INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

2021, 34
Heather M. Hill
Editor
Peer-reviewed

Inducing Ethanol Tolerance in Free-Flying Honey Bees (*Apis mellifera* L.)

Laura L. Stephenson,[1] Ana M. Chicas-Mosier,[2] Timothy E. Black,[3] Harrington Wells,[4] and Charles I. Abramson[1*]

[1]Laboratory of Comparative Psychology and Behavioral Biology, Oklahoma State Universsity, Stillwater OK, USA
[2]Department of Entomology and Plant Pathology, Auburn University, Auburn AL, USA
[3]Department of Psychology, Weber State University, Ogden UT, USA
[4]Department of Biology, University of Tulsa, OK, USA

Ethanol dependency affects the health of more than 15 million adults in the United States of America. Honey bees have been used as a model for ethanol studies because of similarities in neural structure to vertebrates and their complex social behaviors. This study compares honey bee free-flight visitation to a food source after exposure to ethanol in aqueous sucrose. Individual bees were followed making six attachment visits to a test-station containing 1M sucrose. After attachment, honey bees were randomly assigned to one of five groups: 0%, 2.5%, 5%, 10% EtOH, or a staged increase in ethanol concentrations (2.5%, 5%, 10%). The results indicated that honey bees tolerate up to 2.5% EtOH without avoidance or altered behavior, and up to 5% EtOH without avoidance but with slower trips. At 10% ethanol, attrition was 75% by the 18th return trip. Bees in the staged increase in concentration group were more likely to return than bees that were offered 10% ethanol in sucrose solution after attachment. The results of this study imply that ethanol-induced tolerance to the effects of ethanol can be achieved in honey bees through incremental increase in EtOH but only in terms of attrition. Other measures of foraging efficiency did not show ethanol-induced tolerance. Understanding how ethanol tolerance develops in bees may provide insight into these processes in humans with minimized ethical considerations.

Keywords: alcohol, ethanol, free-flying, honey bees, tolerance

The 2015 United States of America National Survey on Drug Use and Health found that more than 15 million people over the age of 18 had an Alcohol Use Disorder (AUD) (NIAAA, 2020). According to the Center for Disease Control and Prevention, the average number of deaths attributable to excessive alcohol use is estimated at more than 93,000 annually (CDC-ARDI, 2019). This crisis is more than a health epidemic; in 2010, the misuse of alcohol cost the United States $107 billion from medical payments, lost productivity, and crime (Sacks et al., 2015). As with many drugs, tolerance to alcohol develops after long-term consumption wherein higher quantities are needed to produce the same effect (American Psychiatric Association, 1994). Development of tolerance is influenced by both physiological and behavioral factors (e.g., Mansfield & Cunningham, 1980; Newlin & Thomson, 1990; Tabakoff et al., 1986; Vogel-Sprott, 1979).

There is evidence that the development of alcohol tolerance is the result of neuro-adaptive changes from frequent exposure (Engel et al., 2016). These neural modifications, such as epigenetic changes to gene expression and enzyme activity, can cause behavioral changes not only to those with AUD but also to their offspring (Engel et al., 2016). Neuro-adaptive changes affect more than ethanol (EtOH) tolerance; therefore, establishing an effective and ethical model for the study of alcohol tolerance is necessary and could have dramatic influences on treatments and therapies for alcoholism.

Popular animal models for studies on the effects of alcohol are the rat (*Rattus spp.*) and fruit fly (*Drosophila spp.*), for which valuable data on the molecular, genetic, and neurologic effects of ethanol have been gleaned (Engel et al., 2016; Kerns et al., 2005; Mahadev & Vemuri, 1998; Moore et al., 1998). Nevertheless, the fruit fly model has significant limitations when it comes to studying tolerance.

Please send correspondence to Charles I. Abramson. Email: Charles.abramson@okstate.edu
http://doi.org/ 10.46867/ijcp.2021.34.00.03

Fruit fly tolerance is developed by exposing flies to ethanol vapors and, after a set interval, counting the number of flies unable to upright themselves. After a set intertrial interval, the method is repeated (Cowmeadow et al., 2005, Cowmeadow et al., 2006, Krishnan et al., 2012). Tolerance is measured by the decreasing number of flies that cannot upright themselves after bouts of EtOH exposure (Engel et al., 2016; Troutwine et al., 2016; Wolf et al., 2002). This is an important model for studying the molecular underpinnings of tolerance but limited in being able to reflect behavioral aspects related to work performance and social interactions (e.g., Tabakoff et al., 1986). However, a positive aspect of the fruit fly model is that the flies are exposed to ethanol on a regular basis in nature and thus, like humans, should not be facing an unnatural challenge.

Like fruit flies, honey bees are naturally exposed to EtOH and have a fully sequenced genome (Bozic et al., 2007; Solignac et al., 2004; Weinstock et al., 2006). Yeast can ferment nectar and inhabits 30-50% of insect-pollinated flowers (Alyokhin et al., 2001). Additionally, yeast occurrence in nectar varies widely inter- and intraspecifically (Pozo et al., 2009). The implications of this are complex when attempting to model intoxication effects on honey bee foragers and are compounded by the fact that yeast are facultative anaerobes and thus may not be producing much EtOH in floral nectar (Kurtzman & Fell, 2006; Piskur, 2014; Visser et al., 1990). Nevertheless, there are recorded cases where EtOH levels in nectar are significant enough to produce noticeable effects on forager behavior (Schaeffer et al., 2017), and honey bees show no natural aversion to EtOH in controlled studies (Abramson et al., 2000). Further, impairment does not lead to conditioned taste aversion (Varnon et al., 2018). That is, associative conditioning does not lead to avoidance of an initially neutral stimulus (odor or taste) that is associated with, but is not the cause of, the detrimental behavioral condition that follows EtOH exposure.

In terms of behavior, honey bees must be able to navigate to and from the hive, maintain social relationships with hive mates, and maintain normal locomotion after ethanol exposure (Abramson et al., 2000; Abramson et al., 2003; Abramson, Kandolf, et al., 2004; Abramson et al., 2005; Mixson et al., 2010; Bozic et al., 2007; Heberlein et al., 2004). Ethanol does affect task performance in both free-flying and harnessed bees. For example, foragers become nonselective with respect to differing rewards associated with blue versus white flowers (Abramson et al., 2005; Chicas-Mosier et al., 2017). Correspondingly, when administered EtOH, harnessed bees show reduced discriminatory ability (Abramson et al., 2000; Mustard et al. 2008). However, as observed in human alcoholism (Weissenborn & Duka, 2000), task efficiency can be highly influenced by context. Flower color constancy by foragers when presented yellow versus blue flowers in a patch is unaffected by EtOH (Abramson et al., 2005). In fact, ability to navigate to the hive fails first. A context effect was also noted by Abramson et al. (2015) when looking at reversal learning under the influence of EtOH. Quantity in conjunction with timing of EtOH consumption with respect to initial leaning produces very different learning curves (Abramson et al., 2015), which may explain the inconsistent results of reversal learning following exposure in the literature (Abramson et al., 2015; Jedema et al., 2011; Wright et al., 2013).

Honey bees are eusocial, and this produces an important dual-outcome of disrupted behavioral tasks: individual and interactive. Studies indicate that exposure to ethanol disrupted several types of honey bee social behavior within the hive. Foragers that consumed nectar containing ethanol reduced waggle dance activity in bees returning to the hive (Bozic et al., 2006). Under both the language (von Frisch 1967) and the odor-transfer models (Wenner & Wells, 1990; Wenner et al., 1969), this reduces recruitment and negatively impacts the colony. In addition to waggle dance impacts, intoxicated foragers, upon returning to the hive, perform the tremble dance more often, which is a reflection of trouble unloading their nectar to hive mates (Bozic et al., 2006; Seeley, 1992). Intoxicated foragers also spend more time performing self-cleaning rituals (Bozic et al., 2006). These behavioral changes may reflect effects on the central nervous system similar to observed effects of food poisoning with sublethal doses of insecticides (Karahan et al., 2015).

The focus of this study was to examine whether ethanol-induced ethanol resistance (ethanol tolerance) can be shown in free-flying honey bees. In honey bees, both baseline ethanol doses that produce no noticeable effects and incapacitation doses of EtOH are well documented (Abramson et al., 2000; Bozic et al., 2007; Maze et al., 2006). We used foraging (work task) as the ethanol-induced ethanol tolerance model rather than using a passive task, as the former is a more environmentally relevant metric. Under this model, the effects of ethanol intoxication on task performance are mitigated by prolonged consumption of EtOH.

The key measures of foraging efficiency are round trip time from the feeding-station to the hive and back (return time, RT), nectar load (μL), and nectar quality (sugar content). Also known to affect foraging net-energy gain is the time it takes a bee to consume a specific volume of nectar (drink time, DT). Consumption rate (μL/sec) depends on nectar viscosity, which is a function of sucrose concentration and temperature. Foraging efficiency is thought to be key to species survival (Stephens & Krebs 1986), which is also true for honey bees (Kim et al. 2011).

Method

Experiments 1 and 2: Individual Foragers

Apis mellifera L. foragers were collected as needed from a feeding station located 15 m from the honey bee colony. The feeding station's feeder was filled once-daily throughout the summer with 1L of 0.5M sucrose solution. Only foragers (>21 days old) were used for this experiment in an effort to minimize age-related variables (Robinson, 1985). Foragers used in the experiment were marked with Testors® paint on the thorax for individual bee identification (White, 1145TT; Blue, 1162TT; Green, 1171TT; and Orange, 1126TT, Vernon Hills, IL USA).

Approximately 10 m from the feeding station, a test station was established (60 mm square table). On the test station was a platform that consisted of a 58-mm diameter gray circular lid with a 40-mm diameter yellow-laminated disk centered on it. After each return trip, the testing platform was rinsed and dried to remove the prior solution. Each honey bee was sacrificed after participating in the study to prevent interference with subsequent bees involved in the experiment.

Experiment 1

Bees were randomly assigned to one of four experimental treatments (12 bees per treatment). Prior to an experimental treatment, the subject bees were trained to the test platform. This was accomplished by simply carrying the bees from the feeding station to the test platform while they were drinking the 0.5M sucrose reward. Bees have been known to have one-trial learning of the new location in this type of training (Amaya-Márquez et al, 2017). Prior to an experiment, each bee was observed for six round trips from the hive and rewarded each time with a 50-μL droplet of 1M sucrose solution in the center of the yellow disk (0% EtOH). This assured that each bee was "crop attached" to the testing station and provided bees with uniform navigational background. The sucrose reward was dispensed using a repeater-pipette.

Each of the four experimental treatments consisted of 18 appetitive-reward trials followed by an extinction test (Table 1). Each trial consisted of one return trip to the test platform from the colony. Treatments all offered bees a 50-μL droplet of 1M sucrose in the center of the yellow disk as an appetitive reward, but differed in EtOH dosage: Treatment 1 = 0% EtOH, Treatment 2 = 2.5% EtOH, Treatment 3 = 5% EtOH, and Treatment 4 = 10% EtOH.

Table 1

Experiments 1 and 2 Treatments

Treatment	Trials 1-6	Trials 7-12	Trials 13-18	Extinction
Experiment 1				
0%	0%	0%	0%	Water
2.5%	2.5%	2.5%	2.5%	Water
5%	5%	5%	5%	Water
10%	10%	10%	10%	Water
Experiment 2				
Staged	2.5%	5%	10%	Water

Note. EtOH dosage given in 10% sucrose solutions. Experiment 1: Treatment 1 = 0% EtOH, Treatment 2 = 2.5% EtOH, Treatment 3 = 5% EtOH, and Treatment 4 = 10% EtOH.

Our earlier controlled studies demonstrated that honey bees show no natural aversion to EtOH even with percentages higher than used in this study (Abramson et al., 2000). Further, impairment caused by EtOH consumption does not lead to conditioned taste-aversion in honey bees (Abramson, Kandolf, et al., 2004; Varnon et al., 2018). However, EtOH consumption has been demonstrated to affect both learning and motor coordination of honey bees (Abramson et al., 2005). Thus, the time it took for the bee to return to the testing station (RT) and the time spent drinking (DT) were recorded for each bee throughout the study as measures of foraging efficiency. Nectar volume (50 μL) and quality (1M sucrose) did not vary among treatments. Honey bee mean crop capacity is close to 60 μL (Wells et al., 1981). Past work has shown that all bees can consume 50 μL and will be satisfied with that reward and return to the hive. Finally, complete failure to return to the feeding station (number returning: NR) was recorded as attrition data. Prior work has also shown that the frequency of honey bees failing to return to a feeding station is alcohol dose-related, and, when they fail to return, they often do not return to the hive (Abramson et al., 2005). In harnessed bee experiments, higher doses of ethanol cause bees to lose their proboscis extension reflex as well as discriminatory abilities (Abramson et al., 2003). Like human ethanol-consumption induced ethanol tolerance, we looked for improvement in RT and DT efficiency over trials. The data were analyzed using a repeated-measures MANOVA (SAS-JMP, Cary NC) with factors EtOH (treatment), trial, and the interaction EtOH × Trial for the variables RT and DT separately.

Immediately after the completion of 18 test trials per treatment, each bee was presented with a behavioral extinction trial. In addition to the training target, a second gray target with no yellow disk (unconditioned stimulus, CS-) was placed on the table within 16 cm of the training target (conditioned stimulus, CS+). The targets were counterbalanced by side to remove locational bias. Both targets were topped with 50 μL of distilled water. The extinction trial was 600 s. The number of times that a bee touched down on each target was recorded for each sequential 30-s interval. Extinction data were analyzed via an ANOVA to determine differences between the cumulative mean number of visits to either the CS+ or CS- by concentration; *t*-tests were used to compare the mean visits to the CS+ and CS- within concentration.

Experiment 2 was similar to Experiment 1 but had only one experimental treatment, which was a staged EtOH increase after each set of 6 trials (Table 1). A crop-attachment phase was completed as in Experiment 1. Following crop-attachment, the experimental phases were: 1M sucrose with 2.5% EtOH for Trials 1 to 6, 1M sucrose with 5% EtOH for Trials 7 to 12, and 1M sucrose with 10% EtOH for Trials 13 to 18. Like in Experiment 1, an extinction test was performed after the 18th trial. We analyzed the effects of Experiment 2 by adding the data to the Experiment 1's data and re-running the repeated-measures MANOVA (SAS-JMP) with factors treatment (EtOH), trial, and the Treatment × Trial interaction in order to see if the pre-treatment altered the significant factors or interactions. The variables RT and DT were tested separately.

Attrition Data (NR) for Experiments 1 and 2 were analyzed using a χ^2 test for difference among treatments (Sokal & Rohlf, 1995). Specifically, we were interested in the first six trials with the 10% EtOH reward and the last six trials of the staged, which was the set with 10% EtOH reward.

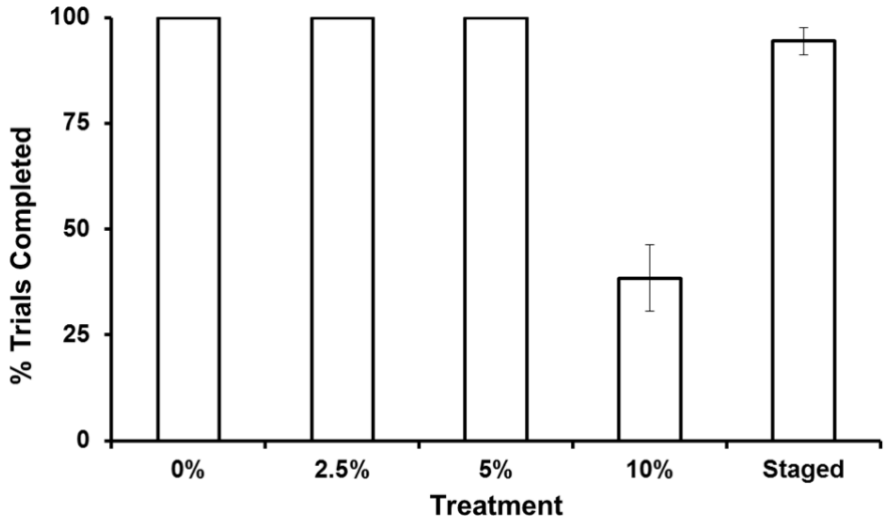
Results

Experiment 1 and 2: Attrition

Considering all bees in Experiment 1 and 2 (N=60), 75% of bees completed all 18 appetitive-reward trials and the extinction test. In fact, all bees of Treatment 1 (0% EtOH), Treatment 2 (2.5% EtOH), and Treatment 3 (5% EtOH) completed the 18 experimental trials and the extinction test. In contrast, not a single bee in Treatment 4 (10% EtOH) completed all 18 experimental trials, and the average number of return trips per bee was just 7 of 18 (Figure 1). In Experiment 2, in which bees received a staged EtOH treatment, 75% of bees completed all 18 appetitive-reward trials and the extinction test (Figure 1). The 10% EtOH bees (Treatment 4) returned significantly fewer times than the remaining treatments of Experiment 1 and the data from Experiment 2 combined ($\chi_1^2 = 6.00$, $p < .025$). As few return trips were made by the 10% EtOH treatment bees, this group of bees was eliminated from the further analysis (RT, DT, Extinction Test).

Figure 1

Forger Attrition



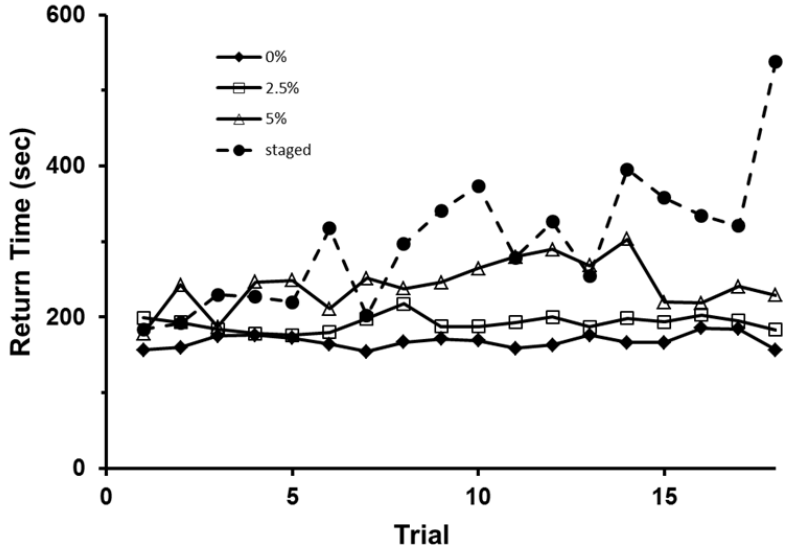
Note. Attrition of foragers is depicted by the average percentage of trials (N = 18 trials) completed by bees (N = 12 bees) in each experimental treatment (mean ± standard error). Experiment 1: Treatment 1 = 0% EtOH, Treatment 2 = 2.5% EtOH, Treatment 3 = 5% EtOH, and Treatment 4 = 10% EtOH. Experiment 2: Staged EtOH with Trials 1-6 2.5% EtOH, Trials 7-12 5% EtOH, and Trials 13-18 10% EtOH.

Experiment 1 and 2: RT and DT

When considering RT for Treatments 1, 2, and 3 (Figure 2), only EtOH was a significant factor, $F(2, 33) = 3.49$, $p = .04$. Bees given 5% EtOH took longer to return back to the experimental area. Neither trial, ($F(17, 17) = 0.85$, $p = .66$, nor EtOH × Trial were significant, $F(34, 34) = 0.98$, $p = .52$. For the same set of treatments, when considering DT (Figure 3), there were no significant factors or interactions, $F(2, 33) = 1.61$, $p = .22$; $F(17, 17) = 0.78$, $p = .69$; $F(34, 34) = 0.96$, $p = .54$.

Figure 2

Forger Round Trip Time



Note. The average time taken by bees to return to the test-station from going to the colony is given for Experiment 2 (staged EtOH dosage) and for Experiment 1 Treatments 1, 2, and 3. Experiment 1: Treatment 1 = 0% EtOH, Treatment 2 = 2.5% EtOH, and Treatment 3 = 5% EtOH. Experiment 2: Staged EtOH with Trials 1-6 2.5% EtOH, Trials 7-12 5% EtOH, and Trials 13-18 10% EtOH. Treatment 4 of Experiment 1 (10% EtOH) is omitted because so few bees were returning.

Experiment 1 and 2 Data Combined

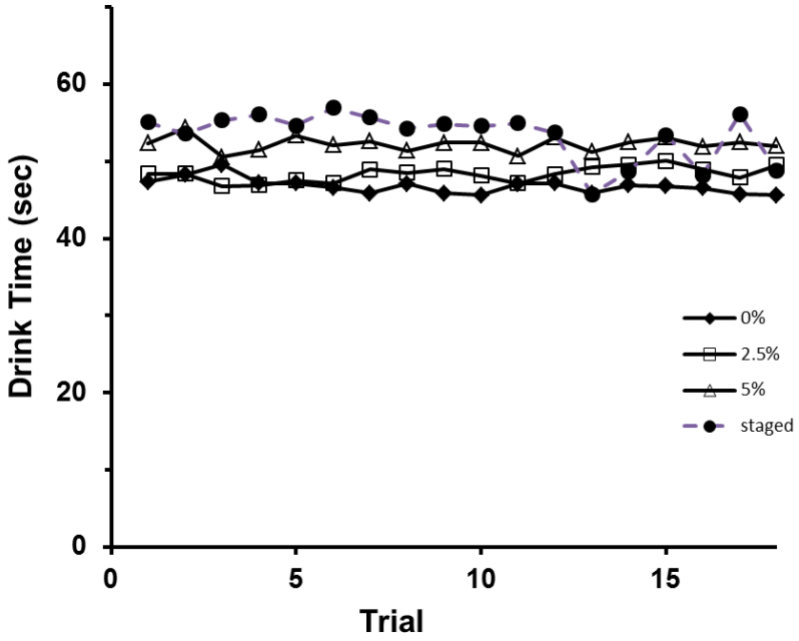
By combining the data from Experiments 1 and 2, we were able to test for novel effects introduced from Experiment 2, which was the staged increase in EtOH in the 1M sucrose reward. Again, Treatment 4 data of Experiment 1 were omitted because the bees given 10% EtOH made so few return trips, with attrition occurring in most bees. In particular, expected from the EtOH-induced EtOH-tolerance model is decreased attrition, and reduced RT and DT because these changes represent increased foraging efficiency.

Considering RT, not only was EtOH a significant factor, $F(3, 41) = 5.16$, $p < .01$, as seen when analyzing the data from Experiment 1 but also significant were trial, $F(17, 25) = 3.00$, $p = .01$, and the Trial × EtOH interaction, $F(51, 75) = 2.00$, $p < .01$. RT increased over the set of trials in the staged treatment (Figure 2) and was particularly noticeable in the last trial of the 10% EtOH stage. If ethanol-induced tolerance had occurred, we would not have expected the sharp rise in RT on Trial 18.

Like the analysis of Experiment 1 DT data, EtOH, $F(3, 41) = 2.28$, $p = .09$, trial, $F(17, 25) = 1.14$, $p = .37$, and the EtOH × Trial interaction, $F(51, 75) = 1.27$, $p = .17$, were not significant. EtOH dosage did not affect how long it took returning foragers to consume the reward, even in the staged EtOH treatment (Figure 3).

Figure 3

Necter Consumption Rate



Note. The average time bees took consuming the test station reward for each trial is given for Experiment 2 (staged EtOH dosage) and for Experiment 1 Treatments 1, 2, and 3. Experiment 1: Treatment 1 = 0% EtOH, Treatment 2 = 2.5% EtOH, and Treatment 3 = 5% EtOH. Experiment 2: Staged EtOH with Trials 1-6 2.5% EtOH, Trials 7-12 5% EtOH, and Trials 13-18 10% EtOH. Treatment 4 of Experiment 1 (10% EtOH) is omitted because so few bees were returning.

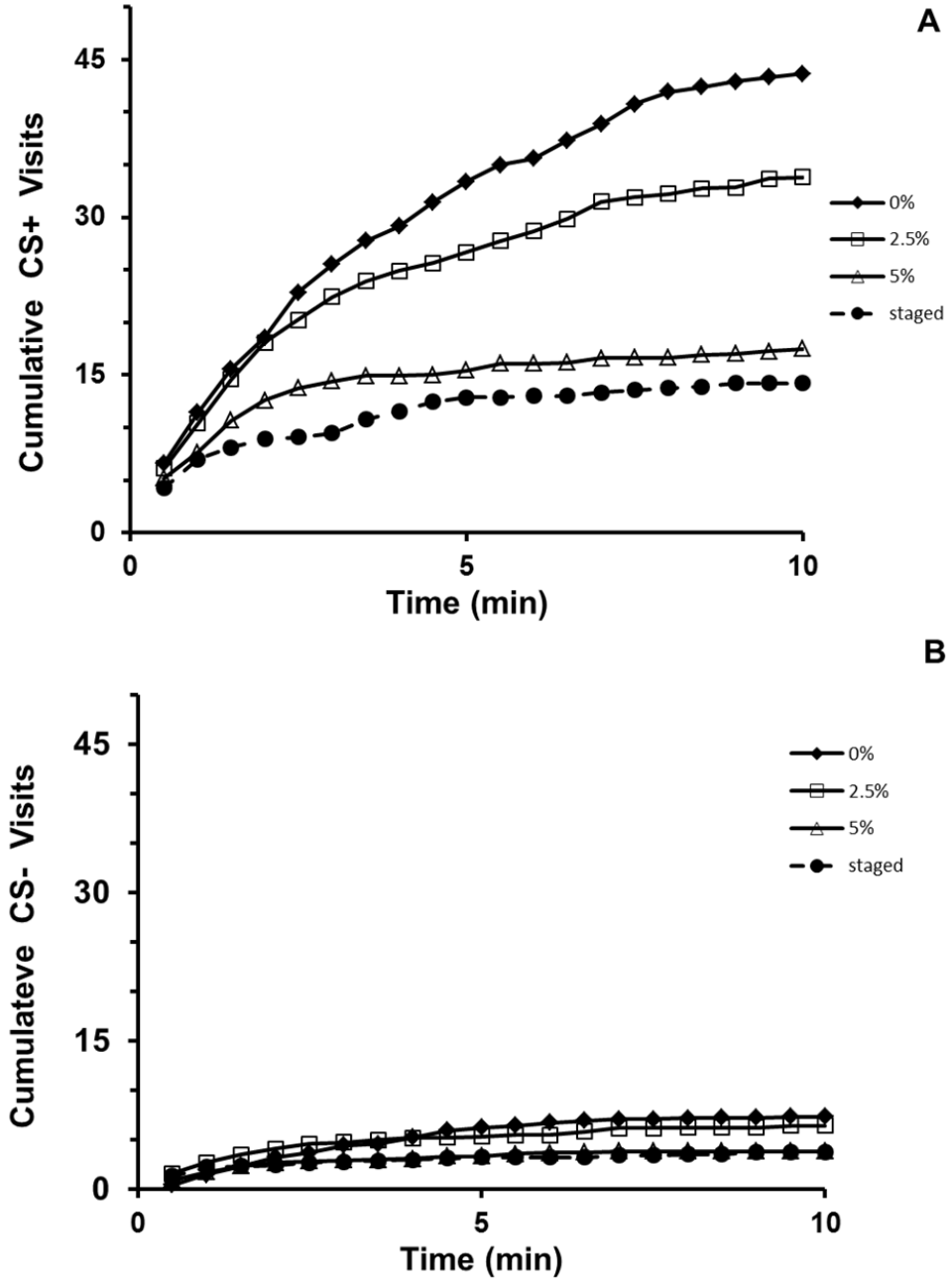
Experiment 1 and 2: Extinction Test

After the 18 appetitive-reward trials, extinction was measured in terms of the number of returns to the platform when the platform offered only water. None of the bees given 10% EtOH completed the 18 trials, and only four bees were sporadically returning when the experiment went to the extinction test. Thus, analysis of the extinction test did not include the 10% EtOH treatment.

Significant differences existed between treatments in the cumulative number of CS+ visits in the extinction test (ANOVA: $F(3, 41) = 19.91$, $p < .01$). However, the 0% and 2.5% EtOH groups did not differ significantly, $F(1, 22) = 3.44$, $p = .08$. The same situation was observed in number of CS- visits (Figure 4). Significant differences existed between treatments in the cumulative number of CS- visits in the extinction test (ANOVA: $F(3, 41) = 3.60$, $p = .02$). Further, the 0% and 2.5% EtOH groups did not differ significantly, $F(1, 22) = 0.38$, $p = .54$. In a comparison of the number of visits to CS+ and CS-, all treatment groups visited the CS+ significantly more often (0% EtOH: $t(21) = 4.7$, $p < .01$; 2.5% EtOH: $t(21) = 3.67$, $p < .01$; 5% EtOH: $t(21) = 2.26$, $p = .02$; Staged EtOH: $t(22) = 2.11$, $p = .02$).

Figure 4

Extinction Test



Note. Cumulative number of visits in the extinction test to the CS+ target (A) and to the CS- target (B) over time shown as average per forager. Staged bees ended the 18 trials of the experiment with 10% EtOH in the reward and that is seen to carry over to the behavior in the extinction test.

Discussion

The results of the study show that bees are able to remain functional in doing tasks when continuously administered EtOH up to about a 5% solution. Like the *Drosophila* studies in which there is an infusion rate where flies can no longer upright themselves (e.g., Cowmeadow et al., 2005; Engel et al., 2016; Wolf et al., 2002), there is a point when honey bee foragers become nonfunctional. In our study, it became apparent at 10% EtOH dosage and was clearly manifested in the attrition rate so much so that our measures of functionality such as RT became meaningless.

EtOH levels through 5% dosage over an extended period of time did not affect basic forager functionality. All foragers made the 18 round trips to the colony even when consuming the 5% EtOH solution. This supports the notion that honey bees are exposed to EtOH in nectar as a result of yeasts, which are often found in this floral reward (Pozo et al., 2009; Schaeffer et al., 2017). Although the yeast inhabiting nectars are facultative anaerobes (Piskur, 2014), conditions must be conducive for EtOH production a significant amount of the time.

We were able to go beyond basic functionality and also look at performance as measures of EtOH tolerance, which turned out to be task specific. RT for foragers given a 2.5% EtOH dosage was indistinguishable from the control group, which had no EtOH in the sucrose solution reward. However, bees given a 5% EtOH dosage took longer to return, but showed no attrition from the EtOH. Nevertheless, DT was unaffected by the 5% EtOH dosage. Thus, ethanol's effect is task specific when examining efficiency. However, we did not observe ethanol induced tolerance. Trial was not a significant factor for either RT or DT. We would have expected these times to decline in the latter trials if EtOH induced tolerance occurred. As an example, Appendix A shows a honey bee receiving 10% ethanol.

The staged increase in EtOH dosage was designed to indicate whether EtOH tolerance could be achieved by building the EtOH dosage consumed over time. Attrition was significantly slower when the 10% EtOH stage (last 6 trials) was compared to only the first 6 trials of 10% EtOH nonstaged experiment. That is, bees that had been consuming EtOH (staged) were able to continue to forage (task) when given 10% EtOH compared to bees that had not been consuming EtOH (first trials of 10% EtOH group). This result fits the ethanol-induced ethanol-tolerance model. The results cannot be reconciled by ethanol mitigation of taste aversion. Honey bees do not display aversion to EtOH, including EtOH concentrations higher than used in this study (Abramson et al., 2000). Also relevant is that ethanol consumption does not lead to conditioned EtOH taste-aversion by honey bees (Abramson, Kandolof, et al., 2004; Varnon et al., 2018). In this respect, honey bees mirror alcoholic humans. Recent work shows that honey bee foragers show higher intoxication resistance to alcohol than nurses (Miler et al., 2020), and this opens the question whether ethanol-induced ethanol resistance is isolated to the foraging cast.

Extinction-trial visits to the CS- target significantly differed among treatments 0%, 2.5%, 5% EtOH and staged, although differences were small. Bees had not been condition to visit the CS- target, and EtOH dosing did little to impair their ability to discriminate the CS+ from the CS-.

In contrast, extinction trial visits to the CS+ target were greatly affected by EtOH dose. The higher the ethanol dose, the fewer times bees touched down on the CS+ target. Ethanol consumption results in foragers losing interest in the now nonrewarding station and may reflect the intolerance seen in humans when drinks are cutoff. Note that the extinction trials occurred after the 18 appetitive-reward trials, and, thus, bees in the staged experiment had just completed 6 trials where they received 10% EtOH reward. Nevertheless, there was not a significant difference between the staged and 5% EtOH groups in total number of landings on the CS+ target, $F(1, 19) = 1.56$, $p = .23$, which may be ethanol-induced tolerance in the staged bees.

Behavioral differences may be indicative of the causal mechanisms of tolerance development. Past research has shown that genes encoding for proteins, such as dynamin (Krishnan et al., 2012), the BK channel protein (Cowmeadow et al., 2005; Cowmeadow et al., 2006), and Heat Shock Protein 70 (Hranitz et al., 2010), are all implicated in the behavioral changes associated with repeated exposure to ethanol.

Our previous work has shown that ethanol disrupts learning (Abramson et al., 2000), interferes with social behavior (Abramson et al., 2003), and increases both pain thresholds and aggression (Abramson, Place, et al., 2004; Giannoni-Guzmán et al., 2014). We have also shown that ethanol influences heat shock proteins (Hranitz et al., 2010). Advancement of the honey bee model for ethanol studies is important to efforts focused on combating alcohol abuse. Recent studies have discovered that building tolerance to alcohol causes neurological adaptations to occur in rats (Engel et al., 2016). Honey bees allow us to study the effects of these neuroadaptations to tolerance in a cost-effective manner and without the complicating factors of past traumas or environmental confounds, which prevent true experimental conditions on human participants. In short, further study of the effects of ethanol tolerance in honey bees can assist in improving long-term outcomes of alcohol-abuse treatment programs.

Acknowledgments

This project was funded by National Science Foundation's Research Experiences for Undergraduates (grant number: 2016-1560389 and 1950805), Partnerships for International Research Experiences (grant number: OISE 1545803), and a National Science Foundation Graduate Research Fellowship (NSF-GRF 1144467).

Data Availability: Data will be available at the following doi: 10.6084/m9.figshare.12896840 upon acceptance and publication.

References

Abramson, C. I., Craig, D. P. A., Varnon, C. A., & Wells, H. (2015). The effect of ethanol on reversal learning in honey bees (*Apis mellifera anatolica*): Response inhibition in a social insect model. *Alcohol*, *49*(3), 245–258. https://doi.org/10.1016/j.alcohol.2015.02.005

Abramson, C. I., Fellows, G. W., Browne, B. L., Lawson, A., & Ortiz, R. A. (2003). Development of an ethanol model using social insects: II. Effect of Antabuse on consumatory responses and learned behavior of the honey bee (*Apis mellifera* L.). *Psychological Reports*, *92*(2), 365–378. https://doi.org/10.2466/pr0.2003.92.2.365

Abramson, C. I., Kandolf, A., Sheridan, A., Donohue, D., Bozic, J., Meyers, J., & Benbasset, D. (2004). Development of an ethanol model using social insects: III. Preference for ethanol solutions. *Pyschological Reports, 94*(1), 227–239. https://doi.org/10.2466%2Fpr0.94.1.227-239

Abramson, C. I., Place, A. J., Aquino, I. S., & Fernandez, A. (2004). Development of an ethanol model using social insects: IV. Influence of ethanol on the aggression of Africanized honey bees (*Apis mellifera* L.). *Psychological Reports*, *94*(3),1107–1115. https://doi.org/10.2466/pr0.94.3c.1107-1115

Abramson, C. I., Sanderson, C,. Painter, J., Barnett, S., & Wells, H. (2005). Development of an ethanol model using social insects: V. Honeybee foraging decisions under the influence of alcohol. *Alcohol*, *36*(3), 187–193. https://doi.org/10.1016/j.alcohol.2005.09.001

Abramson, C. I., Stone, S. M., Ortez, R. A., Luccardi, A., Vann, K. L., Hanig, K. D., & Rice, J. (2006). The development of an ethanol model using social insects I: Behavior studies of the honey bee (*Apis mellifera* L.). *Alcoholism: Clinical and Experimental Research, 24*(8), 1153–1166. https://doi.org/10.1111/j.1530-0277.2000.tb02078.x

Alyokhin, A. V., Mille, C., Messing, R. H., & Duan, J. J. (2001). Selection of pupation habitats by oriental fruit fly larvae in the laboratory. *Journal of Insect Behavior*, *14*(1), 57–67. https://doi.org/10.1023/A:1007849629409

Amaya-Márquez, M., Abramson, C. I., & Wells, H. (2017). Use of flower color-cue memory by honey bee foragers continues when rewards no longer differ between flower colors. *Journal of Insect Behavior, 30*, 728–740. https://doi.org/10.1007/s10905-017-9653-x

American Psychiatric Association. (1994). Diagnostic and Statistical Manual of Mental Disorders 4th edition.

Bozic, J., Abramson, C. I., & Bedencic, M. (2006). Reduced ability of ethanol drinkers for social communication in honeybees (*Apis mellifera carnica* Poll.). *Alcohol*, *38*(3), 179–183. http://doi.org/10.1016/j.alcohol.2006.01.005

Bozic, J., DiCesare, J., Wells, H., & Abramson, C. I. (2007). Ethanol levels in honeybee hemolymph resulting from alcohol ingestion. *Alcohol*, *41*(4), 281–284. https://doi.org/10.1016/j.alcohol.2007.04.003

Center for Disease Control and Prevention. (2019). *Alcohol and Public Health: Alcohol Related Disease Impact (CDC-ARDI)*. https://nccd.cdc.gov/DPH_ARDI/default/default.aspx

Chicas-Mosier, A. M., Cooper, B. A., Melendez, A. M., Pérez, M., Oskay, D., & Abramson, C. I. (2017). The effects of ingested aqueous aluminum on floral fidelity and foraging strategy in honey bees (*Apis mellifera*). *Ecotoxicology and Environmental Safety*, *143*, 80–86. https://doi.org/10.1016/j.ecoenv.2017.05.008

Cowmeadow, R. B., Krishnan, H. R., & Atkinson, N. S. (2005). The slowpoke gene is necessary for rapid ethanol tolerance in *Drosophila*. *Alcoholism: Clinical and Experimental Research*, *29*(10), 1777–1786. https://doi.org/10.1097/01.alc.0000183232.56788.62

Cowmeadow, R. B., Krishnan, H. R., Ghezzi, A., Al' Hasan, Y. M., Wang, Y. Z., & Atkinson, N. S. (2006). Ethanol tolerance caused by slowpoke induction in *Drosophila*. *Alcoholism: Clinical and Experimental Research, 30*(5), 745–753. https://doi.org/10.1111/j.1530-0277.2006.00087.x

Engel, G. L,. Marella, S., Kaun, K. R., Wu, J., Adhikari, P., Kong, E. C., & Wolf, F. W. (2016). Sir2/Sirt1 links acute inebriation to presynaptic changes and the development of alcohol tolerance, preference, and reward. *Journal of Neuroscience*, *36*(19), 5241–5251. https://doi.org/10.1523/jneurosci.0499-16.2016

Giannoni-Guzmán, M. A., Giray, T., Agosto-Rivera, J. L., Stevison, B. K., Freeman, B., Ricci, P., Brown, E. A., & Abramson, C. I. (2014). Ethanol-induced effects on sting extension response and punishment learning in the western honey bee (*Apis mellifera*). *PLoS One*, *9*(7), 1–8. https://doi.org/10.1371/journal.pone.0100894

Heberlein, U., Wolf, F. W., Rothenfluh, A., & Guarnieri, D. J. (2004). Molecular genetic analysis of ethanol intoxication in *Drosophila melanogaster*. *Integrative and Comparative Biology*, *44*(4), 269–274. https://doi.org/10.1093/icb/44.4.269

Hranitz, J. M., Abramson, C. I., & Carter, R. P. (2010). Ethanol increases HSP70 concentrations in honeybee (*Apis mellifera* L.) brain tissue. *Alcohol*, *44*(3), 275–282. https://doi.org/10.1016/j.alcohol.2010.02.003

Jedema, H. P., Carter, M. D., Dugan, B. P., Gurnsey, K., Olsen, A. S., & Bradberry, C. W. (2011). The acute impact of ethanol on cognitive performance in rhesus macaques. *Cerebral Cortex, 21*(8), 1783–1791. https://doi.org/10.1093/cercor/bhq244

Karahan, A., Cakmak, I., Hranitz, J. Karaca, I., & Wells, H. (2015). Sublethal imidacloprid effects on honey bee flower choices when foraging. *Ecotoxicology*, *24*(9), 2017–2025. https://doi.org/10.1007/s10646-015-1537-2

Kerns, R. T., Ravindranathan, A., Hassan, S., Cage, M. P., York, T., Sikela, J. M., Williams, R. W., & Miles, M. F. (2005). Ethanol-responsive brain region expression networks: Implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. *Journal of Neuroscience 25*(9), 2255–2266. https://doi.org/10.1523/jneurosci.4372-04.2005

Kim, W., Gilet, T., & Bush, J. W. M. (2011). Optimal concentrations in nectar feeding. *Proceedings of National Academy of Sciences, 108*(40), 16618–16621. https://doi.org/10.1073/pnas.1108642108

Krishnan, H. R., Al-Hasan, Y. M., Pohl, J. B., Ghezzi, A., & Atkinson, N. S. (2012). A role for dynamin in triggering ethanol tolerance. *Alcoholism Clinical and Experimental Research*, *36*(1), 24–34. https://doi.org/10.1111/j.1530-0277.2011.01587.x

Kurtzman, C. P., & Fell, J. W. (2006). Yeasts systematics and phylogeny—Implication of molecular identification methods for studies in ecology. In *Biodiversity and ecophysiology of yeasts* (pp. 11-30). Springer.

Mahadev, K., & Vemuri, M. C. (1998). Effect of ethanol on chromatin and nonhistone nuclear proteins in rat brain. *Neurochemical Research, 23*(9), 1179–1184. https://doi.org/10.1023/a:1020778018149

Mansfield, J. G., & Cunningham, C. L. (1980). Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. *Journal of Comparative and Physiological Psychology*, *94*(5), 962–969. http://doi.org/10.1037/h0077824

Maze, I. S., Wright, G. A., & Mustard, J. A. (2006). Acute ethanol ingestion produces dose-dependent effects on motor behavior in the honey bee (*Apis mellifera*). *Journal of Insect Physiology*, *52*(11-12), 1243–1253. http://doi.org/10.1016/j.inphys.2006.09.006

Miler, K., Stec, D., Kaminska, A., Pardyak, L., & Kuszewska, K. (2020). Alcohol intoxication resistance and alcohol dehydrogenase levels differ between the honeybee castes. *Apidologie*, *52*, 230–241. https://doi.org/10.1007/s13592-020-00812-y

Mixson, T. A., Abramson, C. I., & Bozic, J. (2010). The behavior and social communication of honey bees (*Apis mellifera carnica* Poll.) under the influence of alcohol. *Psychological Reports*, *106*(3), 701–717. https://doi.org/10.2466/pr0.106.3.701-717

Moore, M. S., DeZazzo, J., Luk, A. Y., Tully, T., Singh, C. M., & Heberlein, U. (1998). Ethanol intoxication in drosophila: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell*, *93*(6), 997–1007. https://doi.org/10.1016/s0092-8674(00)81205-2

Mustard, J. A., Wright, G. A., Edgar, E. A., Mazade, R. E., Wu, C., & Lillvis, J. L. (2008). Acute ethanol ingestion impairs appetitive olfactory learning and odor discrimination in the honey bee. *Neurobiology of Learning and Memory*, *90*(4), 633–643. https://doi.org/10.1016/j.nlm.2008.07.017

National Institute on Alcohol Abuse and Alcoholism (NIAAA). (2020, February) *Alcohol Facts and Statistics*. https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-facts-and-statistics.

Newlin, D. B., & Thomson, J. B. (1990). Alcohol challenge with sons of alcoholics: A critical review and analysis. *Psychological Bulletin*, *108*(3), 383–402. https://doi.org/10.1037/0033-2909.108.3.383

Piskur, J. (2014). *Molecular mechanisms in yeast carbon metabolism.* Springer-Verlag.

Pozo, M. J., De Vega, C., Canto, A., & Herrera, C. M. (2009). Presence of yeasts in floral nectar is consistent with the hypothesis of microbial-mediated signaling in plant-pollinator interactions. *Plant Signaling & Behavior*, *4*(11), 1102-1104. http://doi.org/10.4161/psb.4.11.9874

Robinson, G. E. (1985). Effects of a juvenile hormone analogue on honey bee foraging behaviour and alarm pheromone production. *Journal of Insect Physiology*, *31*(4), 277–282. https://doi.org/10.1016/0022-1910(85)90003-4

Sacks, J. J., Gonzales, K. R., Bouchery, E. E., Tomedi, L. E., & Brewer, R. D. (2015). 2010 national and state costs of excessive alcohol consumption. *American Journal of Preventitive Medicine*, *49*(5), e73–e79. https://doi.org/10.1016/j.amepre.2015.05.031

Schaeffer, R. N., Mei, Y. Z., Andicoechea, J., Manson, J. S., &. Irwin, R. E. (2017). Consequences of a nectar yeast for pollinator preference and performance. *Functional Ecology*, *31*(3), 613–621. http://doi.org/10.1111/1365-2435.12762

Seeley, T. D. (1992). The tremble dance of the honey bee: Message and meanings. *Behavioral Ecology and Sociobiology*, *31*(6), 375–383. http://doi.org/10.1007/BF00170604

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry*, 3rd ed. W.H. Freeman & Company.

Solignac, M., Vautrin, D., Baudry, E., Mougel, F., Loiseau, A., & Cornuet, J. M. (2004). A microsatellite-based linkage map of the honeybee, *Apis mellifera* L. *Genetics*, *167*(1), 253-262. https://doi.org/10.1534/genetics.167.1.253

Stephens, D. W., & Krebs, J.R. (1986). *Foraging theory.* Princeton University. Press. https://doi.org/10.1515/9780691206790

Tabakoff, B., Cornell, N., & Hoffman, P. L. (1986). Alcohol tolerance. *Annals of Emergency Medicine*, *15*(9), 1005–1012. https://doi.org/10.1016/s0196-0644(86)80119-6

Troutwine, B. R., Ghezzi, A., Pietrzykowski, A. Z., & Atkinson, N. S. (2016). Alcohol resistance in Drosophila is modulated by the Toll innate immune pathway. *Genes, Brain and Behavior*, *15*(4), 382–394. https://doi.org/10.1111/gbb.12288

Varnon, C. A., Dinges, C. W., Black, T. E., Wells, H., & Abramson, C. I. (2018). Failure to find ethanol-induced conditioned taste aversion in honey bees (*Apis mellifera* L.). *Alcoholism: Clinical and Experimental Research*, *42*(7), 1260–1270. https://doi.org/10.1111/acer.13761

Visser, W., Scheffers, W. A., Batenburg-van der Vegte, W. H., & Van Dijken, J. P. (1990). Oxygen requirements of yeasts. *Applied and Environmental Microbiology*, *56*(12), 3785–3792. https://doi.org/10.1128/AEM.56.12.3785-3792.1990

Vogel-Sprott, M. D. (1979). Acute recovery and tolerance to low doses of alcohol: Differences in cognitive and motor skill performance. *Psychopharmacology*, *61*(3), 287–291. https://doi.org/10.1007/BF00432274

Von Frisch, K. (1967). *The dance language and orientation of bees.* The Belknap Press of Harvard University Press.

Weinstock, G. M., Robinson, G. E., Gibbs, R. A., Worley, K. C., Evans, J. D., Maleszka, R., Robertson, H. M., Weaver, D. B., Beye, M., Bork, P., Elsik, C. G., Hartfelder, K., Hunt, G. J., Zdobnov, E. M., Amdam, G. V., Bitondi, M. M. G, Collins, A. M,. Cristino, A. S., Michael, H., … Havlak, P. (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature*, *443*(7114), 931–949. https://doi.org/10.1038/nature05260

Weissenborn, R., & Duka, T. (2000). State-dependent effects on explicit memory: the role of semantic associations. *Psychopharmacology*, *149*(1), 98–106. http://doi.org/10.1007/s002139900349

Wells, H., Wells, P. H., & Smith, D. M. (1981). Honey bee responses to reward size and colour in an artificial flower patch. *Journal of Apicultural Research*, *20*(3), 172–179. https://doi.org/10.1080/00218839.1981.11100493

Wenner, A. M., & Wells, P. H. (1990). *An anatomy of a controversy: The question of a 'language' among bees*. Columbia University Press. https://doi.org/10.7312/wenn90330

Wenner, A. M., Wells, P. H., & Johnson, D. L. (1969). Honeybee recruitment to food sources: Olfaction or language? *Science*, *164*(3875), 84–86. https://doi.org/10.1126/science.164.3875.84

Wolf, F. W., Rodan, A. R., Tsai, L. T., & Heberlein, U. (2002). High-resolution analysis of ethanol-induced locomotor stimulation in *Drosophila*. *Journal of Neuroscience*, *22*(24), 11035–11044. https://doi.org/10.1523/JNEUROSCI.22-24-11035.2002

Wright, M. J., Jr, Glavis-Bloom, C., & Taffe, M. A. (2013). Acute ethanol reduces reversal cost in discrimination learning by reducing perseverance in adolescent rhesus macaques. *Alcoholism: Clinical and Experimental Research*, *37*(6), 952–960. https://doi.org/10.1111/acer.12050

Financial conflict of interest: No stated conflicts.
Conflict of interest: No stated conflicts.

Submitted: *April 9th, 2021*
Resubmitted: *May 21st, 2021*
Accepted: *May 28th, 2021*