Habituation but not classical conditioning of the disturbance hiss of the hissing cockroach (*Gromphadorhina portentosa*) (#110520)

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Habituation but not classical conditioning of the disturbance hiss of the hissing cockroach (*Gromphadorhina portentosa*)

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This paper explores learned changes in the disturbance hiss of the hissing cockroach, *Gromphadorhina portentosa*. ~~Despite extensive research on learning in other cockroaches, studies with this species are rare. Of the natural behaviors of *G. portentosa*, the disturbance hiss is also seldom investigated.~~ Two experiments were conducted to address these deficits. The first experiment investigated habituation to repeated tactical stimulus delivered to the cerci. The effect of sex and heat were also assessed in a group design. This experiment found typical habituation trends, with males showing higher rates of hissing, and heated cockroaches showing marginally higher rates of hissing. Similar, but less pronounced results were seen with probability of movement. The second experiment explored classical conditioning by presenting an olfactory stimulus prior to, and along with, tactile stimulation. After conditioning, the olfactory stimulus and a second novel olfactory stimulus were presented on opposite ends of the apparatus to determine if there was conditioned preference. No evidence of conditioned response was observed in this experiment. Hissing and movement were observed during and after tactile stimulation, but responses were not observed before trials or during olfactory stimulus presentations. No preference between novel and conditioned odor was observed in the preference test. These findings confirm habituation in *G. portentosa* but highlight challenges in eliciting conditioned responses, emphasizing the need for further research to enhance understanding of insect learning and behavior.

Habituation but not Classical Conditioning of the Disturbance Hiss of the Hissing Cockroach (*Gromphadorhina portentosa*)

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Abstract

This paper explores learned changes in the disturbance hiss of the hissing cockroach, *Gromphadorhina portentosa*. Despite extensive research on learning in other cockroaches, studies with this species are rare. Of the natural behaviors of *G. portentosa*, the disturbance hiss is also seldom investigated. Two experiments were conducted to address these deficits. The first experiment investigated habituation to repeated tactical stimulus delivered to the cerci. The effect of sex and heat were also assessed in a group design. This experiment found typical habituation trends, with males showing higher rates of hissing, and heated cockroaches showing marginally higher rates of hissing. Similar, but less pronounced results were seen with probability of movement. The second experiment explored classical conditioning by presenting an olfactory stimulus prior to, and along with, tactile stimulation. After conditioning, the olfactory stimulus and a second novel olfactory stimulus were presented on opposite ends of the apparatus to determine if there was conditioned preference. No evidence of conditioned response was observed in this experiment. Hissing and movement were observed during and after tactile stimulation, but responses were not observed before trials or during olfactory stimulus presentations. No preference between novel and conditioned odor was observed in the preference test. These findings confirm habituation in *G. portentosa* but highlight challenges in eliciting conditioned responses, emphasizing the need for further research to enhance understanding of insect learning and behavior.

Introduction

This paper investigates if the hissing cockroach, *Gromphadorhina portentosa*, can alter its disturbance hiss through learning. *G. portentosa* is the most well-known of several Madagascar hissing cockroach species in the tribe Gromphadorhini. These large, wingless cockroaches produce several hissing sounds by forcing air from their respiratory spiracles (Nelson, 1979; Nelson & Fraser, 1980). Adult males can produce agonistic and courtship hisses, and older nymphs and adults of both sexes can produce a disturbance hiss. While the agonistic and courtship hisses have been thoroughly investigated (e.g., Clark & Moore, 1995a, 1995b, 1995c; Fraser & Nelson, 1984a, 1985b), the disturbance hiss, although well-known, is seldom studied. It may be elicited by either visual or tactile stimuli. A weak stimulus may elicit a single hiss, while a strong stimulus can cause a long series of hisses as the animal flees (Nelson & Fraser, 1980). The adaptive function of the disturbance hiss remains unclear. It may function to startle predators or otherwise inhibit predation attempts. Alternatively, it may act as a warning for other cockroaches, as research shows they do respond to the sounds of other types of hisses (Nelson & Fraser, 1980).

There is growing interest in the behavior and learning abilities of *G. portentosa*, both as subjects of experimental research and as practical species for hands-on teaching opportunities. This can be seen through student thesis projects (e.g., Albaitis, 2022; Gunnarsson, 2013; Harri-Dennis, 2016) and numerous presentations at behavior-focused conferences such as the Association of Behavior Analysis International. The interest in *G. portentosa* as practical model for behavior mirrors interest in this species as a model for anatomy (Heyborne, Fast, Goodding, 2013), immunology (Chua et al., 2017), and general science teaching (Fisher & Lorenz-Reaves, 2018; Wagler & Wagler, 2011, 2019, 2021).

Unfortunately, published work on learning in this species is exceedingly rare. There are only two published studies. Davis and Heslop (2004) reported habituation of the disturbance hiss in response to handling in ten out of twelve cockroaches (another eight never hissed), with four showing a recovery of hissing in the presence of a novel handler. Additionally, Dixon et al. (2016) demonstrated individual food preferences in seven cockroaches using a reinforcement procedure. While these studies are useful, the small number of publications is in great contrast to other cockroach species. Topics include studies of habituation (Varnon & Adams, 2021; Zilber-Gachelin & Chartier, 1973a, 1973b), classical conditioning (Lent & Kwon, 2004; Varnon, Barrera & Wilkes, 2022; Watanabe et al, 2003; Watanabe & Mizunami, 2007), operant conditioning (Arican et al, 2020; Garren, Sexauer & Page, 2013), spatial learning (Brown & Strausfeld, 2009), learned helplessness (Brown & Stroup, 1988), memory formation (Hosono, Matsumoto & Mizunami, 2006), and neuroscience (Matsumoto et al, 2013; Sato, Matsumoto and Mizunami, 2023). While most work involves *Periplaneta americana* or *Blattella germanica*, two well-known pests (Schall, Gautier & Bell, 1984), several studies involve non-pest species that may be more practical for many laboratories, such as *Blaberus cranifer* (Zilber-Gachelin & Chartier, 1973a, 1973b), *Blaberus discoidalis* (Harley, English & Ritzmann, 2009), *Eublaberus posticus* (Varnon & Adams, 2021; Varnon, Barrera & Wilkes, 2022), *Nauphoeta cinerea* (Kou et al. 2019; Longo, 1964) and *Rhyparobia maderae* (Garren, Sexauer & Page, 2013). Clearly, there is potential to study cockroach behavior and learning. What is missing is research with *G. portentosa*, ~~both in terms of the disturbance hiss and in terms learning~~. This paper describes two experiments designed to fill some of these gaps.

The first experiment explored habituation of the disturbance hiss. Habituation is highly conserved and occurs across a range of animals, including rodents (Davis, 1974), songbirds

(Vincze et al., 2016), snakes (Place & Abramson, 2008), bees (Varnon et al., 2021), and single-celled organisms (Rajan et al., 2023). While often studied in cockroaches, Davis and Heslop (2004) is the only investigation of habituation in *G. portentosa*. The current experiment ~~confirms and~~ expands existing findings. Cockroaches were presented with repeated tactile stimuli to observe habituation of the disturbance hiss and related behavior. The effects of heat and sex on responding were also investigated.

The second experiment investigated classical conditioning. Like habituation, classical conditioning is a widespread phenomenon that has been documented across taxa, including rats (Rescorla, 1968), pigeons (Hittesdorf & Richards, 1978), fish (Tennant & Bitterman, 1975), and bees (Abramson et al., 2015). Unlike habituation, there are many species-specific trends in classical conditioning, especially when surrounding phenomena like autoshaping (Jenkins & Moore, 1973, Palm & Powell, 1985, Timberlake & Grant, 1975, Wasserman, 1973) and conditioned taste aversion (Braveman, 1975; Garcia & Koelling, 1966; Ratcliffe et al., 2003; Varnon et al., 2018). Although there are many demonstrations of classical conditioning in cockroaches, there is no documentation of classical conditioning in *G. portentosa*, nor are there reports of olfactory aversive learning in cockroaches similar to those found in other insects such as ants (Desmedt et al., 2017), bees (Caracaud et al., 2009; Tedjakumala & Giurfa, 2013; Vergoz et al., 2007) and fruit flies (Busto et al., 2010). In this experiment, an olfactory conditioned stimulus (CS) was presented alongside a tactile unconditioned stimulus (US) to determine if a disturbance hiss would emerge as a conditioned response (CR).

Materials & Methods

Subjects

Adult hissing cockroaches (*Gromphadorhina portentosa*) were used as subjects for this experiment (n = 160). Prior to collection, cockroaches lived in large breeding colonies. Founding members were obtained from Fluker's Farms (Port Allen, LA) and Rainbow Mealworms (Compton, CA). Each colony was maintained at 23˚C and 57% relative humidity in ventilated plastic bins (52 x 36 x 36 cm) with a layer of ReptiBark substrate (Zoo Med Laboratories; San Luis Obispo, CA; zoomed.com). A thin layer of petroleum jelly was applied to the top of the bins to prevent young cockroaches from escaping. Colonies were fed dry dog food (Purina One, Nestlé Purina PetCare; St. Louis, MO), apples, and water ad libitum. Wooden and cardboard shelters were provided for the cockroaches to hide in and climb on. Colonies were maintained on a 12:12 hour day:night cycle, with two red 3-watt light bulbs (Feit Electric; Pico Rivera, CA) providing illumination for the experimenters. Red lights do not disrupt the behavior of cockroaches as they cannot easily see red wavelengths (Brisco & Chittka, 2001). After participating in the experiment, subjects were retired to a colony reserved for experimentally experienced subjects.

Experiment 1: Habituation

Subjects (n = 80) were randomly selected from a breeding colony one day before the experiment began. Subjects were sexed, weighed, and placed in individual apparatuses. Each apparatus was a ventilated plastic bin (14 x 12 x 7.5 cm) with a thin layer of ReptiBark substrate. The presence of hissing when the subject was collected and moved to the apparatus was recorded. Subjects were allowed a one-day acclimation period in the apparatus.

Subjects were divided into groups based on a temperature manipulation and sex. Heated subjects received the same procedure as unheated subjects, except that their apparatus was placed on an Intellitemp Reptile Heat Mat (Big Apple Pet Supply; Boca Raton, FL). The surface temperature of each subject was recorded after the one-day acclimation period with a CH-1022-NCIR infrared thermometer (Engineering Products; Loganville, GA) held 2 cm away from the mid abdomen. Temperature was recorded again after the experiment was complete. The average temperature was 20.86 ˚C for unheated subjects and 32.84 ˚C for heated subjects. Unheated and heated groups were further divided by sex, leading to a 2 x 2 design with four groups of 20 subjects each.

The experiment began after the one-day acclimation period and consisted of 14 trials for each subject, starting one hour after the end of the dark cycle. During each trial, the experimenter presented a tactile stimulus consisting of tapping the cerci with a small, flexible metal wire. This stimulus was selected as pilot work indicated it produced hissing reliably without requiring excessive force. After presenting the tactile stimulus, two observers independently recorded behavior for 10 seconds, then the subject was left undisturbed for a six-minute intertrial interval. After all trials were complete, subjects were recollected and placed in the colony for experimentally experienced cockroaches. The presence of hissing during recollection was also recorded.

Two responses were recorded for each trial: 1) the number of hisses, and 2) the presence of movement. Hissing was defined as any audible hiss vocalization that occurred within 10 seconds of the stimulus presentation. Duration, count, and intensity of hissing varied but were not recorded. Movement was defined as any time the subject took a step forward within 10 seconds of the stimulus presentation. Subjects were generally stationary before each trial, but movement was not recorded if subjects were moving at the start of the trial.

Experiment 2: Classical Conditioning

Subjects (n = 80) were randomly selected from a breeding colony the day of the experiment. Subjects were sexed, weighed, and then placed in individual clear plastic runway apparatuses (23.5 x 8.1 x 7.5 cm). The upper rim of the runway was lined with petroleum jelly to prevent escape. Between conditioning trials, the runway was placed on an Intellitemp heat mat. During conditioning trials, the runway was placed on a paper grid covering a second heat mat between two fume extractors (Xytronic-USA; Shingle Springs, CA). The fume extractors were used to remove lingering odors, a standard precaution for insect olfactory conditioning procedures (Matsumoto et al., 2012). The heat mats maintained an average apparatus temperature of 31.48˚C. The paper grid underneath the apparatus contained marks that divided the runway into five 4.7 cm cells used to record movement. A camera positioned above the runway recorded each trial for later scoring by two independent observers.

Each subject participated in eight conditioning trials following a one-hour acclimation period. During each trial, the runway was moved into the conditioning arena, and the subject was observed for a 30-second pre-trial interval. Next, the CS was delivered. The experimenter held a two-by-two cm filter paper soaked in 0.02 mL of orange or peppermint extract (McCormick; Hunt Valley, MA) between the subject's antenna for either three or six seconds, depending on group assignment. The odor used was counterbalanced across subjects. The filter paper was attached to a metal ring worn by the experimenter so that the CS odor was permanently affixed to the experimenter's hand. The subject was observed for the CS interval, then the unconditioned stimulus (US) was presented. The US consisted of gently lifting the subject one cm by the thorax and upper abdomen for three seconds. If the subject dislodged itself, it was quickly lifted again and held until the three second US duration ended. During this time, the CS odor remained present. The subject's behavior was recorded during this three-second interval, as well as a 30-second post-trial interval. The runway was then returned to its position on the first heat mat for a 30-minute intertrial interval. During the pre-trial, CS, US, and post-trial intervals, the number of hisses and the amount of movement, defined as number of cell crossings, were recorded.

Several modifications of methods ~~(~~compared to Experiment 1~~)~~ were made to facilitate hissing and reduce the likelihood of habituation to the US. These methods were selected based on Thompson and Spencer's (1966) principles of habituation and Groves and Thompson's (1970) dual process theory. First, the acclimation period was reduced to one hour. As collection may be a stressful experience, subjects were deliberately provided less time to recover, as agitated state may lead to sensitization instead of habituation. Second, the US was more intense. Habituation is more likely to occur to weak stimuli, so the gentle lift was used as a more ecologically valid mock predation attempt that was safe for the animals, but more intense than a cerci touch. Finally, the intertrial interval was longer, as longer intertrial intervals delay the rate of habituation.

A five-minute preference assessment was conducted 30 minutes after the final conditioning trial. Drops of orange extract and peppermint extract were presented on opposite sides of the runway, with one acting as the CS and the other as a novel odor. The duration subjects spent in each cell of the runway was recorded. The preference assessment was used to determine if an aversion developed to the CS odor outside of the initial conditioning method, as has been shown with bees (Carad et al., 2009). After completing the preference assessment, subjects were placed in a retirement bin, and their response to recollection was recorded.

Analysis

All regression analyses were conducted using the StatsModels package included in the Anaconda distribution of Python. Generalized estimating equations (Hardin & Hilbe, 2003) repeated measures regressions with exchangeable covariance structures were used to analyze rate and probabilities of behavior. For rate of behavior, Gaussian links were used. Logistic links were used for probability of behavior. Other analyses (dependent *t*-test and Pearson's *r* correlation) were conducted using the SciPy package.

Results

Experiment 1: Habituation

A simple overview of the hissing behavior of cockroaches before, during, and after the experiment is summarized in Table 1. The data reveal that unheated subjects were less likely to hiss compared to heated subjects, and females were less likely to hiss than males. A decrease in hissing across trials suggests habituation, further supported by the tendency for hissing to recover during recollection, indicating that the decrease was not due to fatigue. Twenty-one percent of cockroaches never hissed at all.

A more detailed view of the rate of hissing during each 10-second trial is presented in Figure 1, with corresponding statistical analysis provided in Table 2. Habituation can be observed for all groups (estimate = -0.007, $p < 0.000$), with unheated females hissing the least and heated males hissing the most. While heat influenced hissing behavior, its effect was relatively small and marginally significant (estimate = 0.026, $p = 0.045$). The role of sex was more substantial (estimate = 0.034, $p = 0.010$). Interactions are not reported as there was no basis in theory or exploratory analysis for learning rate to be affected by heat or sex, and initial explorations of between heat and sex showed weak unsignificant interactions.

Figure 2 shows the probability of movement during each trial, with Table 3 providing corresponding statistical analysis. As with rate of hissing, habituation of movement is readily observed both in the graphs and the analysis (estimate = -0.118, $p < 0.000$). Unlike with hiss rate, there was no effect of heat on probability of movement (estimate = 0.326, $p = 0.235$), and the difference between sexes was negligible (estimate = 0.524, $p = 0.057$).

Experiment 2: Classical Conditioning

The hissing behavior of cockroaches before, during, and after the experiment is summarized in Table 4. The percentage of subjects hissing at collection was notably higher than in Experiment 1. A general trend of habituation was observed, with hissing decreasing across the experiment and recovering during recollection. Eleven percent of cockroaches never hissed.

Figure 3 shows a detailed view of the rate of hissing during the pre-trial, CS, US, and post-trial intervals. Hissing never occurred during the 10-second pre-trial interval and only occurred once during the CS presentation. Hisses occurred regularly when the US was presented and continued to a lesser extent in the post-trial interval. The nearly complete lack of response to the CS, despite continual response to the US, suggests that no classical conditioning occurred. As in Experiment 1, males hissed more than females, and the tendency to respond to stimulation habituated across trials.

Statistical analysis for the rate of hissing during the US and post-trial intervals is provided in Table 5. No analysis was conducted for the pre-trial and CS intervals due to the lack of response. An initial model included trial, sex, CS duration, and CS odor as parameters, but CS duration and CS odor were removed due to high p values. The analyses confirm what can be seen in Figure 3; a significant decrease occurs across trials (p values < 0.000) and males respond more than females (p values < 0.000).

The rate of movement during the pre-trial, CS, US, and post-trial intervals is shown in Figure 4. Movement was very rare during the pre-trial and CS intervals. When the US was presented, movement occurred but nearly ceased in the post-trial interval. Movement elicited by the US appeared to habituate across trials. Similar to Experiment 1, there appeared to be little difference in movement behavior between sexes. The near lack of movement during the CS interval, and its similarity to the pre-trial interval again suggests a lack of classical conditioning.

Table 6 shows statistical analysis for the rate of movement during the US and post-trial intervals. As with hiss rate, analysis was not conducted for pre-trial and CS intervals and CS duration and CS odor were removed from initial models. The analyses confirm the response decrease across trials (p values < 0.000). Interestingly, the effect of sex was borderline. It was not significant during US presentation ($p = 0.087$) but was significant for the post-trial interval ($p < 0.000$). Given the mixed significance and the very low rate of movement in the post-trial interval, this effect should be interpreted with caution.

Results of the preference assessment conducted after the final conditioning trial are shown in Figure 5. Generally, cockroaches were inactive during this assessment, as they were during pre-trial intervals. Many never moved at all (52.5% male, 60.0% female). Subjects spent the most time near the two extreme ends of the runway, regardless of odor. Dependent t-tests confirmed there was no difference in the duration subjects spent within 4.7 cm of the CS odor compared to the novel odor ($t(79) = -0.337$, $p = 0.734$), nor was there a difference in the duration subjects spent within 9.4 cm of the CS odor compared to the novel odor ($t(79) = -0.345$, $p = 0.731$).

Pearson's r correlation was used to explore the relationship between the total number of hisses and total amount of movement emitted during conditioning trials for each subject. The analysis revealed a strong and statistically correlation (*r* = 0.554, $p < 0.000$), indicating that individuals that hissed more frequently also tended to exhibit higher levels of movement. A similar analysis was not possible for Experiment 1 due to the binary nature of the movement data.

Discussion

Habituation

Both experiments demonstrate habituation of the disturbance hiss and movement in response to aversive stimuli. While important to document, as this is only the third demonstration of learning in this species (and tribe), it is not an especially surprising finding given the highly conserved nature of habituation. However, differences between these experiments and those of Davis and Heslop (2004) provide valuable insights. First, in the present experiments, habituation was observed over the course of a single day, with intertrial intervals of 6 or 30 minutes. In Davis and Heslop (2004), a two-minute continuous stimulus was presented once or twice daily, six days a week. Together this suggests that *G. portentosa* is capable of both short-term and long-term habituation, which may be distinct processes (Rankin et al., 2009). Second, Davis and Heslop (2004) reported that 40% of subjects never hissed, much larger than the 11% to 21% observed in the present experiments. There are a number of possibilities for this discrepancy, including sex and methodological variations. However, these findings converge on an interesting point; not all hissing cockroaches emit the disturbance hiss. Further investigations may consider if this is an individual trait or context dependent. In Experiment 2, the total amount of hissing was strongly correlated with the amount of movement, suggestion investigations of individual factors may be a promising direction.

Future research on habituation should systematically explore the principles of habituation and sensitization outlined by Thompson and Spencer (1966) and Groves and Thompson (1970). The fact that a higher rate of hissing was observed in Experiment 2 is a promising indication of the importance of factors such as intertrial-interval, stimulus intensity, and agitated state. Other species relevant factors may also be investigated. For instance, Varnon and Adams (2021) found the presence of food in habituation procedures momentarily inhibited startle responses in *Eublaberus posticus* cockroaches. Future work could also expand studies of habituation beyond the disturbance hiss and startle responses. Notably, habituation may be a substantial factor in reinforcer satiation (Murphy et al., 2003) and exploration (Poucet, Durup & Thinus-Blanc, 1988; Wong et al. 2012). Given the territorial behavior of male *G. portentosa*, which includes the agonistic hiss, research on habituation of conspecific aggression would also be a promising and biologically relevant topic. In some species, habituation has been suggested to be a mechanism for reduced aggression for known rivals (Bee & Gerhardt, 2001; Petrinovich & Peeke, 1973).

Classical Conditioning

The lack of classical conditioning in Experiment 2 was unexpected given the numerous studies on classical conditioning in cockroaches, other insects, and animals in general. There are several possibilities to consider. First, it is possible that classical conditioning cannot occur during habituation to the US. This appears unlikely. While there are no studies that specifically investigate classical conditioning during habituation, there is also no theoretical reason to exclude this possibility. Additionally, quantitative models such as the Rescorla-Wager model of classical conditioning (Rescorla & Wagner, 1972) actually predict that CR will develop during US habituation. Figure 6 illustrates Rescorla-Wagner model predictions based on the UR observed in experiment 2. The UR line was derived from the analysis in Table 5, excluding sex as a factor. Conditioned responses are plotted at several levels of CS salience (α) with a consistent learning rate (β = 1), assuming a one-to-one correspondence between US expectancy and hiss rate. Note that even with a small salience of 0.1, conditioned responses still emerge.

A second possibility is that the olfactory CS used were not sufficiently detectable. While specific olfaction work is needed, this explanation also seems unlikely. Research shows cockroaches, including *G. portentosa,* have excellent olfactory abilities (Leibensperger et al., 1985; Persoons & Ritter, 1979; Schall, Gauiter & Bell, 1984) which are often used in classical conditioning research (Arican et al., 2020; Garren, Sexauer & Page, 2013; Hosono, Matsumoto & Mizunami, 2006; Matsumoto et al., 2013; Sato, Matsumoto & Mizunami, 2023; Varnon, Barerra & Wilkes, 2022; Watanabe et al., 2003; Watanabe & Mizunami, 2007). The specific odors used in this experiment have even been successfully used in other cockroach conditioning experiments (Varnon, Barrera & Wilkes, 2022; Watanabe et al., 2003).

Another consideration is that perhaps these specific methods do not produce a conditioned response. This possibility is more likely and warrants future research. Experiment 2 demonstrated that a three-second or six-second CS followed by an overlapping three-second US then a 30-minute intertrial interval was not sufficient to develop a conditioned response. These temporal parameters are typical of cockroach conditioning research, as well as those of probiscis extension response conditioning in bees (Frost, Shutler & Hillier, 2012). It is possible, however, that a variation of these stimulus timings or other methods may prove successful. For example, restrained conditioning procedures work well for honey bees (Frost, Shutler & Hillier, 2012; Varnon et al., 2018), but are challenging for bumble bees and stingless bees, necessitating unrestrained modifications (Muth et al., 2017; Amaya-Márquez et al., 2019).

The final, and most interesting possibility is that this particular CS-US association may not be effective at developing hissing or movement as a CR. Such exceptions to the general rules of learning are not uncommon. A number of species-specific constraints on learning have been documented for both classical and operant conditioning. For example, Garcia and Koelling (1966) demonstrated that rats can learn to associate illness with taste or smell, but not visual stimuli. Guinea pigs, however, learn to associate both color and taste with illness (Braveman, 1975). Honey bees and vampire bats also have challenges learning to associate toxic substances with odor and taste (Ratcliffe et al., 2003; Varnon et al., 2018). In the operant realm, Bitterman (1965) described a number of differences in how animals respond in serial reversal and probability matching experiments, and Craig and Abramson (2015) discussed how different species produce qualitatively distinct response patterns in fixed interval procedures. With respect to aversive conditioning, species-specific defensive reactions are known to inhibit learning certain responses (Bolles, 1970; Crawford & Masterson, 1982; Smith, Gustavson & Gregor, 1972; Smith & Keller, 1970). It is possible, that for hissing cockroaches, hissing and fleeing are species-appropriate responses to a predation attempt, but not to stimuli that predict predation. Instead, tonic immobility may be a more adaptive response when potential threats are anticipated but not yet present (Gallup, 1974, 1977). In other words, it may not be beneficial for a cockroach to hiss or flee if it has not yet been located by a predator. Detecting conditioned immobility in hissing cockroaches may be challenging, however, given their inactive nature. Note that subjects in Experiment 2 almost never moved during both pre-trial and CS intervals. Interestingly, despite a substantial body of work on olfactory aversive conditioning in other insects, there is limited corresponding research in cockroaches. The closest approximation is the use of salt solutions in differential taste conditioning procedures (Varnon, Barrera & Wilkes, 2022; Watanabe et al., 2003). Most procedures that focus on aversive learning follow the headless cockroach leg position method of Horridge (1962), with a few studies using intact cockroaches (Brown & Stroup, 1988). Conditioned stimuli are not used in this work. Only a few studies explore the association of shock with other cues such as light or location (Longo, 1964; Lovell & Eisenstein, 1973; Szymanski, 1912). Clearly, more research in aversive conditioning is needed across cockroach species.

Conclusions

These experiments aim to stimulate additional research on hissing cockroaches in behavior analysis. The work fills some important gaps, but also highlights the need for additional studies with *G. portentosa*, the disturbance hiss, and cockroach aversive conditioning. Given the lack of conditioning work with this species, it is crucial to report even null results, as describing differences and even a lack of behavioral abilities is an important component of research (Avarguès-Weber & Giurfa, 2013) and necessary to avoid publication bias and file-drawer effects often found in science (Rosenthal, 1979). Although research has repeatedly shown that learning is impacted by the ecological and evolutionary history of a species, even comparative research often focuses only on a small number of model organisms, leading to the neglect of entire orders and classes of animals (Beach, 1950; Varnon, Lang & Abramson, 2018; Varnon & Moore, 2024; Vonk, 2021). Continual work with *G. portentosa* offers an excellent opportunity to correct this deficit. Additionally, such research supports not only a scientific understanding of behavior, but also provides information crucial to furthering interest in cockroaches and other invertebrates as models to teach the next generation of scientists (Abramson, 1986; Dixon et al., 2016; Matthews & Matthews, 1997; Proctor & Jones, 2021).

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References

Abramson, C. A., 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. (1986). Invertebrates in the classroom. *Teaching of Psychology, 13*(1), 24-29. https://doi.org/10.1207/s15328023top1301_6

Albaitis, M. R. (2022). Conditioned territory defense in Madagascar hissing cockroaches (*Gromphadorhina portentosa*). [Honors thesis, Bucknell University].

Amaya-Márquez, M., Tusso, S., Hernández, J., Jiménez, J. D., Wells, H., & Abramson, C. I. (2019). Olfactory learning in the stingless bee *Melipona eburnea* friese (Apidae: Meliponini). *Insects, 10*(11), 412. https://doi.org/10.3390/insects10110412

Arican, C., Bulk, J., Deisig, N., & Nawrot, M. P. (2020). Cockroaches show individuality in learning and memory during classical and operant conditioning. *Frontiers in Physiology, 10*, 1539. https://doi.org/10.3389/fphys.2019.01539

Avarguès-Weber, A., & Giurfa, M. (2013). Conceptual learning by miniature brains. *Proceedings of the Royal Society B: Biological Sciences, 280*, 2013190. https://doi.org/10.1098/rspb.2013.1907

Beach, F. A. (1950). The snark was a boojum. *American Psychologist, 5*(4), 115-124. https://doi.org/10.1037/h0056510

Bee, M. A., & Gerhardt, H. C. (2001). Habituation as a mechanism of reduced aggression between neighboring territorial male bullfrogs (*Rana catesbeiana*). *Journal of Comparative Psychology, 115*(1), 68-82. https://doi.org/10.1037/0735-7036.115.1.68

Bitterman, M. E. (1965). Phyletic differences in learning. *American Psychologist, 20*(6), 396. https://doi.org/10.1037/h0022328

Bolles, R. C. (1970). Species-specific defense reactions and avoidance learning. *Psychological Review, 77*, 32-48. https://doi.org/10.1037/h0028589

Braveman, N. S. (1975). Relative salience of gustatory and visual cues in the formation of poison-based food aversions by guinea pigs (*Cavia porcellus*). *Behavioral Biology, 14*, 189-199. https://doi.org/10.1016/S0091-6773(75)90187-X

Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology, 46*, 471-510. https://doi.org/10.1146/annurev.ento.46.1.471

Brown, G. E., & Stroup, K. (1988). Learned helplessness in the cockroach (*Periplaneta americana*). *Behavioral and Neural Biology, 50*, 246-250. https://doi.org/10.1016/s0163-1047(88)90921-1

Brown, S., & Strausfeld, N. (2009). The effect of age on a visual learning task in the American cockroach. *Learning & Memory, 16*, 210-223. https://doi.org/10.1101/lm.1241909

Busto, G. U., Cervantes-Sandoval, I., & Davis, R. L. (2010). Olfactory learning in *Drosophila*. *Physiology, 25*(6), 338-346. https://doi.org/10.1152/physiol.00026.2010

Carcaud, J., Roussel, E., Giurfa, M., & Sandoz, J.-C. (2009). Odour aversion after olfactory conditioning of the sting extension reflex in honeybees. *Journal of Experimental Biology, 212*(5), 620-626. https://doi.org/10.1242/jeb.026641

Chua, J., Fisher, N. A., Falcinelli, S. D., DeShazer, D., & Friedlander, A. M. (2017). The Madagascar Hissing Cockroach as an Alternative Non-mammalian Animal Model to Investigate Virulence, Pathogenesis, and Drug Efficacy. *Journal of Visualized Experiments*: JoVE, (129), 56491. https://doi.org/10.3791/56491

Clark, D. C., & Moore, A. J. (1995a). Genetic aspects of communication during male-male competition in the Madagascar hissing cockroach: Honest signaling of size. *Heredity, 75*, 198-205. https://doi.org/10.1038/hdy.1995.124

Clark, D. C., & Moore, A. J. (1995b). Social communication in the Madagascar hissing cockroach: Features of male courtship hisses and a comparison of courtship and agonistic hisses. *Behaviour, 132*(5/6), 401-417. https://doi.org/10.1163/156853995X00630

Clark, D. C., & Moore, A. J. (1995c). Variation and repeatability of male agonistic hiss characteristics and their relationship to social rank in *Gromphadorhina portentosa*. *Animal Behaviour, 50*, 719-729. https://doi.org/10.1016/0003-3472(95)80132-4

Craig, D. P. A., & Abramson, C. I. (2015). A need for individual data analyses for assessments of temporal control: Invertebrate fixed interval performance. *International Journal of Comparative Psychology, 28*(1). https://doi.org/10.46867/ijcp.2015.28.02.10

Crawford, M., & Masterson, F. A. (1982). Species-specific defense reactions and avoidance learning. *Pavlovian Journal of Biological Science, 17*, 204-214. https://doi.org/10.1007/BF03001275

Davis, H., & Heslop, E. (2004). Habituation of hissing by Madagascar hissing cockroaches (*Gromphadorhina portentosa*): Evidence of discrimination between humans? *Behavioural Processes, 67*(3), 539-543. https://doi.org/10.1016/j.beproc.2004.08.003

Davis, M. (1974). Sensitization of the rat startle response by noise. *Journal of Comparative and Physiological Psychology, 87*(3), 571. https://doi.org/10.1037/h0036985

Desmedt, L., Baracchi, D., Devaud, J.-M., Giurfa, M., & d'Ettorre, P. (2017). Aversive learning of odor–heat associations in ants. *Journal of Experimental Biology, 220*(24), 4661-4668. https://doi.org/10.1242/jeb.161737

Dixon, M. R., Daar, J. H., Gunnarsson, K., Johnson, M. L., & Shayter, A. M. (2016). Stimulus preference and reinforcement effects of the Madagascar hissing cockroach (*Gromphadorhina portentosa*): A case of reverse translational research. *The Psychological Record, 66*, 41-51. https://doi.org/10.1007/s40732-015-0149-9

Fisher, N., & Lorenz-Reaves, A. (2018). Teaching with Live Insects. *Science and Children, 56*(4), 32–39. https://doi.org/10.2505/4/sc18_056_04_32

Fraser, J., & Nelson, M. C. (1984a). Communication in the courtship of a Madagascan hissing cockroach. I. Normal courtship. *Animal Behaviour, 32*, 194-203. https://doi.org/10.1016/S0003-3472(84)80337-1

Fraser, J., & Nelson, M. C. (1984b). Communication in the courtship of a Madagascan hissing cockroach. II. Effects of deantennation. *Animal Behaviour, 32*, 204-209. https://doi.org/10.1016/S0003-3472(84)80338-3

Frost, E. H., Shutler, D., & Hillier, N. K. (2012). The proboscis extension reflex to evaluate learning and memory in honeybees (*Apis mellifera*): Some caveats. *Naturwissenschaften, 99*, 677-686. https://doi.org/10.1007/s00114-012-0955-8

Gallup, G. G. Jr. (1974). Animal hypnosis: Factual status of a fictional concept. *Psychological Bulletin, 81*, 836-853. https://doi.org/10.1037/h0037227

Gallup, G. G. (1977). Tonic immobility: The condition of fear and predation. *The Psychological Record, 27*, 41-61. https://doi.org/10.1007/BF03394432

Garcia, J., & Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic Science, 4*, 123-124. https://doi.org/10.3758/BF03342209

Garren, M. V., Sexauer, S. B., & Page, T. L. (2013). Effect of circadian phase on memory acquisition and recall: Operant conditioning vs. classical conditioning. *PLoS ONE, 8*(3), e58693. https://doi.org/10.1371/journal.pone.0058693

Groves, P. M., & Thompson, R. F. (1970). Habituation: A dual-process theory. *Psychological Review, 77*(5), 419-450. https://doi.org/10.1037/h0029810

Gunnarsson, K. F. (2013). Using Madagascar hissing cockroaches as research subjects in behavior analysis. [Master's thesis, Southern Illinois University Carbondale, Department of Rehabilitation Services].

Hardin, J. W., & Hilbe, J. M. (2003). *Generalized Estimating Equations* (1st ed.). New York: Chapman and Hall/CRC.

Harley, C. M., English, B. A., & Ritzmann, R. E. (2009). Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*. *Journal of Experimental Biology, 212*(10), 1463-1476. https://doi.org/10.1242/jeb.028381

Harri-Dennis, E. A. (2016). Antennal depression as a consummatory response in the Madagascar hissing cockroach. [Master's thesis, St. Cloud State University, School of Health and Human Services].

Heyborne, W. H., Fast, M., & Goodding, D. D. (2012). The Madagascar hissing cockroach: a new model for learning insect anatomy. The *American biology Teacher, 74*(3), 185-189.

Hittesdorf, W. M., & Richards, R. W. (1978). Aversive second-order conditioning in the pigeon. *The Psychological Record, 28*, 605-613. https://doi.org/10.1007/BF03394578

Horridge, G. A. (1962). Learning of leg position by the ventral nerve cord in headless insects. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 157*, 33-52. https://doi.org/10.1098/rspb.1962.0061

Hosono, S., Matsumoto, Y., & Mizunami, M. (2006). Interaction of inhibitory and facilitatory effects of conditioning trials on long-term memory formation. *Learning & Memory, 32*, 669-678. https://doi.org/10.1101/lm.043513.116

Jenkins, H. M., & Moore, B. R. (1973). The form of the auto-shaped response with food or water reinforcers. *Journal of the Experimental Analysis of Behavior, 20*(2), 163-181. https://doi.org/10.1901/jeab.1973.20-163

Kou, R., Hsu, C. C., Chen, S. C., Chang, P. Y., & Fang, S. (2019). Winner and loser effects in lobster cockroach contests for social dominance. *Hormones and Behavior, 107*, 49-60. https://doi.org/10.1016/j.yhbeh.2018.12.004

Leibensperger, L. B., Traniello, J. F. A., & Fraser, J. M. (1985). Olfactory cues used by female *Gromphadorhina portentosa* during mate choice. *Annals of the Entomological Society of America, 78*, 629-634. https://doi.org/10.1093/aesa/78.5.629

Lent, D. D., & Kwon, H. (2004). Antennal movements reveal associative learning in the American cockroach *Periplaneta americana*. *Journal of Experimental Biology, 207*(2), 369-375. https://doi.org/10.1242/jeb.00736

Longo, N. (1964). Probability-learning and habit-reversal in the cockroach. *American Journal of Psychology, 77*(1), 29-41. https://doi.org/10.2307/1419269

Lovell, K. L., & Eisenstein, E. M. (1973). Dark avoidance learning and memory disruption by carbon dioxide in cockroaches. *Physiology & Behavior, 10*(5), 835-840. https://doi.org/10.1016/0031-9384(73)90050-4

Matsumoto, C. S., Kuramochi, T., Matsumoto, Y., Watanabe, H., Nishino, H., & Mizunami, M. (2013). Participation of NO signaling in formation of long-term memory in salivary conditioning of the cockroach. *Neuroscience Letters, 541*, 4-8. https://doi.org/10.1016/j.neulet.2013.01.010

Matsumoto, Y., Menzel, R., Sandoz, J. C., & Giurfa, M. (2012). Revisiting olfactory classical conditioning of the proboscis extension response in honey bees: A step toward standardized procedures. *Journal of Neuroscience Methods*, *211*(1), 159-167.

Matthews, R. W., Flage, L. R., & Matthews, J. R. (1997). Insects as teaching tools in primary and secondary education. *Annual Review of Entomology, 42*, 269-289. https://doi.org/10.1146/annurev.ento.42.1.269

Murphy, E. S., McSweeney, F. K., Smith, R. G., & McComas, J. J. (2003). Dynamic changes in reinforcer effectiveness: theoretical, methodological, and practical implications for applied research. *Journal of Applied Behavior Analysis, 36*(4), 421-438. https://doi.org/10.1901/jaba.2003.36-421

Muth, F., Cooper, T. R., Bonilla, R. F., & Leonard, A. S. (2017). A novel protocol for studying bee cognition in the wild. *Methods in Ecology and Evolution, 9*(1), 78-87. https://doi.org/10.1111/2041-210X.12852

Nelson, M. C. (1979). Sound production in the cockroach, *Gromphadorhina portentosa*: The sound-producing apparatus. *Journal of Comparative Physiology A, 132*, 27-38. https://doi.org/10.1007/BF00292773

Nelson, M. C., & Fraser, J. (1980). Sound production in the cockroach, *Gromphadorhina portentosa*: Evidence for communication by hissing. *Behavioral Ecology and Sociobiology, 6*, 305-314. https://doi.org/10.1007/BF00292773

Palm, L. J., & Powell, R. W. (1985). Crows and pigeons differ under autoshaping. *Bulletin of the Psychonomic Society, 23*(4), 430-432. https://doi.org/10.3758/BF03330206

Persoons, C. J., & Ritter, F. J. (1979). *Pheromones of Cockroaches*. Amsterdam: Elsevier.

Petrinovich, L., & Peeke, H. V. (1973). Habituation to territorial song in the white-crowned sparrow (*Zonotrichia leucophrys*). *Behavioral Biology*, *8*(6), 743-748.

Place, A. J., & Abramson, C. I. (2008). Habituation of the rattle response in western diamondback rattlesnakes, *Crotalus atrox*. *Copeia, 2008*(4), 835-843. https://doi.org/10.1643/CE-06-246

Poucet, B., Durup, M., & Thinus-Blanc, C. (1988). Short-term and long-term habituation of exploration in rats, hamsters and gerbils. *Behavioural Processes, 16*(3), 203-211. https://doi.org/10.1016/0376-6357(88)90040-X

Proctor, D., & Jones, M. (2021). Cockroaches to the rescue: A new approach to reintroduce animal labs to the psychology undergraduate curriculum. *Scholarship of Teaching and Learning in Psychology, 7*(3), 237-242. https://doi.org/10.1037/stl0000235

Rajan, D., Makushok, T., Kalish, A., Acuna, L., Bonville, A., Almanza, K. C., et al. (2023). Single-cell analysis of habituation in *Stentor coeruleus*. *Current Biology, 33*(2), 241-251. https://doi.org/10.1016/j.cub.2022.11.010

Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C. F., & Thompson, R. F. (2009). Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory, 92*(2), 135-138. https://doi.org/10.1016/j.nlm.2008.09.012

Ratcliffe, J. M., Fenton, M. B., & Galef, B. G. Jr. (2003). An exception to the rule: Common vampire bats do not learn taste aversions. *Animal Behaviour, 65*, 385-389. https://doi.org/10.1006/anbe.2003.2059

Rescorla, R. A. (1968). Probability of shock in the presence and absence of CS in fear conditioning. *Journal of Comparative and Physiological Psychology, 66*(1), 1-5. https://doi.org/10.1037/h0025984

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical Conditioning II: Current Research and Theory* (pp. 64-99). New York: Appleton-Century-Crofts.

Rosenthal, R. (1979). File drawer problem and tolerance for null results. *Psychological Bulletin, 86*, 638-641. https://doi.org/10.1037/0033-2909.86.3.638

Sato, C., Matsumoto, Y., & Mizunami, M. (2023). Roles of octopamine neurons in the vertical lobe of the mushroom body for the execution of a conditioned response in cockroaches. *Neurobiology of Learning and Memory, 203*, 107778. https://doi.org/10.1016/j.nlm.2023.107778

Schall, C., Gautier, J. Y., & Bell, W. J. (1984). Behavioural ecology of cockroaches. *Biological Reviews, 59*, 209-254. https://doi.org/10.1111/j.1469-185X.1984.tb00408

Smith, R. F., Gustavson, C. R., & Gregor, G. L. (1972). Incompatibility between the pigeons' unconditioned response to shock and the conditioned key-peck response. *Journal of the Experimental Analysis of Behavior, 18*(1), 147-153. https://doi.org/10.1901/jeab.1972.18-147

Smith, R. F., & Keller, F. R. (1970). Free-operant avoidance in the pigeon using a treadle response. *Journal of the Experimental Analysis of Behavior, 13*(2), 211-214. https://doi.org/10.1901/jeab.1970.13-211

Szymanski, J. S. (1912). Modification of the innate behavior of cockroaches. *Journal of Animal Behavior, 2*(2), 81-90. https://doi.org/10.1037/h0071345

Tedjakumala, S. R., & Giurfa, M. (2013). Rules and mechanisms of punishment learning in honey bees: The aversive conditioning of the sting extension response. *Journal of Experimental Biology, 216*(16), 2985-2997. https://doi.org/10.1242/jeb.086629

Tennant, W. A., & Bitterman, M. E. (1975). Blocking and overshadowing in two species of fish. *Journal of Experimental Psychology: Animal Behavior Processes, 1*(1), 22-29. https://doi.org/10.1037/0097-7403.1.1.22

Thompson, R. F., & Spencer, W. A. (1966). Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review, 73*, 16-43. https://doi.org/10.1037/h0022681

Timberlake, W., & Grant, D. L. (1975). Auto-shaping in rats to the presentation of another rat predicting food. *Science, 190*(4215), 690-692. https://doi.org/10.1126/science.190.4215.690

Varnon, C. A., & Adams, A. T. (2021). Habituation of the light-startle response (LSR) of orange head cockroaches (*Eublaberus posticus*): Effects of acclimation, stimulus duration, presence of food, and intertrial interval. *Insects, 12*(4), 1-15. https://doi.org/10.3390/insects12040339

Varnon, C. A., Barrera, I. E., & Wilkes, I. N. (2022). Learning and memory in the orange head cockroach (*Eublaberus posticus*). *PLoS ONE, 17*(8), e0272598. https://doi.org/10.1371/journal.pone.0272598

Varnon, C. A., Vallely, N. V., Beheler, C. R., & Coffin, C. A. (2021). The disturbance leg-lift response (DLR): An undescribed behavior in bumble bees. *PeerJ, 9*, 1-22. https://doi.org/10.7717/peerj.10997

Varnon, C. A., & Moore, M. K. (2024). Reconsidering the subject and object of comparative cognition. *Comparative Cognition and Behavior Reviews*. https://doi.org/10.3819/CCBR.2024.190019

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

Varnon, C. A., Lang, H., & Abramson, C. I. (2018). Automated research in comparative psychology: Limitations and new directions. *International Journal of Comparative Psychology, 31*, 1-17. https://doi.org/10.46867/ijcp.2018.31.01.08

Vergoz, V., Roussel, E., Sandoz, J.-C., & Giurfa, M. (2007). Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. *PLoS ONE, 2*(3), e288. https://doi.org/10.1371/journal.pone.0000288

Vincze, E., Papp, S., Preiszner, B., Seress, G., Bókony, V., & Liker, A. (2016). Habituation to human disturbance is faster in urban than rural house sparrows. *Behavioral Ecology, 27*(5), 1304-1313. https://doi.org/10.1093/beheco/arw047

Vonk, J. (2021). The journey in comparative psychology matters more than the destination. *Journal of Comparative Psychology, 135*(2), 156-167. https://doi.org/10.1037/com000027

Wagler, R., & Wagler, A. (2011). Arthropods: attitude and incorporation in preservice elementary teachers. *International Journal of Environmental and Science Education*, *6*(3), 229-250.

Wagler, R., & Wagler, A. (2019). Replicating Roaches. *The Science Teacher*, *86*(9), 26-31.

Wagler, R., & Wagler, A. (2021). Fear and loathing of cockroaches. *American Entomologist, 67*(1), 34-38.

Wasserman, E. A. (1973). Pavlovian conditioning with heat reinforcement produces stimulus-directed pecking in chicks. *Science, 181*(4102), 875-877. https://doi.org/10.1126/science.181.4102.875

Watanabe, H., & Mizunami, M. (2007). Pavlov's cockroach: Classical conditioning of salivation in an insect. *PLoS ONE, 2*(6), e529. https://doi.org/10.1371/journal.pone.0000529

Watanabe, H., Kobayashi, Y., Sakura, M., Matsumoto, Y., & Mizunami, M. (2003). Classical olfactory conditioning in the cockroach *Periplaneta americana*. *Zoological Science, 20*(12), 1447-1454. https://doi.org/10.2108/zsj.20.1447

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., et al. (2010). Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). *Behavioural Brain Research, 208*(2), 450-457. https://doi.org/10.1016/j.bbr.2009.12.023

Zilber-Gachelin, N. F., & Chartier, M. P. (1973a). Modification of the motor reflex responses due to repetition of the peripheral stimulus in the cockroach. I. Habituation at the level of an isolated abdominal ganglia. *Journal of Experimental Biology, 59*, 359-381. https://doi.org/10.1242/jeb.59.2.359

Zilber-Gachelin, N. F., & Chartier, M. P. (1973b). Modification of the motor reflex responses due to repetition of the peripheral stimulus in the cockroach. II. Conditions of activations of the motoneurons. *Journal of Experimental Biology, 59*, 383-399. https://doi.org/10.1242/jeb.59.2.383

Figure 1

Rate of hissing for nonheated and heated subjects in Experiment 1.

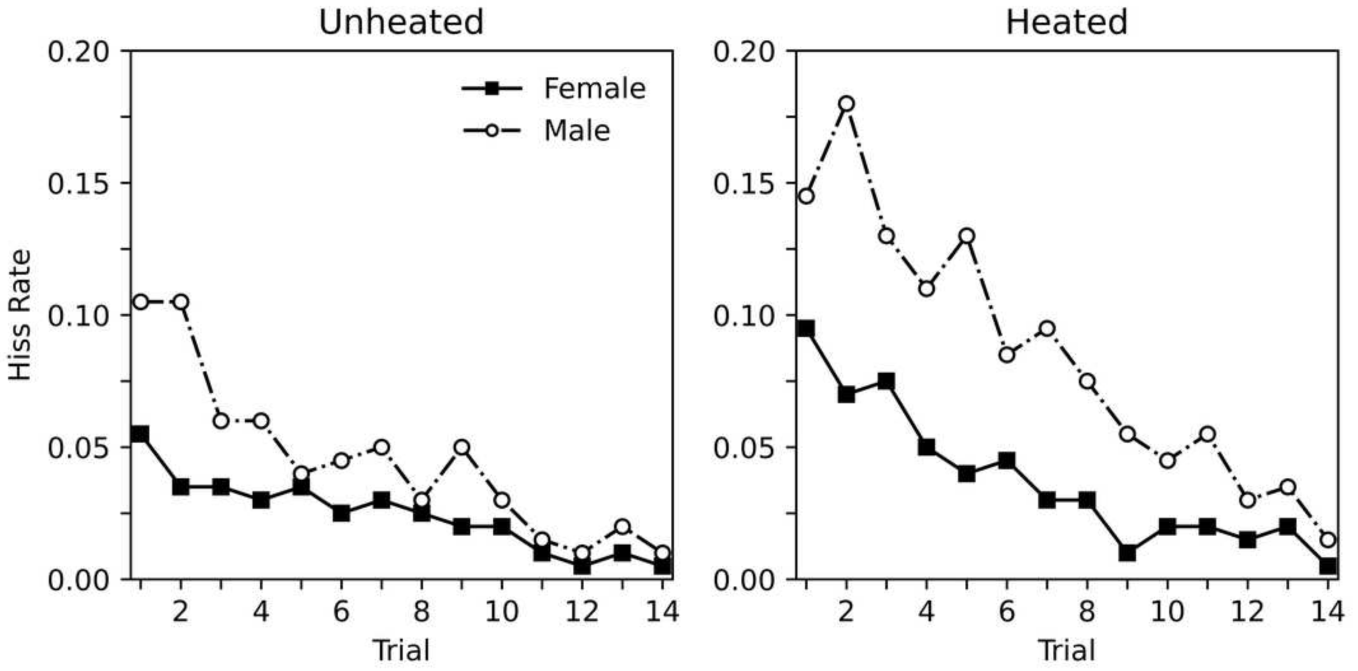

Figure 2

Probability of movement for nonheated and heated subjects in Experiment 1.

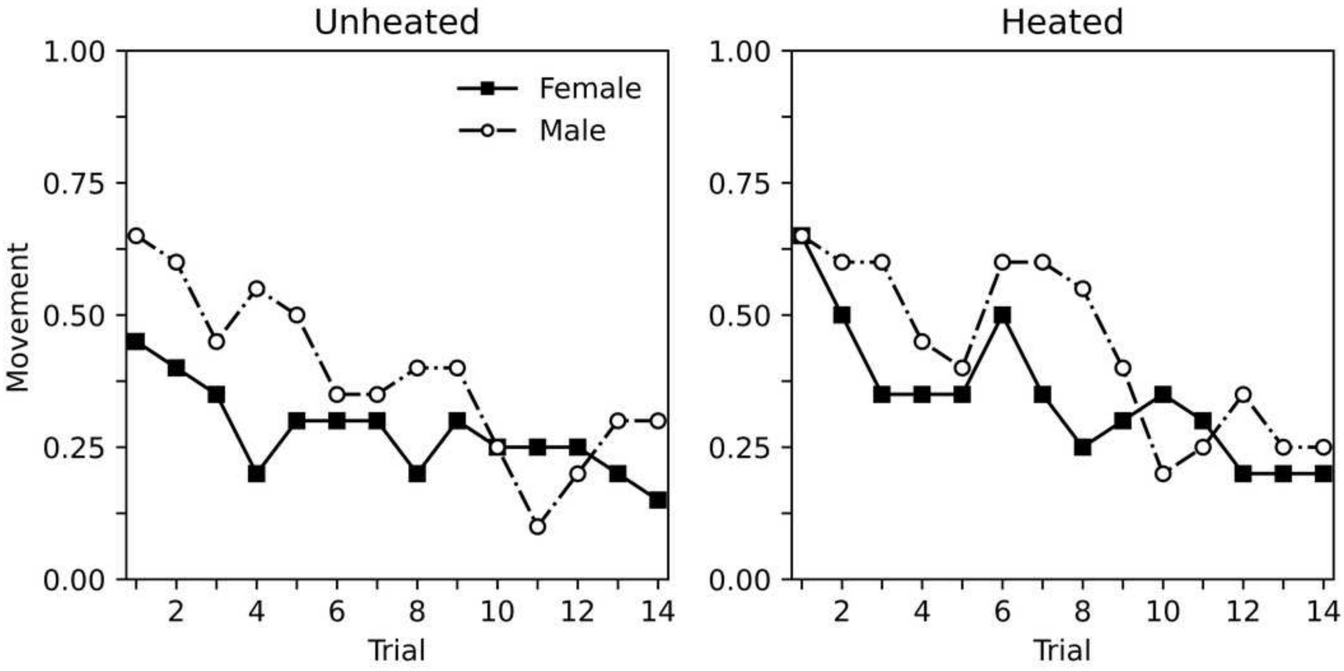

Figure 3

Rate of hissing during the pre-trial, CS, US, and post-trial intervals for the 3-second and 6-second CS groups in Experiment 2.

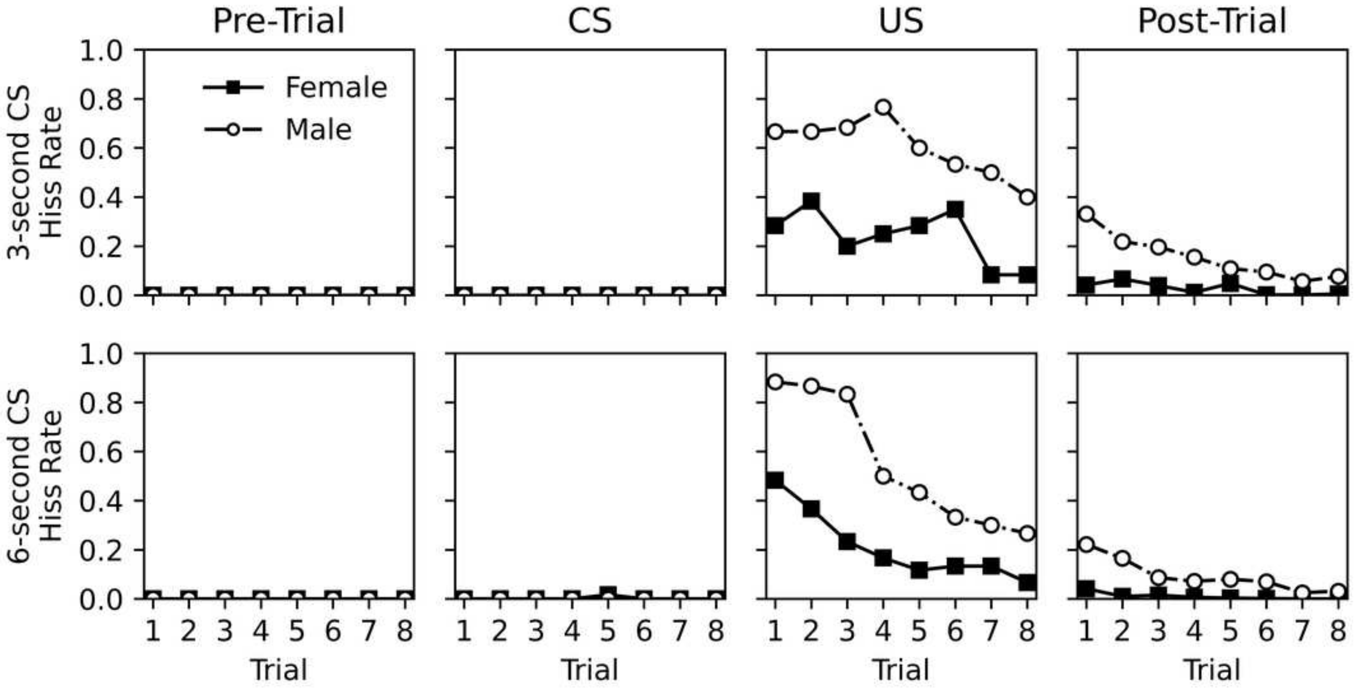

Figure 4

Rate of movement during the pre-trial, CS, US, and post-trial intervals for the 3-second and 6-second CS groups in Experiment 2.

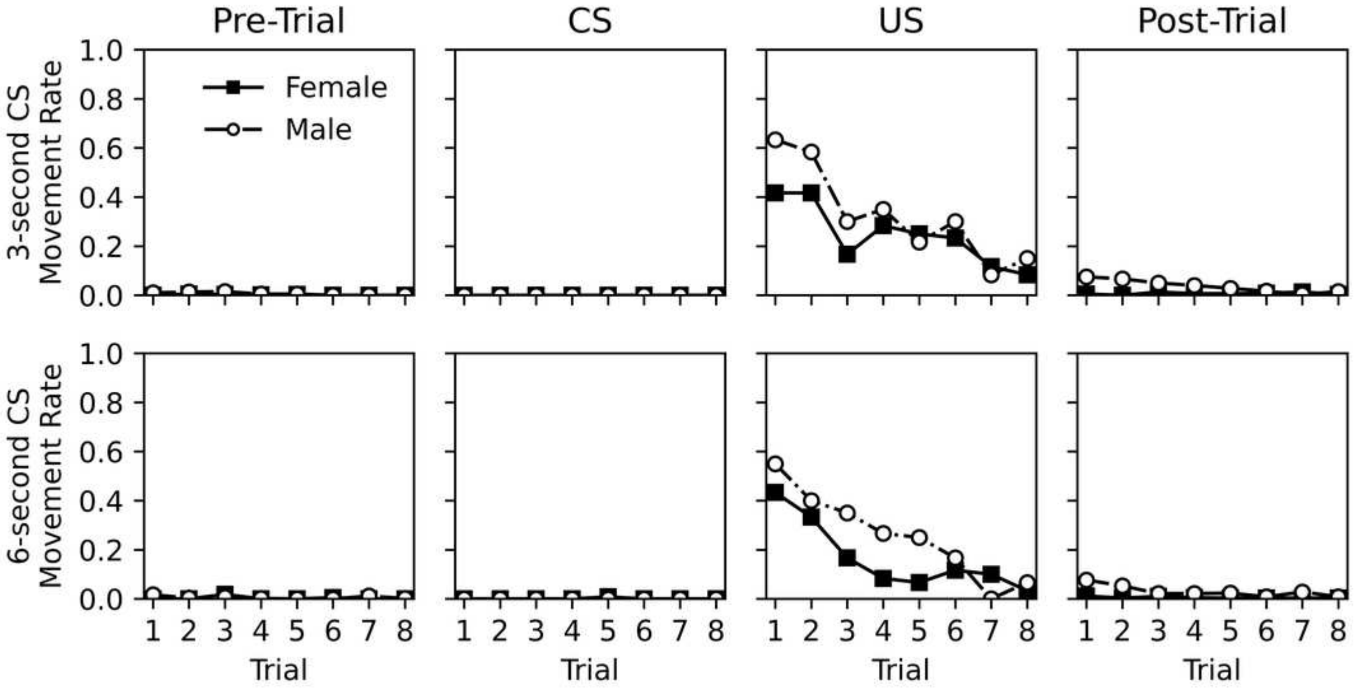

Figure 5

Distance from the CS odor in the preference assessment of Experiment 2. These distances can be reversed to show the duration subjects spent near the novel odor.

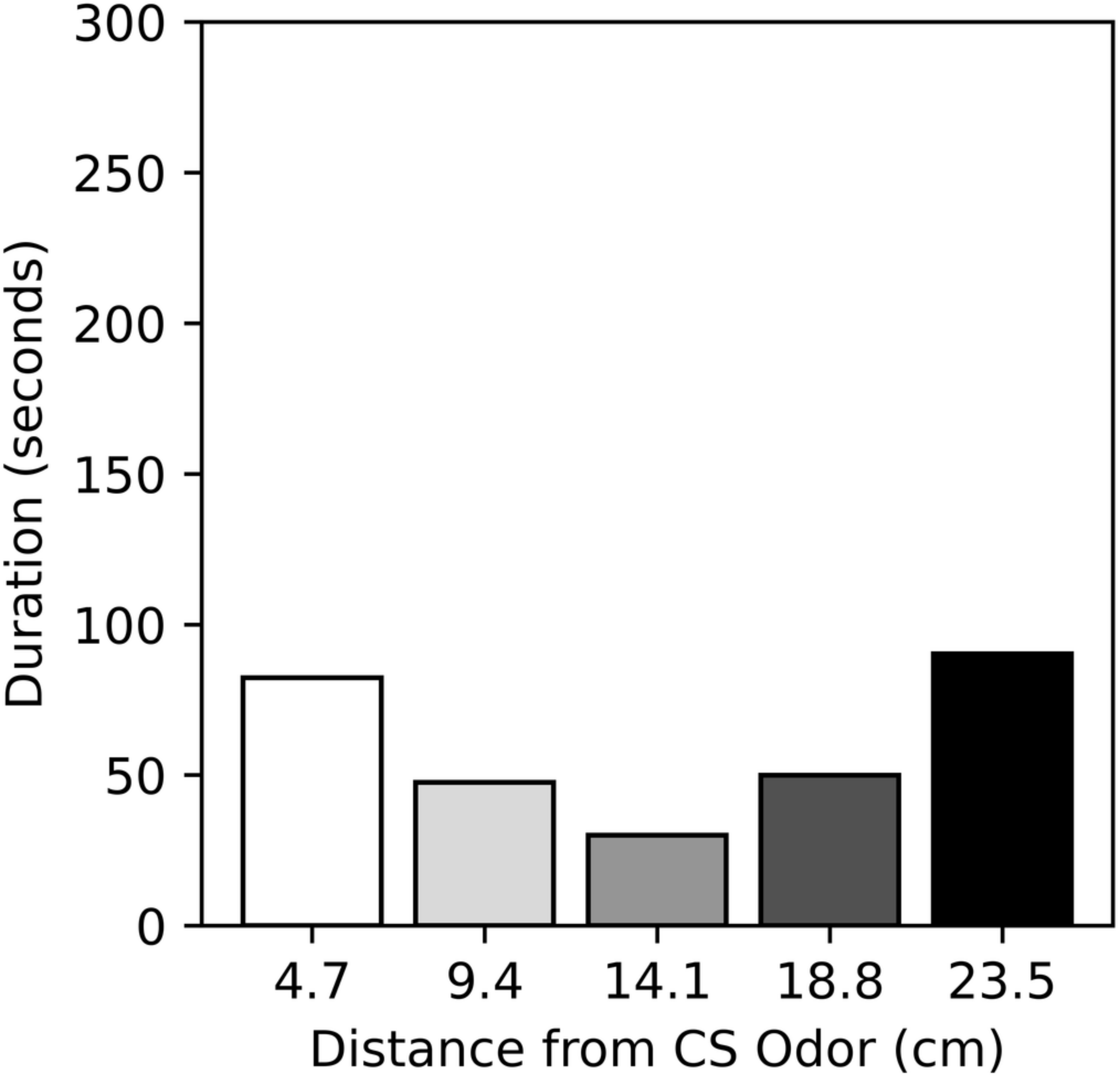

Figure 6

Rescorla-Wagner model predictions for hiss rate at several CS saliences (α). The UR line was derived from analysis in Table 5, excluding sex as a factor.

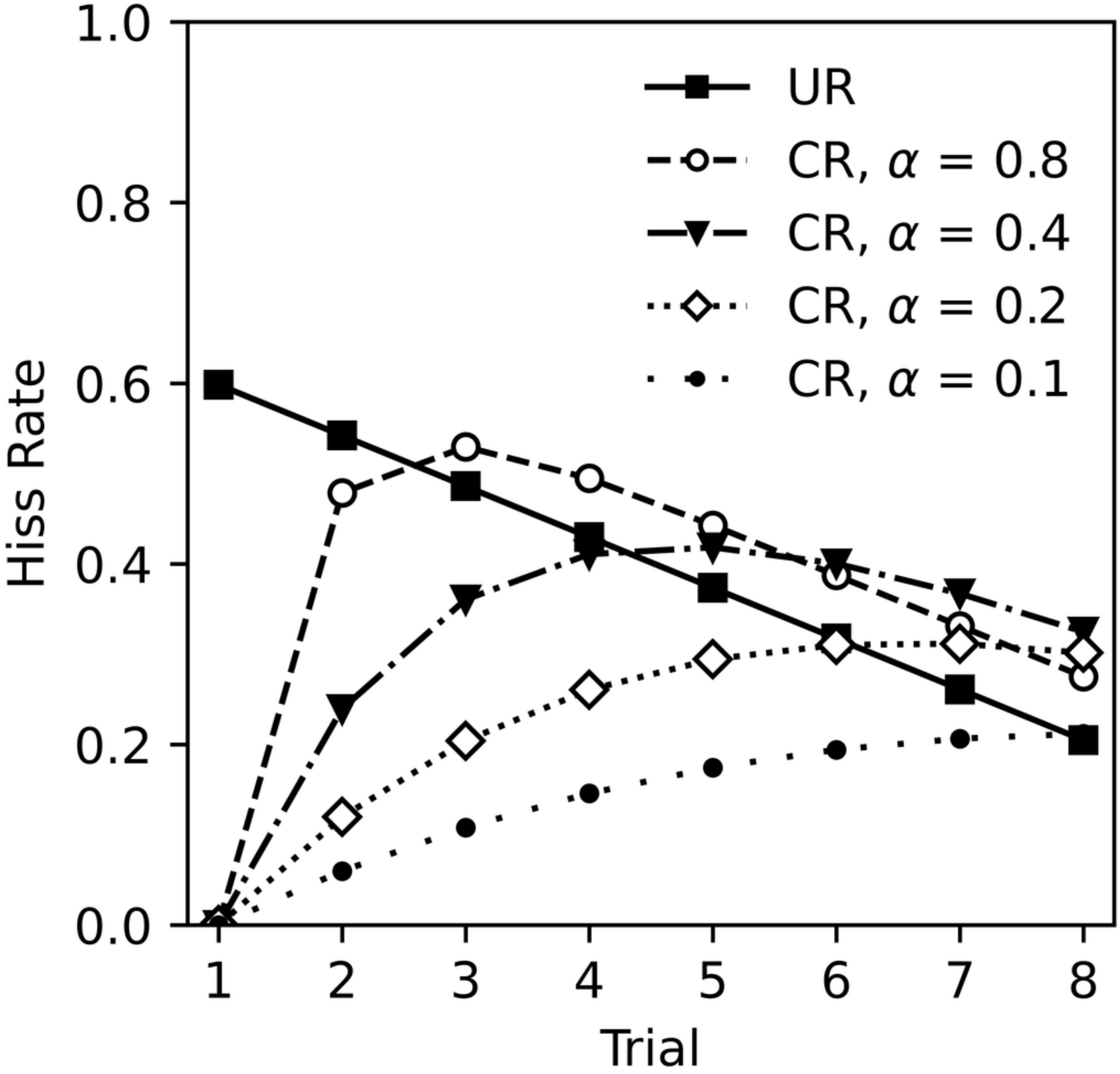

Table 1(on next page)

Experiment 1 Percent of Cockroaches Hissing

Table 1. Experiment 1 Percent of Cockroaches Hissing

Group	**Collection**	**Trial 1**	**Trial 14**	**Recollection**	**Never**
Unheated Female	40.00	30.00	05.00	15.00	30.00
Unheated Male	50.00	40.00	10.00	35.00	25.00
Heated Female	30.00	50.00	05.00	20.00	25.00
Heated Male	55.00	70.00	15.00	55.00	05.00
All Unheated	45.00	35.00	07.50	25.00	27.50
All Heated	42.50	60.00	10.00	37.50	15.00
All Female	35.00	40.00	05.00	17.50	27.50
All Male	52.50	55.00	12.50	45.00	15.00
All Cockroaches	43.75	47.50	08.75	31.25	21.25

Table 2(on next page)

Experiment 1 Hiss Rate Regression Analysis

Table 2. Experiment 1 Hiss Rate Regression Analysis

Parameter	Estimate	Standard Error	95% Confidence Intervals		*p*-Value
Intercept	0.068	0.013	0.043	0.093	0.000
Trial	-0.007	0.001	-0.009	-0.005	0.000
Heat	0.026	0.013	0.001	0.052	0.045
Sex	0.034	0.013	0.008	0.060	0.010

Note. Unheated females are included in the intercept.

Table 3(on next page)

Experiment 1 Movement Probability Regression Analysis

Table 3. Experiment 1 Movement Probability Regression Analysis

Parameter	**Estimate**	**Standard Error**	**95% Confidence Intervals**		***p*-Value**
Intercept	-0.164	0.262	-0.676	0.349	0.531
Trial	-0.118	0.018	-0.154	-0.083	0.000
Heat	0.326	0.275	-0.213	0.866	0.235
Sex	0.524	0.275	-0.015	1.063	0.057

Note. Unheated females are included in the intercept.

Table 4(on next page)

Experiment 2 Percent of Cockroaches Hissing

Table 4. Experiment 2 Percent of Cockroaches Hissing

Group	Collection	Trial 1	Trial 8	Recollection	Never
3s CS Female	60.00	45.00	15.00	50.00	25.00
3s CS Male	60.00	75.00	35.00	70.00	10.00
6s CS Female	70.00	50.00	10.00	45.00	10.00
6s CS Male	70.00	90.00	30.00	70.00	00.00
All 3s CS	60.00	60.00	25.00	60.00	17.50
All 6s CS	70.00	70.00	20.00	57.50	05.00
All Female	65.00	47.50	12.50	47.50	17.50
All Male	65.00	82.50	32.50	70.00	05.00
All Cockroaches	65.00	65.00	22.50	58.75	11.25

Table 5(on next page)

Experiment 2 Hiss Rate Regression Analysis

Table 5. Experiment 2 Hiss Rate Regression Analysis

US Hiss Rate

Parameter	Estimate	Standard Error	95% Confidence Intervals		*p*-Value
Intercept	0.479	0.066	0.350	0.608	0.000
Trial	-0.056	0.008	-0.071	-0.041	0.000
Sex	0.351	0.087	0.181	0.522	0.000

Post-Trial Hiss Rate

Parameter	Estimate	Standard Error	95% Confidence Intervals		*p*-Value
Intercept	0.010	0.023	0.054	0.146	0.000
Trial	-0.018	0.004	-0.027	-0.009	0.000
Sex	0.106	0.030	0.048	0.164	0.000

Note. Females are included in the intercept.

Table 6(on next page)

Experiment 2 Movement Rate Regression Analysis

Table 6. Experiment 2 Movement Rate Regression Analysis

US Movement Rate

Parameter	Estimate	Standard Error	95% Confidence Intervals		*p*-Value
Intercept	0.471	0.060	0.354	0.588	0.000
Trial	-0.059	0.008	-0.075	-0.043	0.000
Sex	0.085	0.050	-0.012	0.183	0.087

Post-Trial Movement Rate

Parameter	Estimate	Standard Error	95% Confidence Intervals		*p*-Value
Intercept	0.027	0.006	0.016	0.038	0.000
Trial	-0.005	0.001	-0.007	-0.002	0.000
Sex	0.027	0.005	0.017	0.038	0.000

Note. Females are included in the intercept.