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The effect of ethanol on reversal learning in honey bees (Apis mellifera anatolica): Response inhibition in a social insect model

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A B S T R A C T

We investigated the effects of ethanol on reversal learning in honey bees (Apis mellifera anatolica). The rationale behind the present experiment was to determine the species generality of the effect of ethanol on response inhibition. Subjects were originally trained to associate either a cinnamon or lavender odor with a sucrose feeding before a reversal of the conditioned stimuli. We administered 15 μL of ethanol at varying doses (0%, 2.5%, 5%, 10%, or 20%) according to group assignment. Ethanol was either administered 5 min before original discrimination training or 5 min before the stimuli reversal. We analyzed the effects of these three manipulations via a recently developed individual analysis that eschews aggregate assessments in favor of a model that conceptualizes learning as occurring in individual organisms. We measured responding in the presence of conditioned stimuli associated with a sucrose feeding, responding in the presence of conditioned stimuli associated with distilled water, and responding in the presence of the unconditioned stimulus (sucrose). Our analyses revealed the ethanol dose manipulation lowered responding for all three measures at increasingly higher doses, which suggests ethanol served as a general behavioral suppressor. Consistent with previous ethanol reversal literature, we found administering ethanol before the original discrimination phase or before the reversal produced inconsistent patterns of responding at varying ethanol doses.

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Introduction

Behavior is readily altered by ethanol consumption (e.g., Dougherty, Marsh, Moeller, Chokshi, & Rosen, 2000; Field, Schoenmakers, & Wiers, 2008). While the effects of ethanol ingestion on simple associative and discrimination learning are well characterized in organisms as diverse as humans, monkeys, rodents, insects, and worms (e.g., Abramson, Wells, & Bozic, 2007; Brown, Calizo, Goodlett, & Stanton, 2007; Fortier et al., 2008; Musselman, Neal-Beliveau, Nass, & Engleman, 2012; Mustard et al., 2008; Pieper & Skeen, 1973, 1975; Wen et al., 1997), the effects of ethanol ingestion on reversal learning remain understudied and unresolved (Wright, Glavis-Bloom, & Taffe, 2013). The present experiment investigated the effect of ethanol on appetitive classically conditioned reversal learning in harnessed honey bee foragers (Apis mellifera anatolica).

Reversal learning is an extension of discrimination conditioning (Harlow, 1949). In reversal learning, an organism is trained to discriminate between two stimuli by pairing one stimulus with a positive outcome and the other stimulus with no outcome (discrimination learning). In studies of classical conditioning, both stimuli are known as a conditioned stimulus (CS); the stimulus associated with the positive outcome (i.e., the unconditioned stimulus) is known as a CS+ and the stimulus associated with no outcome is known as the CS−. In the course of training, the organism learns to respond to the CS+ but does not respond to the CS−. After the initial discrimination is learned, the roles of the stimuli are reversed: the CS+ becomes the CS−, and the CS− becomes the CS+. In some versions of the reversal experiment, the organism is exposed to several reversals (Harlow, 1949). Thus, reversal learning encompasses any circumstance wherein an individual is trained to respond differently to two cues and is then trained to respond in a reverse manner to the same cues during a subsequent phase of the experiment.

Few experiments have been conducted on the effect of ethanol consumption in reversal learning, and these investigations almost

exclusively have used non-human primates and rodent models. In adult rhesus monkeys, reversal learning has been reported to be impaired when subjects consume ethanol (Jedema et al., 2011). This observation is in accordance with a human study of alcoholics; a history of chronic alcoholism was found to be positively correlated with impaired reversal learning (Fortier et al., 2008).

In contrast, no difference was found by Wright et al. (2013) when comparing reversal to simple discrimination errors when adolescent rhesus monkeys consumed ethanol 3–24 h before training. In fact, Wright et al. (2013) observed an improvement in reversal learning compared to discrimination learning for subjects administered ethanol less than 90 min before the reversal tests. This finding does not state ingesting ethanol had no effect on rhesus monkey learning; there was a decrease in discrimination ability for subjects exposed to ethanol (Wright et al., 2013). A systematic reduction in both discrimination and reversal learning was observed in monkeys tested from 3 to 24 h after consuming ethanol, and a relatively smaller effect on reversal learning occurred in the monkeys that had recently ingested ethanol 90 min prior to the testing. The age of the monkeys may account for the differences between the Jedema et al. (2011) and Wright et al. (2013) studies. Based on the work related to ethanol-induced brain damage, we would have expected a greater decrement in juvenile monkeys than adults (reviewed in Riley & McGee, 2005).

Rats have also been used as an animal model to study potential effects of ethanol consumption on discrimination conditioning and reversal learning, but these investigations utilized a different method. Ethanol was delivered in a binge-like period to weanlings, but the rats were not tested until 3 weeks after last consuming ethanol (Brown et al., 2007). Under this scenario, rats showed an ethanol dose-related impairment in both discrimination conditioning and reversal learning, with higher weanling ethanol dosages resulting in more severe impairment (Brown et al., 2007). Weanling age was critical for this permanent ethanol effect and is highly correlated to time of administration in weanlings (Goodlett & Lundahl, 1996).

Only one previous study utilized an invertebrate model to study the effects of ethanol consumption on reversal learning. Free-flying honey bee foragers were the experimental organism using color discrimination cues (Abramson, Sanderson, Painter, Barnett, & Wells, 2005). When presented with blue and white flowers as the conditioning cues, a reversal effect could not be demonstrated because ethanol consumption impaired simple discrimination learning. When presented with blue and yellow flowers as the conditioning cues, a reversal effect could not be demonstrated because bees did not show learning even though they demonstrated acute discrimination ability (Abramson et al., 2005). Here, we again use honey bees as the experimental organism, but under a very different experimental paradigm; we used harnessed bees and odor cues.

The rationale behind the present experiment is to provide data on the influence of ethanol on reversal learning and thus response inhibition in an insect model (Chandra, Hosler, & Smith, 2000). Wright et al. (2013) recently suggested the reversal-learning design could be utilized to investigate ethanol-induced behavior because the reversal design can tease apart simple associative learning from the ability to inhibit previously learned responses when new contingencies are encountered. Insects are an excellent model system to do this because the behavioral and molecular effects of ethanol have been characterized for insect models (Scholz & Mustard, 2013). *Drosophila* have been extensively used as an ethanol model by a variety of laboratories (e.g., Peru Y Colón de Portugal et al., 2013; Singh & Heberlein, 2000) to investigate such effects as tolerance (e.g., Berger, Heberlein, & Moore, 2006; Scholz, Ramond, Singh, & Heberlein, 2000), locomotion (e.g., Wolf, Rodan, Tsai, &

Heberlein, 2002), and addiction (Devineni & Heberlein, 2009). Complementing the *Drosophila* work, honey bees have been used as an ethanol model of aggression, communication, locomotion, learning, taste preferences, and social behavior (e.g., Abramson et al., 2000, 2007; Mustard et al., 2008).

One major advantage of honey bees for this study is that they have been used extensively in reversal-learning studies (e.g., Chandra et al., 2000; Komischke, Giurfa, Lachnit, & Malun, 2002), while *Drosophila* reversal learning has not been extensively investigated (e.g., Tully et al., 1990). Additionally, all of these relatively complex social behaviors are contained in an organism with fewer than 1 million neurons (Abramson et al., 2000); recent mapping of the honey bee genome reveals that honey bees are an ideal learning and ethanol insect model (Chandra, Hunt, Cobey, & Smith, 2001).

Methods

Subjects

Honey bees (*Apis mellifera anatolica*) were collected from outdoor laboratory feeders containing 1.5 M sucrose solution at approximately 7:00 AM on the day before data collection. Honey bees collected at such feeders are known as forager bees and are optimal subjects in learning experiments (Dinges et al., 2013). Forager bees are typically between 21 and 30 days of age and have experience in tasks such as associating odors with a reward and learning to navigate (Giurfa, 2007). Each bee was captured in a glass vial and was then placed in an ice-water bath, thus causing the bee to become immobile. When the bee became inactive, it was immediately removed from the vial and placed into a metal restraining harness. To secure the bee in the harness, a strip of duct tape was placed between the subject's head and the thorax and fastened to the sides of the harness. After the bee warmed up and became active, it was administered a 1.5 M sucrose solution until it would no longer extend its proboscis. The bees were then left in the harness until the day of conditioning the following morning. On the day of conditioning, only bees that extended their proboscis to sucrose stimulation of the antennae were used as subjects in the experiments. This pretest was administered 30 min before training and was necessary to ensure all subjects had the same motivation to feed at the time of training (for further details on this method, see Abramson, Aquino, Silva, & Price, 1997).

Proboscis extension response conditioning

We used the classical conditioning of proboscis extension as our conditioning protocol (Abramson, Sokolowski, & Wells, 2011; Bitterman, Menzel, Fietz, & Schäfer, 1983). In this conditioning protocol, an olfactory conditioned stimulus (CS) is paired with a 1.5 M sucrose feeding, the unconditioned stimulus (US). After several CS/US pairings, proboscis extension is elicited by the previously neutral or ineffective CS.

One CS consisted of cinnamon odor (Gilbertie's, Southampton, NY) and the second CS consisted of lavender odor (Gilbertie's, Southampton, NY). These odors were selected because previous research indicated that bees respond to these stimuli in a similar manner (Abramson et al., 2010). The CS odor was first transferred to a 1 cm^2 piece of filter paper (Whatman #4) by dipping a wooden dowel in the odor and lightly applying the odor to the filter paper; this filter paper was then secured to the plunger of a 20 cc plastic syringe with an uncoated metal thumbtack, thereby making an "odor cartridge". Previous work demonstrated this procedure produces reliable results consistent with automated methods (Abramson & Boyd, 2001).