

Limited evidence for learning in a shuttle box paradigm in crickets (*Acheta domesticus*)

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Abstract

Aversive learning has been studied in a variety of species, such as honey bees, mice, and non-human primates. Since aversive learning has been found in some invertebrates and mammals, it will be interesting to know if this ability is shared with crickets. This paper provides data on aversive learning in male and female house crickets (*Acheta domesticus*) using a shuttle box apparatus. Crickets are an ideal subject for these experiments due to their well-documented learning abilities in other contexts and their readily quantifiable behaviors. The shuttle box involves a two-compartment shock grid in which a 'master' cricket can learn to avoid the shock by moving to specific designated locations, while a paired yoked cricket is shocked regardless of its location and therefore cannot learn. Baseline control crickets were placed in the same device as the experimental crickets but did not receive a shock. Male and female master crickets demonstrated some aversive learning, as indicated by spending more time than expected by chance in the correct (no shock) location during some parts of the experiment, although there was high variability in performance. These results suggest that there is limited evidence that the house crickets in this experiment learned how to avoid the shock. Further research with additional stimuli and other cricket species should be conducted to determine if house crickets and other species of crickets exhibit aversive learning.

Keywords

aversion, Avoidance behavior, comparative, invertebrate learning, Orthoptera

Introduction

Aversive learning is crucial to an individual's survival. One example of aversive learning is taste aversion, which is an important defense against potential poisoning (Logue 1985, Bernstein 1999). We would expect many species to develop aversive learning since it is crucial to learn whether something is aversive rather than make the potentially fatal error of not learning from previous experience(s). As a result, aversive learning is found across many species, such as goldfish, terrestrial mollusks, mice, coyotes, non-

human primates, and humans (Gelperin 1975, Garcia et al. 1985, Logue 1985, Manteifel and Karelina 1996, Wright et al. 2010, Goltseker and Barak 2018).

Insects also demonstrate aversive learning (Abramson et al. 1977, Dethier 1980, Abramson 1986). Specifically, researchers have studied insect taste aversion related to foraging choices (Dethier 1980, Bernays 1993). Dethier (1980) first studied food aversion in polyphagous insects using two woolly bear caterpillars. These caterpillars exhibited aversive learning to petunias after recovering from acute illness linked to the consumption of the plant (Dethier 1980). Additionally, the grasshopper *Schistocerca americana* (Drury, 1773) exhibited taste aversion depending on the palatability of the food (Bernays and Lee 1988), although individuals of this species did not exhibit aversive learning following nicotine hydrogen tartrate poisoning when the food was highly palatable broccoli (Bernays and Lee 1988). In comparison, when presented with a less palatable food such as spinach, grasshoppers exhibited aversive learning (Bernays and Lee 1988). Honey bees also exhibit aversive learning, as demonstrated in escape, punishment, and avoidance paradigms (Abramson 1986), and harvester ants can learn to go to a specific area to terminate and passively avoid vibration (Abramson et al. 1977).

Shock is commonly used as an aversive stimulus in learning experiments (Garcia and Koelling 1966). Researchers have used shock as a stimulus in aversive learning experiments with rats (Garcia and Koelling 1966), humans (Lovibond et al. 2008), honey bees (Abramson 1986, Nuñez et al. 1997, Agarwal et al. 2011), and fruit flies (Tully and Quinn 1985). Nuñez and colleagues (1997) used shock to stimulate the stinging response in honey bees following previous injection of isopentyl acetate, which is the main component of the honey bee alarm pheromone. In a different experiment, Tully and Quinn (1985) used electrical shock pulses paired with a conditioned odor to determine whether trained fruit flies could learn to avoid the shock based on the paired odor stimulus.

Another way that shock is administered in experiments is through the use of an apparatus called the shuttle box—one of the oldest and most widely used apparatuses for the study of learning and memory (Warner 1932). A shuttle box is a chamber in which an organism can move back and forth ('shuttle') to avoid an aversive stimulus (Abramson 1986). A shuttle box can also be used to train an organism to move towards an attractive stimulus that is delivered to one side of the chamber (Abramson 1986). One of the earliest uses of the shuttle box was by Warner (1932), who studied avoidance learning in rodents. Over the years, shuttle boxes have been built for many organisms, including aquatic animals (Horner et al. 1961) and insects such as cockroaches, ants, and honey bees (Abramson et al. 1982, Abramson 1986). The shuttle box is designed primarily to study aversive conditioning such as escape, punishment, and both unsignaled and signaled avoidance (Abramson 1986). However, a shuttle box can also be used to study time allocation (DeCarlo and Abramson 1989), place learning (Agarwal et al. 2011), learned helplessness (Dinges et al. 2017), and caste differences in learning using social species like honey bees (Dinges et al. 2013). Moreover, with slight modifications, the shuttle box can be used to deliver appetitive stimuli such as food and odors (Abramson et al. 1982, DeCarlo and Abramson 1989).

Crickets are ideal for studying aversive learning because this group of species exhibits a variety of learned behaviors, ranging from associative learning of olfactory cues (Matsumoto and Mizunami 2000, 2002), to spatial learning (Wessnitzer et al. 2008, Doria et al. 2019), and even social learning (Coolen et al. 2005, Ebina and Mizunami 2020). For instance, the Mediterranean field cricket (*Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae)) has been shown to learn the association between specific odors with paired rewards and punishments (Matsumoto and Mizunami 2000). *G. bimaculatus* has also been shown to have good olfactory memory, with some associations being remembered for at least six weeks (Matsumoto and Mizunami 2002). Other work on memory in *G. bimaculatus* has shown that caffeine can improve long-term memory in this species (Sugimachi et al. 2016).

Crickets also exhibit spatial learning and memory. *G. bimaculatus* were placed in a stadium similar to a Morris water maze in which the traditional water and the hidden platform were replaced with a hot metal surface possessing a cool area on the platform's surface (Wessnitzer et al. 2008). The time that it took for *G. bimaculatus* to find the cool spot decreased with experience, indicating spatial learning (Wessnitzer et al. 2008). Texas field crickets (*Gryllus texensis* Cade & Otte, 2000) have also been tested in radial-arm mazes, where they had to remember which arm contained a food reward (Doria et al. 2019). The Texas field cricket's ability to learn has been linked to thigmotaxis (crickets' movement towards or away from a physical stimulus) (Doria et al. 2019). *G. bimaculatus* has also been used in prediction error theory experiments using visual and olfactory stimuli (Terao et al. 2015). The crickets were trained on either an olfactory or visual stimulus; after the training, they were given a combined visual/olfactory stimulus before being tested on the stimulus that they were not initially tested on. The crickets that initially learned by a visual pattern were less capable of finding the reward when only olfactory stimuli were available, even though they ran several trials with both stimuli combined (Terao et al. 2015).

There is also some evidence for social learning in crickets, although this has been less explored. One social learning experiment involved naive *Nemobius sylvestris* (Bosc, 1792) learning anti-predator behaviors from more experienced conspecifics (Coolen et al. 2005). This was done by placing naive crickets with demon-

strators and placing an odor that demonstrators had learned to associate with a predator in a container. The demonstrators would burrow into the leaf litter when exposed to that odor, followed by the naive crickets, whereas on their own the naive crickets did not display any anti-predator behavior (Coolen et al. 2005). *G. bimaculatus* is also capable of associating the presence of conspecifics with rewards (Ebina and Mizunami 2020).

No studies of cricket learning have employed a shuttle box. The shuttle box has two major advantages that make it worth exploring as a test paradigm in crickets: it is automated, and it can be used to test a wide range of learning behaviors. The automation of the shuttle box is a major advantage since it allows for the apparatus to be used consistently and repeatedly with a variety of species and experimental designs. Furthermore, as there have been many shuttle box experiments with a wide variety of organisms, it will be interesting to compare cricket behavior in the shuttle box to that of other species to gain insight into species differences in learning. It would also open the door to the 'psychological' study of cricket behavior, as many interesting psychological phenomena such as social learning and spatial memory can be explored (see above).

In the present study, we tested the suitability of using the shuttle box for behavioral studies of learning using house crickets *Acheta domesticus* (Linnaeus, 1758) (Orthoptera: Gryllidae). Crickets have many benefits as model organisms for behavioral studies. They are usually easy to maintain and exhibit a wide range of interesting behaviors, including social behaviors and learning. In addition, crickets are short-lived and have clear developmental markers (e.g., wing development, chirping, and development of an ovipositor). These traits allow researchers to use individuals that are all at the same life stage, identify the males and females, and help to minimize differences between subjects.

Methods

Subjects.—Subjects consisted of 130 house crickets [females $n = 78$, males $n = 52$ (*Acheta domesticus*)] collected from colonies maintained for laboratory purposes sourced from Fluker Farms, Louisiana. Crickets were sorted into two different communal containers based on sex. In the containers, crickets had a source of cover (piece of egg carton or cardboard), ground up chicken feed in petri dishes, and distilled water in a Falcon tube closed with a cotton ball. The food and water were refilled every 48 hours. Crickets were housed in this manner until they were needed for the experiment. Only mature crickets (crickets with fully developed wings) were tested.

Apparatus.—The present experiment made use of a modified shuttle box apparatus (see Fig. 1 and Suppl. material 1: Cricket Shuttle box Video), similar to that used by Dinges et al. (2013). The apparatus consisted of two separate compartments with external dimensions of 200 mm × 60 mm × 25 mm and internal dimensions of 140 mm × 20 mm × 10 mm. Each compartment contained a series of 55 shock grid pins and a set of two infrared LED and receptor pairs. Pins were 1 mm in diameter and placed 2.5 mm apart. Pins were placed so that when subjects contacted consecutive pins—completing the circuit—shock was applied to the grid, which would be felt by the animal. Once an animal was introduced to a compartment, a piece of clear Plexiglas measuring 145 mm × 25 mm × 5 mm was placed on top of the compartment to ensure that the crickets remained in contact with the grid. When each cricket was in its compartment, it was unable to see or communicate with the cricket in the other compartment.



Fig. 1. The shuttle box apparatus with propeller controller in the upper left-hand corner. In this image, one of the plexiglass covers (A) has been removed so that the shock grid can be seen more easily. In the experiments, one cricket was placed into the compartment labeled A and one cricket was placed into the compartment labeled B. A piece of filter paper was placed under the left-hand side of the shuttle box to illustrate the midpoint for the two halves of the compartment. Once the cricket was inside the compartment, the plexiglass was placed over the compartment to ensure that the cricket maintained contact with the grid.

Each compartment was then connected to a control box containing a Propeller Experiment Controller (Parallax Inc., Rocklin, CA; Varnon and Abramson 2013, 2018) and an external variable DC power supply for the shock grid. Experimental parameters (location of the shock based on the position of the master cricket: left half near, left half away, right half near, and right half away) were set using the control panel and automatically implemented through the Propeller controller. When master crickets (see below) crossed into the side of the compartment paired with shock, electricity was applied to the grids of both compartments. This ensured that the yoked subjects experienced shock at the same time and duration as the master subjects, regardless of their position in the compartment. Shock terminated upon the master subject entering the safe side of the apparatus.

Behavioral assay.—Preliminary experiments were conducted to determine the appropriate voltage and amplitude. During the preliminary experiments, individual crickets were shocked at various voltages and amplitudes. Crickets were observed for behaviors

(such as digging) that indicate the presence of a noxious stimulus (i.e., shock). Observations were made about the ability of the individuals to enact behaviors to escape the shock.

For the formal experiment, same-sex cricket pairs were captured from the container using a plastic shot glass and a notecard. Each individual was placed in a separate compartment. Once they were in the shuttle box, a three-minute habituation phase started where no shock was administered. The habituation phase provided crickets with time to recover from any stress associated with the transfer from the container to the apparatus. After the habituation phase, and once both crickets crossed the midline in either direction, the experimental trial began. During the experimental trial, shock was delivered at 12 VDC and 0.5 A.

The formal trial duration was 9 minutes. Results of previous research using this setup with honey bees (*Apis mellifera* Linnaeus, 1758) found that experimental fatigue occurs after 5 minutes (Black et al. 2018), so we tested for longer than this to see if crickets exhibit a similar behavioral pattern to the honey bees. After each cricket pair completed the trials, the shuttle box was cleaned with 90% EtOH and a Kimwipe (Kimtech Science) to remove chemical cues. Crickets were only used once. A Propeller Experiment Controller (Varnon and Abramson 2013, 2018) automatically recorded cricket position, time of movement between the two sides, and onset or offset of the shock.

Crickets were randomly assigned to the role of ‘master,’ ‘yoked,’ or ‘baseline.’ Crickets were placed in the apparatus with one cricket in each compartment. The baseline cricket pair served as an experimental control in which no cricket received a shock. The master and yoked cricket pairs were the experimental pairs where one cricket’s behavior (master) determined whether both crickets were shocked. For these pairs, the shuttle box was assigned to administer shock on one of the two sides of the grid in each compartment (i.e., either on the half away from or towards the researcher). The side that was associated with the shock was randomly chosen and did not change during the trial. If the master cricket moved to the side that was shocked, then both crickets received the shock. Once the master cricket left the side corresponding to the shock, both crickets stopped receiving the shock. The yoked cricket was unable to observe or communicate with the master cricket and therefore received the shock regardless of what side of the compartment it was on.

The reason for the pairing between the master and the yoked cricket was to set up a situation where one cricket had the opportunity to learn (the master cricket) and the other (yoked cricket) was essentially a control that experienced the same conditions, including the experience of electric shock, but could not learn because there was no consistent association between its behavior and the shock. Master crickets could learn to avoid the side associated with the shock, while yoked crickets would not be able to associate either side with a shock. As a result, the side preferences of the yoked crickets should resemble those of the baseline crickets: neither of these crickets should show a bias towards one side or the other. In contrast, if the master crickets are able to learn the association, they should spend less time on the side associated with the shock than either the baseline or the yoked cricket.

The data came from a 9-minute trial that was divided into 60-second intervals for data management purposes. The amount of time that was spent on the side that was not linked to shock (hereafter ‘correct side’) was calculated for nine 60-second intervals starting at 0 s and ending at 540 s. The proportion of time spent on the correct side was calculated in 60-second intervals by dividing the actual amount of time spent on the correct side by 60 s.

All the data were analyzed using R version 4.1.0 (R Core Team 2021) and R Studio version 1.4 (R Core Team 2021). A suite of one sample *t*-tests with Bonferroni corrections were used to determine whether the performances (proportion of time spent on the correct side for each 60 s interval) of the experimental groups for the male and female crickets differed from chance (0.50). The requirements for the parametric tests were satisfied. A Bonferroni adjusted α value of 0.003 was used. Then, a linear mixed model (LMM) was conducted using the lmer function in lme4 version 1.1-27 package (Bates et al. 2015) to determine the effects of time point (i.e., 60-second intervals) and the interaction between experimental role and sex on the amount of time spent on the correct side.

Results

The first two sets of *t*-tests were used to compare the percentage of time the male and female shock-free control (e.g., baseline) crickets spent on the correct side compared to random chance (50%) (Table 1; Figs 2, 3). Neither the male nor female baseline crickets exhibited significant differences from chance in the amount of time spent on the correct side (Table 1; Figs 2, 3). These results indicate that the baseline crickets are representative of normal behavior when in the apparatus without aversive conditions (Black et al. 2021).

Two additional sets of *t*-tests were conducted on the male and female yoked crickets to see if the percentage of time they spent on the correct side differed from chance (Table 2; Figs 2, 3). Like the baseline crickets, the yoked crickets did not exhibit significant differences from chance (Table 1; Figs 2, 3). In comparison, the master crickets exhibited statistically significant differences at the 60 s time

point for the males and the 480 s time point for the females (Table 3; Figs 2, 3). These results indicate that at the 60 s time point and at the 480 s time point, the males and the females, respectively, spent significantly more time on the correct side compared to chance.

The results from the LMM showed that time point was a significant predictor of amount of time spent on the correct side, with crickets spending more time on the correct side as the experiment progressed (Table 4). The interactions between experimental role and sex for master and yoked were not significant (Table 4). Overall, these results suggest that the amount of time that crickets spent on the correct side differed depending on the time point.

The findings from the first LMM suggest that cricket learning occurred at different time points, as seen by the difference in male (beginning of trial) and female (end of trial) learning (Fig. 3). The second LMM with only the master crickets did not show that time point or sex were significant predictors of amount of time spent on the correct side (Table 5). Additionally, the interaction between time point and sex was not significant. These findings suggest that the amount of time the male and female crickets spent on each side did not differ over time.

Simultaneous pairwise comparisons using Tukey’s HSD test indicated that the difference between the master group and the yoked control was statistically significant (Table 6). Tukey’s HSD test did not indicate significant differences between the master group and the shock-free baseline control group (Table 6). There was also no significant difference between the yoked control and the shock-free control group (Table 6). These results suggest that learning occurred for the master crickets when they were compared to the yoked crickets, but not when they were compared to the baseline crickets.

Table 1. One sample *t*-test results for male and female no shock control (baseline) crickets compared to random chance (0.5). *M* = proportion of time spent on the correct side; females *df* = 17; males *df* = 9; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		t	p	Males		t	p
	M	SD			M	SD		
60	0.55	0.33	0.63	0.538	0.75	0.25	3.13	0.012
120	0.56	0.35	0.67	0.513	0.57	0.30	0.76	0.464
180	0.35	0.32	-2.03	0.058	0.50	0.29	-0.00	1.000
240	0.43	0.37	-0.74	0.471	0.59	0.38	0.77	0.461
300	0.51	0.41	0.11	0.916	0.44	0.40	-0.50	0.629
360	0.44	0.40	-0.62	0.544	0.39	0.40	-0.83	0.429
420	0.59	0.45	0.85	0.407	0.35	0.35	-1.31	0.224
480	0.67	0.42	1.74	0.100	0.49	0.38	-0.04	0.966
540	0.54	0.40	0.43	0.672	0.54	0.46	0.31	0.766

Table 2. One sample *t*-test results for male and female yoked control crickets compared to random chance (0.5). *M* = proportion of time spent on the correct side; females *df* = 29; males *df* = 20; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		t	p	Males		t	p
	M	SD			M	SD		
60	0.45	0.37	-0.81	0.427	0.46	0.27	-0.71	0.488
120	0.53	0.41	0.40	0.692	0.42	0.32	-1.18	0.251
180	0.41	0.37	-1.33	0.194	0.44	0.36	-0.79	0.439
240	0.53	0.40	0.46	0.652	0.51	0.45	0.06	0.950
300	0.60	0.42	1.35	0.188	0.42	0.42	-0.91	0.373
360	0.55	0.41	0.70	0.489	0.44	0.44	-0.61	0.551
420	0.46	0.43	-0.54	0.591	0.51	0.47	0.13	0.897
480	0.60	0.42	1.25	0.222	0.54	0.45	0.41	0.688
540	0.60	0.44	1.24	0.226	0.53	0.41	0.29	0.771

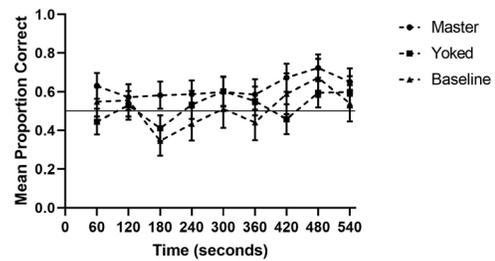


Fig. 2. Proportion of mean time spent on correct side of the apparatus for female crickets during each 60 s interval. Female *N* values: Baseline = 18, Yoked = 30, and Master = 30. The bars on the data points represent the standard errors. The solid line at 0.5 indicates chance.

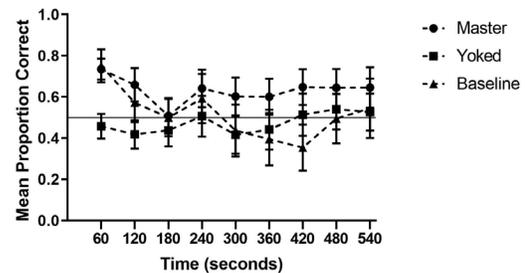


Fig. 3. Proportion of mean time spent on correct side of the apparatus for male crickets during each 60 s interval. Male *N* values: Baseline = 10, Yoked = 21, Master = 21. The bars on the data points represent the standard errors. The solid line at 0.5 indicates chance.

Table 3. One sample *t*-test results for male and female behavioral (master) crickets compared to random chance (0.5). *M* = proportion of time spent on the correct side; females *df* = 29; males *df* = 20; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		<i>t</i>	<i>p</i>	Males		<i>t</i>	<i>p</i>
	<i>M</i>	<i>SD</i>			<i>M</i>	<i>SD</i>		
60	0.63	0.37	1.94	0.062	0.73	0.23	4.620	0.000*
120	0.57	0.36	1.10	0.280	0.66	0.37	1.972	0.063
180	0.58	0.38	1.17	0.250	0.51	0.40	0.089	0.930
240	0.59	0.39	1.24	0.224	0.64	0.42	1.555	0.136
300	0.60	0.42	1.29	0.206	0.60	0.42	1.108	0.281
360	0.59	0.43	1.10	0.282	0.60	0.40	1.142	0.267
420	0.67	0.40	2.37	0.025	0.65	0.40	1.690	0.106
480	0.72	0.38	3.22	0.003*	0.64	0.42	1.596	0.126
540	0.65	0.39	2.13	0.042	0.65	0.45	1.474	0.156

Table 4. Results of the LMM model to test the effects of time point, experimental role, and sex on time spent on the correct side. Significant predictors: * $p < 0.05$ and ** $p < 0.001$.

Independent	Predictor	Estimate	SE	<i>t</i> -value	95% CI	<i>p</i>	
Percent of time spent on correct side	Intercept	29.17	3.73	7.83	21.87	36.48	<0.001**
	Time Point	0.01	0.00	2.04	0.00	0.01	0.042*
	Exp. Role [Master]	6.67	4.56	1.45	-2.33	15.66	0.146
	Exp. Role [Yoked]	0.72	4.56	0.16	-8.27	9.71	0.875
	Sex [Male]	1.28	7.25	0.18	-12.93	15.50	0.860
	Exp. Role [Master]	-1.12	8.41	-0.13	-17.61	15.37	0.894
	*Sex [Male]						
	Exp. Role [Yoked] *Sex [Male]	-5.14	8.4	-0.61	-21.63	11.35	0.541

Table 5. Results of the master cricket LMM model to test the effects of time point, experimental role, and sex on time spent on the correct side. Significant predictors: * $p < 0.05$ and ** $p < 0.001$.

Independent	Predictor	Estimate	SE	<i>t</i> -value	95% CI	<i>p</i>	
Percent of time spent on correct side	Intercept	35.02	3.35	10.45	28.45	41.59	<0.001**
	Time Point	0.01	0.01	1.50	-0.00	0.02	0.134
	Sex [Male]	3.36	5.09	0.66	-6.62	13.33	0.510
	Time Point	-0.01	0.01	-1.23	-0.03	0.01	0.220
	*Sex [Male]						

Table 6. Results of the LMM model Tukey post-hoc pairwise comparisons for experimental role. * $p < 0.05$, *df* = 126.

Exp. Role 1	Exp. Role 2	Estimate	SE	<i>t</i> -value	<i>p</i>
Baseline	Master	-6.11	4.21	-1.45	0.32
Baseline	Yoked	1.85	4.21	0.44	0.90
Master	Yoked	7.95	3.02	2.64	0.03*

Discussion

The series of experiments presented in this study had two goals. One goal was to investigate whether house crickets, *A. domesticus*, exhibit aversive learning. The other goal was to determine if the shuttle box is a suitable apparatus for studying aversive learning with crickets. The results show that the male master crickets' behavior exhibited learning at the beginning of the experimental trials, while the female master crickets' behavior exhibited learning towards the end of the experimental trials (Table 1). As in experi-

ments with other species of crickets (Matsumoto and Mizunami 2000, 2002, Doria et al. 2019, Wessnitzer et al. 2008), both the male and female house crickets in our experiments exhibited behavior indicative of learning (Tables 3, 4).

The master male crickets exhibited learning early in the experiment because they spent significantly more time than chance on the correct side. In comparison, the master female crickets exhibited learning later in the experiment, as they spent significantly more time than chance on the correct side. For both males and females, the yoked control pairs and no shock control pairs all performed similarly and were no different from the chance expectation of 50% (Fig. 2). While the amount of time spent on the correct side for both the male master and female master, yoked controls, and no-shock baseline control crickets all appear to be similar, the results from the LMM indicated that there was a significant difference between the master and yoked experimental roles but not between the master and the baseline crickets (Fig. 3). The performance of the controls was as expected: their behavior did not differ from chance. The master crickets, however, were expected to perform better than chance, but, at most, they performed better than chance for only small portions of the experiment.

Generally, honey bees hit around 60–75% on performance in this assay and maintained that performance over time, which has been taken as evidence for aversive learning (Dinges et al. 2013). The crickets from this experiment demonstrated similar behavioral patterns, with the average proportion of time spent on the correct side by the male and female master crickets ranging from 0.56 to 0.75 (Table 3; Figs 2, 3). The reason for the statistical differences between the crickets and the bees might be due to greater variance in performance by the crickets. Although on average the master male and female crickets spent around 60–75% of their time on the correct side throughout the trials (Table 3), the standard errors of the mean proportion of time on the correct side for the master crickets are quite large, indicating substantial variance within the data (Figs 2, 3).

Unlike the honey bees (Black et al. 2018), the crickets' behavior does not suggest that they experienced experimental fatigue or a decrease in their performances. The average proportion of time spent on the correct side for the master crickets in the experiment decreased somewhat for the males at 180 s and then increased at 240 s and remained relatively stable (Fig. 3). The master female crickets' proportion of time spent on the correct side stayed relatively stable and increased at 420 and 480 s. Overall, despite the slight decrease for the males, the proportion of time spent on the correct side for both males and females appeared to be on average better than chance (50%); however, these findings were not statistically significant (Table 3), suggesting no evidence for learning when considering performance across the entire duration of the experiment.

Crickets in the shuttle box responded to the shock by exhibiting digging behavior (Suppl. material 2: Cricket Digging Behavior Video). We observed the crickets digging with their front legs in response to the shock. Anecdotal observations of this behavior indicated that crickets displayed differences in digging behavior during the experimental trials when they were shocked. An experiment by Coolen et al. (2005) found that crickets exhibited burrowing behaviors such as digging when certain odors and cues associated with wolf spiders were put in their containers. We believe that the crickets in our experiment exhibited this digging response in the presence of the shock due to it being an aversive stimulus.

One improvement to the design that may enhance the ability of crickets to learn would be the addition of visual or olfac-

tory stimuli. Previous research showed that individuals of other species are able to orient and can learn that if they are getting shocked, the shock will cease when they move to the other side of the arena. The addition of other cues could enhance learning but are not necessary for learning to occur (Dinges et al. 2013). Findings from previous experiments demonstrate that crickets can use visual and olfactory stimuli in learning experiments (Doria et al. 2019, Matsumoto and Mizunami 2000, Terao et al. 2015). Additional stimuli might, therefore, provide the crickets with information that would allow them to learn the association with the shock more easily.

Another possible improvement would be to replicate this experiment using a cricket species other than *A. domesticus*. Previous learning experiments in crickets have focused on species in the genus *Gryllus*, e.g., *Gryllus bimaculatus* (Doria et al. 2019; Matsumoto and Mizunami 2000) and *Gryllus texensis* (Terao et al. 2015), and there may be species differences in learning ability. The house crickets that we used were purchased from a cricket farm and have likely been in captivity for many generations. As a result, they may have been selected for fast growth, aggression in foraging, and disease resistance, which could affect performance relative to natural populations of other species.

The use of the shuttle box as described here is promising. We were able to demonstrate the predicted avoidance behavior in a majority of our animals in the master group. However, there are still some unanswered questions that must be addressed before the apparatus can gain wide applicability. These questions include appropriate spacing between the shock bars and variations in shock intensity. We believe that these are relatively minor issues and easily addressed in future studies. For example, consider a lever press situation for crabs. Abramson and Feinman (1990) found that restraining them with clamps produced poor results, but enclosing the crabs in a small box produced effective lever pressing. A similar situation was found with the proboscis conditioning of stingless bees. Restraining stingless bees in tubes did not produce any proboscis conditioning, but putting them in small bottles where they made contact with the stimuli through a screen produced rapid learning (Amaya-Márquez et al. 2019). A similar modification may be needed if the shuttle box is to be useful. One potential idea is to create a 'one-way' or 'circular' shuttle box where the cricket is always going in the same direction and therefore avoids entering a compartment where it just received a shock.

This study provided important information about the learning abilities of house crickets and the suitability of using a shuttle box. Our experiment tested the house crickets' ability to learn through aversive stimuli (i.e., shock). The behavior of both the female and male master cricket demonstrated limited aversive learning. Previous research has provided evidence of the learning abilities of crickets in other contexts. Further investigation into the learning abilities of house crickets and other cricket species through modifications of this aversive learning paradigm might provide more evidence on whether house crickets and other cricket species can learn through aversive conditioning using a shuttle box.

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