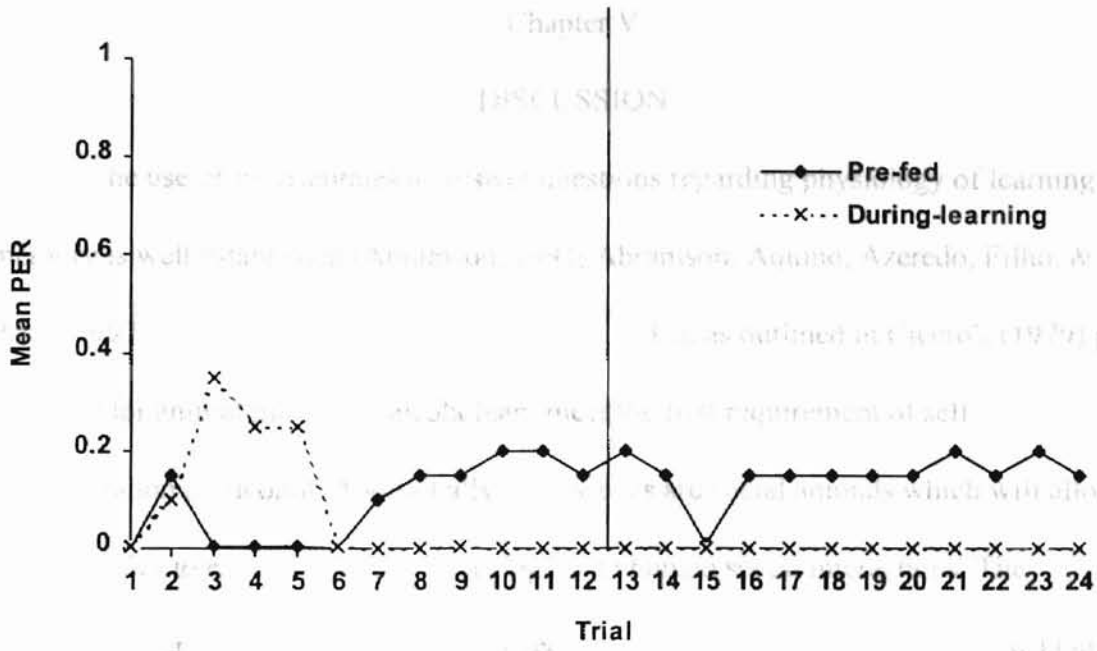

Figure 20. Mean proportion of honey bees responding to the 20% alcohol solutions over the 12 acquisition and 12 extinction trials. The switch from acquisition to extinction occurred on Trial 13. PER = Proboscis Extension Response.

Chapter V

DISCUSSION

The use of invertebrates to answer questions regarding physiology of learning and memory is well established (Abramson, 1994; Abramson, Aquino, Azeredo, Filho, & Price, 1997). European honey bees (*Apis mellifera* L.), as outlined in Cicero's (1979) pre-requisite for animal models of alcoholism, meet the first requirement of self-administration of alcohol. Additionally, honey bees are social animals which will allow future studies to examine behaviors analogous to human social interactions. The preliminary series of experiments conducted in our laboratory demonstrated that 1) under harnessed conditions, honey bees will readily consume 1%, 5%, 10%, and 20% alcohol, 2) sucrose stimulation, as well as sensory bypass, elicits excitation and feeding of 95% alcohol, 3) alcohol consumption decreases locomotion, 4) honey bees readily consume fruit juice and fruit flavored wine, and 5) honey bees will self-administer alcohol at an artificial feeder.

The fruit and wine results support the hypothesis that honey bees will consume fermented fruit in naturalistic environments. Further investigation with a sealed observation hive is necessary to analyze the self-administration of fermented nectar by foraging honey bees. Experiment 10 of the preliminary studies indicated that honey bees will self-administer 5% alcohol solutions in a free flying environment. If the results obtained from an observation hive indicate that honey bees will self-administer fermented fruit when free to choose a nonalcoholic food source and an alcohol solution, questions regarding the effects of alcohol consumption may be analyzed. For instance, the effect the alcohol has upon the individual colony members and the colony as a whole may be

examined. Additionally, examination of the effect of alcohol consumption on the social structure, egg laying by the queen, larvae development, and life span are possible.

The results of Experiment 2 indicate that honey bees consume alcohol solutions regardless of mixing agent, sucrose or water. This provides support for the hypothesis that the honey bees are not consuming the alcohol mixed with sucrose for the caloric benefits. In contrast, it appears that regardless of mixing agent, sucrose or water, the honey bees are consuming the solution for the alcohol. Further study is necessary to determine if the honey bees become addicted to the alcohol.

The current experiments provided evidence that alcohol consumption negatively impacts the ability to acquire new behavior. The Pre-fed and During-learning experiments analyzed consumption of alcohol behavior in the honey bee by measuring consumption in self-administration tests and measuring acquisition of learned behavior. As hypothesized, Experiment 3 and Experiment 4 found that honey bees who consumed alcohol five minutes prior to the learning trials (pre-fed) and honey bees who consumed alcohol as the unconditioned stimulus (during learning) both exhibited impaired learning. That is, alcohol consumption disrupted the acquisition of new behavior by interfering with learning. However, the pre-fed honey bees appeared to acquire new behavior better than the honey bees who consumed the alcohol during the learning trials which suggested that the amount of time between consumption and learning is important to acquisition of new behavior. This was tested in Experiment 5.

Experiment 5 analyzed the variation of time that elapsed between consumption and the commencement of learning. That is, the results of Experiment 3 and Experiment 4 were analyzed in a separate statistical procedure. The results indicated that alcohol

consumed five minutes prior to the learning trials was not as detrimental to the learning process as alcohol that was consumed during the learning trials. Additionally, the concentration of the alcohol solution also influenced the ability to acquire new behavior. That is, the stronger the alcohol concentration of the solution, the less acquisition of learning occurred. However, a ceiling effect for learning was also found. Regardless of time the alcohol was consumed (five minutes prior to the learning trials or during the learning trials), an alcohol concentration level of 20% resulted in no significant difference. The data suggested that the honey bees from both groups were too intoxicated to learn any new behavior.

Previous research has indicated that rapid bioassay methods are successful in detecting adulterated beeswax (Aquino, Abramson, & Payton, 1999). However, future research is necessary to understand how the honey bees metabolize the alcohol. The results may provide a better awareness of the ability to prevent alcohol consumption through the use of alcohol inhibiting drugs.

Disulfiram, also referred to as Antabuse, inhibits the neurological reuptake of serotonin (Alvarado, Contreras, Segovia-Riquelme, & Mardones, 1990). These drugs block the metabolism of alcohol and results in a greater concentration of acetaldehyde in the synapse which has toxic consequences for the individual. When the individual consumes alcohol after taking these drugs, they experience unpleasant physical symptoms such as nausea. The expectation of becoming violently nauseous is thought to deter alcohol consumption. Similarly, several animal studies have shown that opiate antagonists such as Naltrexone will decrease alcohol consumption.(Myers, Borg, & Mossberg, 1986; Volpicelli, Alterman, Hayashida, O'Brien, 1992).

Currently, no animal model exists which meets all of the requirements set forth by Cicero (1979). A mouse model of alcoholism was developed by Rijk, Crabbe, and Rigter (1982) and adheres to the first three requirements set forth by Cicero, 1) the animal must voluntarily self-administer, 2) tolerance to alcohol should be demonstrated following a period of continuous consumption, and 3) dependence on alcohol, as demonstrated by withdrawal symptoms, should be demonstrated following a period of continuous consumption. However, this mouse model has not tested biomedical complications such as liver and brain damage from chronic ethanol vapor inhalation.

A honey bee model meets Cicero's (1979) requirement of oral self-administration. However, more research is needed to determine tolerance, dependence, and biomedical complications associated with chronic alcohol consumption such as brain and neuronal damage. Another advantage of honey bee models of alcohol consumption is the ability to analyze behavioral, in addition to, neurological, biological, and physiological aspects of alcohol consumption. These results, combined with known honey bee genetics information, support the development of an alcohol model using honey bees. This alcohol model may provide insights into effects of alcohol consumption, addiction, and deterrence of alcohol consumption in the human.

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