STUDIES OF LEARNED HELPLESSNESS IN HONEY BEES (*APIS MELLIFERA LIGUSTICA*)

By

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Abstract: The current study provides evidence of learned helplessness in the honey bee (*Apis mellifera* L.). Bees received either avoidable or unavoidable shock during a discriminative compartment restriction task in an automated shuttle box. Decreased avoidance behavior was observed when bees received unavoidable shock prior to avoidable shock tests, conserving a non-preference response pattern. Prior training with avoidable shock created a preference that was conserved when shock was later unavoidable. Length of the training time impacted how pronounced the conserved behavior was in subsequent tests. Unlike existing learned helplessness studies in other animals, no decrease in general activity was observed. These findings identify honey bees as a unique model organism to explore the process of learned helplessness.

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CHAPTER I

INTRODUCTION

Learned helplessness is one of the most well-known psychological phenomena (Garber & Seligman, 1980; Seligman, 1992). The original demonstration, Overmier and Seligman (1967), established that dogs exposed to bouts of inescapable shock perform poorly when subsequently permitted to escape shock. This study not only contributed to new approaches and insights in treating depression, but also is one of the few clinical studies that can be said to have stimulated comparative psychologists to explore the generality of such an effect across the phylogenetic scale.

Learned helplessness appears to be a ubiquitous behavioral phenomena that may be a fundamental neurological process. It has been observed in isolated or incomplete components of nervous systems such as headless insects (Horridge, 1962), individual ganglia (Eisenstein & Cohen, 1964), and spinal rats (Buerger & Chopin, 1976). These and other findings are reviewed in Eisenstein, Carlson, and Harris's (1997) ganglionic model of learned helplessness, which suggests that the brain is not required to produce learned helplessness. A related finding is that experience with escapable shock can reduce the effects of learned helplessness, producing an immunization effect (Brown, Howe, & Jones, 1990; Seligman & Maier, 1967). The immunization effect is also found across taxa, although it has not been observed in headless insects or isolated ganglia (Eisenstein & Carlson, 1997). See Table 1 for an overview of comparative work in learned helplessness and immunization.

Learned helplessness is a fundamental, wide-spread behavioral phenomena, but recent research has neglected this topic. Figure 1 shows a notable decrease in learned helplessness studies since the 1980s. This is unfortunate considering the clinical implications learned helplessness has to human disorders such as depression and post-traumatic stress disorder (Garber & Seligman, 1980; Miller & Seligman, 1975; Seligman, 1992).

The purpose of the present investigation is to demonstrate the usefulness of the honey bee (*Apis mellifera* L.*)* as a model organism to study learned helplessness. Honey bees may be especially suited for learned helplessness research as much is known about their natural history, social structure and physiology (Crane, 1999; Seeley, 1995). Additionally, many automated techniques can be used with honey bees that are similar to vertebrate methods (Scheiner *et al*., 2010), enabling many cross-species comparisons. Perhaps most importantly, honey bees are becoming a popular model organism to study many behavioral processes including ethanol-induced behavior (Abramson, Craig, Varnon, & Wells, 2015), addiction (Søvik & Barron, 2013), decision-making (Cakmak *et al*., 2010), and perception of time (Craig, Varnon, Sokolowski, Wells, & Abramson, 2014). In the present experiments, we demonstrate that honey bees can also be used to study learned helplessness and immunization to learned helplessness using shock as an aversive stimulus and color as a discriminative stimulus.

CHAPTER II

LITERATURE REVIEW

In their classic study, Overmier and Seligman (1967) found dogs exposed to uncontrollable and inescapable shock failed escape and avoidance tests when control was later returned to the animals. In contrast, animals trained from the outset with an escape/avoidance contingency quickly learned the task. The debilitating effects of inescapable shock on the ability to later learn escape or avoidance is called “learned helplessness” (Maier, 1970; Maier, Seligman, & Soloman, 1969; Overmier, 1968; Overmier & Seligman, 1967; Seligman, 1975).

Learned helplessness has been studied in cats (Seward & Humphrey, 1967), cockroaches (Brown, Busby, & Klopfenstein, (1992); Brown, Howe, & Jones (1990); Brown, Hughes, & Jones, 1988; Brown & Stroup, 1988; Brown, Anderson, & Scruggs, 1994), dogs (Overmier, 1968; Overmier & Seligman, 1967; Seligman & Groves, 1970; Seligman & Maier, 1967; Seligman, Maier, & Geer, 1968), gerbils (Brown & Dixon, 1983), goldfish (Nash, Martinez, Dudeck, & Davis, 1983; Padilla, Padilla, Ketterer, & Giacalone, 1970; Brown, Smith, & Peters, 1985), humans (Hiroto, 1974; Thornton & Jacobs, 1971; Hokanson, DeGood, Forrest, & Brittain, 1971; Fosco & Geer, 1971; Glass & Singer, 1972; Hiroto & Seligman, 1975; Klein, Fencil-Morse, & Seligman, 1976;

Krantz, Glass, & Snyder, 1974; Roth, 1973; Roth & Bootzin, 1974; Roth & Kubal 1975; Thornton & Jacobs, 1971), mice (Braud, Wepman, & Russo, 1969), rats (Maier, Albin, & Testa, 1973; Seligman & Beagley, 1975; Seligman, Rosellini, & Kozak, 1975), and slugs (Brown, Davenport, & Howe, 1995; Brown, Davenport, & Howe, 1994). Even though learned helplessness is deep seated and ubiquitous behavior, interest in learned helplessness has been steadily decreasing since the 1980s. Despite the decrease in interest over the years, the theory of learned helplessness remains as a viable interpretation of seemingly maladaptive behavior with broad clinical applications. The behavioral analogs between learned helplessness and human depression are of particular note.

Hiroto (1974) investigated learned helplessness in humans utilizing a triadic design typical of animal learned helplessness research. The triadic design consisted of an escape condition group that could escape an aversive noise by a button press, an inescapable condition group yoked to the escape condition group that could not escape the noise, and a group that received no noise. They found participants trained in the inescapable condition failed to acquire escape behaviors when control of the stimulus was later given to them. Furthermore, Hiroto added an extra condition to the experiment: half of the participants in each group were told the task was a test of skill, the other was told the task was purely driven by chance. With this manipulation, the influence of “locus of control,” either internal (skill) or external (chance), was varied in the investigation. Hiroto found those with an external locus of control became helpless more readily than did those with an internal locus of control. Attribution was thus manipulated and created expectations of contingency and non-contingency, respectively. Attributional perception is key to resisting or developing helplessness symptoms (Garber & Seligman, 1980).

Such perceptions of environment and control are important determinants of human depression.

For humans, expectation of uncontrollability is sufficient to produce the behavioral and psychological deficits typical of depressive symptoms (Abramson & Martin, 1982; Abramson, *et al*., 1978; Garber & Seligman, 1980; Peterson & Seligman, 1984; Seligman, 1975). Hyland (1987) describes learned helplessness in relation to the control theory of depression as an error between perception of control and actual control. The control theory of depression is characterized by perceptual input, how well that perceptual input meets a reference criterion, and how sensitive the person is to errors between the perceptual input and reference criterion. Control theory incorporates a control loop that compares the perceptual input (perceptions of environmental contingencies) to a reference criterion (goal) and in the event of an error, guides behavior to reduce error between perceptual input and the reference criterion. In the case of learned helplessness, the reference criterion is control of an outcome and the perceptual input is the perception of environment-behavior interaction. Continued errors between reference criterion and perceptual input can result in depressive symptoms, especially in individuals primed to externalize control.

The type of control mismatch produced by learned helplessness is associated with deficits in motivational, cognitive, and emotional faculties. Maier and Seligman (1976) describe these deficits in learned helplessness trained subjects as such: 1) Motivation to respond to continued aversive stimuli seems to decrease with repeated exposures. 2) In the event that a response is performed to terminate the aversive stimulus in control tests, the subject has difficulty associating the response and the resulting escape. 3) Emotional balance is disturbed; depression and anxiety result and may present in a variety of measures. These descriptions of internal processes are products of learned helplessness training in humans. These deficits may also be inferred to occur in other animals such as dogs and cats, and such inferences may be accurate. However, these internal processes are unnecessary when considering the behavioral product of learned helplessness training.

The learned helplessness effect has often been couched in such internal processes as ‘feelings of helplessness’ and ‘feelings of hopelessness.’ Descriptors of internal processes such as these may apply to human and higher vertebrate learned helplessness, but these terms may be inappropriate for analogous behaviors in simpler systems. Learned helplessness has been produced in both vertebrates and invertebrates with strikingly similar results. The prevalence of such ubiquity calls to question cognitive interpretations of the learning phenomenon. Learned helplessness has been produced in headless cockroaches and locusts (Horridge, 1962), isolated ganglia in the cockroach prothoracic legs (Eisenstein & Cohen, 1964), and even spinal rats (Buerger & Chopin, 1976). It is unlikely that a brainless animal is capable of ‘feelings of helplessness.’

Eisenstein and Carlson (1997) reviewed the use of the term ‘learned helplessness’ and offered a more behaviorally neutral, albeit longer, name for these simpler systems: “learned decrease in avoidance/escape behavior” (LDE/A). The brain is not required for LDE/A to occur. Furthermore, it is important to note that not all criteria of learned helplessness have been produced in “brainless systems,” but LDE/A has been observed in these systems. Currently, no investigation has yet reported on immunization in headless insects or isolated ganglia. Immunization to learned helplessness, or “resistance to learned decrease in avoidance/escape behavior," may be key to distinguishing between instances of true learned helplessness, replete with the associated cognitive descriptors, and the "cognition free" description LDE/A. We incorporate an immunization test in the current study; however, some criteria of learned helplessness may need to be revised for new organisms.

As previously mentioned, LDE/A has been shown to be ubiquitous across taxa and has been interpreted as learned helplessness in many; however, the organisms investigated thus far have been solitary invertebrates or solitary/social vertebrates. There have been no investigations of social bees and social wasps. This leads to a somewhat biased basis for the description and interpretation of learned helplessness behavior. Learned helplessness by its current description, fits adaptive behaviors such as conservation-withdrawal (Engel & Schmale, 1972), tonic immobility (Suarez & Gallup 1976; Gallup, 1977), and thanatosis/death feigning (Holmes, 1908). These are all adaptive behaviors that occur in the face of threating, traumatic, or uncontrollable situations that involve passivity, freezing, or just general inaction. These behaviors are well documented and all described as a withdrawal from action and involve passivity and stillness as a defense. In the event of predation, tonic immobility may allow an animal to blend into the background and avoid being detected (Gallup, 1977). Conservation-Withdrawal may serve to conserve energy in the event of uncontrollable outcomes or inconsistent resource availability (Menahem, 1994). Thanatosis or "death feigning" is described as a deceptive measure to dissuade predators or blend in to surroundings by assuming a deathlike posture (Holmes, 1908). Thanatosis has been studied for over a century and appears in a variety of vertebrates and invertebrates. These animals include amphibians (e.g., Gargaglioni *et al*. 2001, Bertoluci *et al*. 2007), birds (e.g., Sargeant & Eberhardt 1975; Rovee *et al*. 1976), fish (e.g., Howe, 1991; Gibran, 2004), mammals (e.g., Francq, 1969; Kimble, 1997), reptiles (e.g., Greene, 1988; Santos *et al*., 2010; Burghardt & Greene, 1988; Harding, 1997), spiders (e.g., Cloudsley-Thompson, 1995), and a staggering array of insects: beetles (Chemsak & Linsley, 1970; Prohammer and Wade, 1981; Allen, 1990; Oliver, 1996; Acheampong & Mitchell, 1997; Miyatake, 2001a,b; Miyatake *et al*., 2004), cicada (Villet, 1999), crickets (Nishino & Sakai, 1996), lepidopterans (Tojo *et al*., 1985; Dudley, 1989; Larsen, 1991), mantids (Edmunds, 1972), odonates (Abbott, 1926), parasitic wasps (King & Leaich, 2006), stick insects (Godden, 1972; Carlberg, 1986), and water bugs (Holmes, 1906). Like learned helplessness investigations, social bees and social wasps are absent in the body of thanatosis research.

The presence or absence of thanatotic behavior in social bees and social wasps may be due to the availability of alternative defensive responses. In the case of thanatosis in the solitary parasitic wasp *Nasonia vitripennis* (King & Leaich, 2006) the propensity of utilizing a sting response as a defensive measure is likely severely reduced, as with other solitary bees and wasps, compared to social hymenoptera. This “reluctance” to sting is most likely due to the metabolic costs of producing venom, and the need to use existing venom stores to provide a suitable host for offspring (Nelsen, Kelln, & Hayes, 2014). Use of the defensive sting reflex of social hymenoptera is not as detrimental to the potential reproductive success of the individual and, as a result, can be used in place of more passive defensing action. In the case of *Polistes* wasps, there is little selective pressure favoring inhibition of the sting reflex in situations of predation. Passive defensive measures beyond fleeing may be of little use to wasps or bees as stinging an aggressor will reduce the probability of future molestation. However, the propensity for a sting defense may be different for sterile worker verses wasp gynes, or reproductive females, when reproductive potential is a factor. The influence of reproductive caste status on costly defensive behavior is brought forth in its purest form through another member of social hymenoptera absent in the list of thanatosis investigations: the honey bee, *Apis mellifera*.

Unlike the other organisms previously mentioned as subjects of learned helplessness and thanatosis investigations, honey bees are eusocial organisms. Honey bees exist in colonies consisting of between 50,000 and 100,000 sterile workers and one reproductive queen. Eusociality is described as a cooperative group consisting of castes characterized by divisions of labor (Wilson & Hölldobler, 2005). In the case of the honey bee, the caste system includes reproductive (queen and drones) and sterile (workers) members. The sterile worker class is further partitioned into nurses, workers, guards, soldiers, and foragers all of which are determined both genetically and through age polyethism (Breed, Robinson, & Page, 1990; Seeley, 1982). The honey bee colony functions as an adaptive unit. Much like the cells of a multicellular organism, the eusocial colony depends on cooperative efforts in both foraging and defense (Seeley, 1995; Seeley, 1997; Breed, Guzman-Novoa, & Hunt, 2004). At the individual level, there is no reproductive potential. This provides a special case for group selection and provides a different ruleset for adaptive individual responses, such as behavior meeting a learned helplessness interpretation.

Passive defensive measures such as tonic immobility or thanatosis may be useful for avoiding costly confrontation or resource expenditure in solitary animals. Solitary animals forced to confront aggressors or utilize venom normally reserved for feeding may limit future reproductive opportunities as a result. In the case of the honey bee, the sting response results in sting autonomy. The honey bee sting apparatus is affixed with recurve barbs that anchor into the skin and allows the sting to remain in the target after the bee flies away, effectively eviscerating herself in the process (Cunard & Breed, 1998). The honey bee dies shortly after stinging, but sting autonomy allows for continued deliverance of venom after the bee is gone, and marks the target with alarm pheromone for continued assault by other bees. Even though the individual bee dies, the effect of her sting is preserved through the aversive conditioning it provides to the victim. The victim of a sting is less likely to pursue honey bees as a future food source. The individual bee, absence reproductive potential herself, provides protection to her sisters (who share 75% of her genes) and brood from potential vertebrate threats. The potential of this aversion is expressed through bee and wasp color and pattern mimicry in the harmless fly family Syrphidae, or Hoverflies. In the case of sterile worker honey bees, there appears to be very little benefit to passive defense measures in high threat situations given alternative options (such as flying away or stinging). Honey bees have various defensive responses to a variety of potential threats (reviewed by Breed, Guzman-Novoa, & Hunt, 2004), but none of these responses include passivity, immobility, or withdrawal.

As a result of prior observations in laboratory settings and yoked responses in prior investigations (Dinges *et al*., 2013), we expect the honey bee may have a different response to uncontrollable aversive stimuli than species previously investigated for learned helplessness and LDE/A behavior. This difference falls primarily to the fourth criteria of learned helplessness outlined in Eisenstein and Carlson (1997): "Following inescapable/unavoidable aversive stimuli training, animals become “passive” when confronted with an escapable/avoidable shock compared to those who previously learned to escape/avoid.” The lack of evidence for thanatosis, described by Eisenstein and Carlson (1997) to be the adaptive origin of learned helplessness, coupled with observations of prior investigations leads us to predict honey bees will not assume a passive response in the face of uncontrollable stimuli. It has been previously shown that the criteria of learned helplessness or LDE/A may need to be amended for a target animal. In the early studies, rats did not exhibit a reduction in escape/avoidance behavior when previously trained with inescapable shock (see Maier & Seligman, 1976 and citations therein). Prior inescapable shock in rats resulted in no more than a slight delay of acquisition of the escape/avoidance behavior. However, an increase in escape/avoidance task difficulty was sufficient to produce learned helplessness or LDE/A. For lever press escape criteria of avoidance, an increased from FR-1 to FR-3 would produce learned helplessness or LDE/A. In the case of shuttle boxes, the rat must move from one side to the other and back again to escape/avoid shock in order for learned helplessness or LDE/A to emerge. The defining criteria of a learned helplessness interpretation must be revised to incorporate animals that do not typically display “passivity.”

The honey bee’s innate drive to explore the compartments of a shuttle box force the bee to inhibit such innate behavior when avoiding or escaping aversive stimuli presented in one compartment but not another. In the case of punishment, forager bees are capable of inhibiting shuttle behavior to restrict their movement to one compartment of a shuttle box but no reduction of general activity occurs (Abramson 1986; Agarwal, *et* *al*., 2011; Dinges *et al*., 2013). The bee continues to shuttle, but restricts their movement to the safe compartment. Bees that are trained to avoid a compartment, master bees, should learn to restrict their movement to the safe compartment; that learned compartment restriction should persist when the bee is made yoked in subsequent phases. Conversely, training with unpaired shock, yoked bees, should produce a pattern of non-preference responses that is conserved when the bee made master in subsequent phases. If these predictions are supported by the data, evidence for learned helplessness will be provided in the form of LDE/A without the general passivity observed in other learned helplessness investigations. This would then suggest learned helplessness may persist in a fashion more fluid than simply withdrawing from action.

CHAPTER III

METHOD

Subjects

Honey bees (*Apis mellifera* L.) maintained at the Oklahoma State University Comparative Psychology and Behavioral Biology Laboratory apiary acted as subjects for the experiment. Forager bees (n = 448) were collected from a feeder containing 50/50 (weight/volume) sucrose solution. Bees collected in this way are typically foragers older than twenty days (Seeley, 1995), and are relatively homogenous in age as bees forage in the last two weeks of life (Seeley, 1982; Winston & Neilson-Punnet, 1982). All foragers were assumed to be experimentally naïve sisters from the same hive. Bees were held in a wire mesh communal carrier for three hours prior to experimental sessions, and allowed access to 50% (weight/volume) sucrose solution *ad libitum*.

Apparatus

A pair of automated shuttle boxes were used to administer experimental contingencies and record responses. The inside of each shuttle box measured 145 × 20 × 5 mm, ensuring that the bees could turn and move, but were always in contact with the top or bottom shock grid. The side walls, front doors and back doors of the each shuttle box were constructed from white high-density polyethylene (HDPE) plastic. Copperplated steel oxyacetylene welding rods were threaded through the walls to create top and bottom shock grids. The rods were positioned 5.0 mm apart (center to center), allowing 3.5 mm gaps between rods. The rods in the shock grids were alternatively wired to either the positive terminal or the ground terminal of an 8.71V, 1A DC power supply. If a bee touched any two adjacent rods during a period of shock activation it would complete the shock circuit and receive an electric shock. Color sheets, cut from Valspar Signature Allen + Roth sample paint swatches, were placed below the bottom shock grid to act as discriminative stimuli. Each shuttle box was partitioned into two compartments; the color sheet under one compartment was blue (AR1226 Ocean Front), the color sheet under the other compartment was yellow (AR1805 Light Rail). This resulted in the each shuttle box being visually divided into blue and yellow compartments. We chose blue and yellow because previous experiments have shown that bees can easily discriminate between these colors in aversive conditioning situations (Agarwal *et al*., 2011; Dinges *et al*., 2013). Clear acrylics shields were placed outside both the top and bottom shock grids to ensure that subjects did not pass between the bars and to protect the color sheets from being damaged. In each shuttle box, two modulated infrared beams were positioned 5.0 mm from the center of the shuttle box to detect the subjects' location. Modulated infrared beams were used to remove the influence of ambient light on subject detection.

Both shuttle boxes were connected to a control unit containing a Propeller Experiment Controller (Varnon & Abramson, 2013) and a user interface. The Propeller Experiment Controller detected the locations of the subjects through the infrared beams installed in each shuttle box, implemented all experimental contingencies including activation of the shock grids, and recorded data to a spreadsheet on an attached micro SD card. The apparatus could therefore be operated independently of a computer or any large equipment. Figure 2 shows a photograph of the shuttle boxes and control unit.

Procedure

Pre-session Preparation. Prior to experimental sessions, the clear acrylic shields were cleaned and a strip of black paper was placed below the bottom acrylic shield. The black paper was used to cover the color sheets before experimental session began. After the acrylic shields were cleaned, two bees were transported from the communal holding container and placed in each shuttle box. Subjects entered the apparatus from either the blue or yellow compartment and this compartment of initial entry was counterbalanced. Once both subject bees were secured in their respective shuttle boxes, a 30-second adaptation began. Experimental sessions began after the adaptation period was over and two additional criteria were met: 1) both subjects were moving and detected by the apparatus, and 2), if a master/yoked session was being conducted, the master entered the correct side of the shuttle box. These pre-session requirements were used to ensure that both subjects were active and recovered from any handling related stress, and to ensure that all master subjects began the session on the correct side of the apparatus. When the control unit indicated that experimental sessions began, the experimenter removed the black paper revealing the color sheets that acted as discriminative stimuli during the session.

Experimental Sessions. Each session was divided into two or three 5-minute phases, depending on the group. During each phase, subjects entered either a *neutral context* condition where neither subject was shocked, or a *master/yoked* condition where the master subject was shocked when entering the incorrect side of the shuttle box and the yoked subject was shocked when the master was shocked, regardless of compartment. For control groups, subjects remained in either master or yoked role across all phases; for experimental groups, subjects switched between master and yoked roles during phase changes. Experimental sessions from each group were conducted concurrently to minimize calendar effects. Each day, sessions were conducted from each group and the order of each day's sessions were pseudo-randomly determined. In the following sections, we describe the groups in terms of their assessments. The overall experimental design can be seen in Table 2.

Compartment and color preferences. To assess pre-existing compartment and color preferences, context neutral sessions were performed; these bees were placed in the shuttle box but did not experience shock. Compartment preferences were assessed using neutral context phases without presenting color sheets. Instead of removing the black paper to reveal the color sheets, the black paper remained in place during experimental sessions. Preexisting preferences for blue and yellow were similarly assessed using neutral context phases, except that the black paper was removed at the start of each session. We assessed compartment and color preferences using a two-phase design and a three-phase design.

Master control. The master control groups focused on the behavior of subjects that remained in a master role for both training and testing phases. The master control subjects always acted in the master role; they never entered a neutral context or yoke phase. The master control groups served as a comparison for other groups. We expected that master subjects would learn to restrict their movement to the correct compartment.

The performance of the master control groups was used to identify change in other groups' behavior as a result of different experience. We used both a two-phase design and a three-phase design for master controls.

Yoked control. The yoked control groups focused on the behavior of subjects that remained in a yoked role for both training and testing phases. The yoked control subjects always acted in the yoked role; they never entered a neutral context or master phase. The yoked control groups served as an additional comparison for other groups. We expected that yoked subjects would not learn to restrict their movement to either compartment resulting in equal time spent in both compartments (150 seconds). Therefore, we refer to this pattern of response as non-preference responding. The performance of the yoked control groups was also used to identify change in other groups' behavior as a result of different experience. We used both a two-phase design and a three-phase design for yoked controls.

Learned helplessness. Learned helplessness was assessed by switching a subject from yoked to master role. These bees were trained as yoked, and then tested as master. We expected that experience with unavoidable shock in the yoked role would cause subjects to have difficulty restricting their movement to avoid shock in the subsequent master role. If our hypothesis is supported, previous experience with unavoidable shock in the training phase (yoked role) would interfere with acquisition of avoidance behavior in the testing phase (master role). Therefore, we expect the master control bees to be better able to avoid shock than the learned helplessness bees. Further, no differences between the learned helplessness bees and yoked control bees are expected. We used both a two-phase design and a three-phase design to assess learned helplessness.

Immunization. Immunization to learned helplessness was assessed by switching a subject from master to yoked role. These bees were trained as master, and then tested as yoked. We expected that experience with avoidable shock in the master role would cause subjects to restrict their movement to the previous correct compartment in the subsequent yoked role. If our hypothesis is supported, previous experience with avoidable shock in the training phase (master role) would create a conserved compartment preference maintained in the testing phase (yoked role). Therefore, we expect the immunization bees to be better able to avoid shock than the yoked control bees. Further, no differences between the immunization bees and master control bees are expected. We used both a two-phase design and a three-phase design to assess learned helplessness.

Analysis

We used R version 3.2.2 (freely available at https://www.r-project.org/), including the nlme, plyr, and multcomp packages, for analysis and post-hoc comparisons. Additionally, we used observation oriented modelling (OOM) (Grice, 2011; Grice *et al*., 2012) to perform additional comparisons without the assumptions of null hypothesis testing (e.g. homogeneity, normality). We primarily analyzed the duration subjects spent in each compartment of the shuttle box and the frequency with which the bees shuttled from one compartment to the other. Bees generally explore and pace between compartments in shuttle box experiments; in aversive condition experiments, bees often learn to restrict their movement to the "safe" compartment (without an aversive stimulus) while maintaining a consistent level of activity. We use the term correct compartment restriction (CCR) to refer to time restricted to the correct compartment of the shuttle box. The "correct" compartment in master testing phases was the no-shock compartment; in yoked testing phases, it was the correct compartment of the paired master bee; in context neutral testing phases, we arbitrarily assigned the yellow compartment as the correct compartment. Our measure of CCR was the total time the bee spent in the correct compartment over the five-minute testing phase. For our analysis, we only considered CCR of the final phase of each experiment (phase 2 of two-phase groups and phase 3 of three-phase groups). To assess potential reduction in movement over the course of the experiment due to fatigue or shock, we also analyzed frequency of movement inside the shuttle box. Although some investigations observed a color-based difference in avoidance behavior (Dinges *et al*., 2013), we found no significant differences between color counterbalances and thus combined color counterbalances for the final analysis.

Statistical assumptions were checked prior to conducting all statistical analyses. The nonparametric version of a test was conducted in the event of a violation of one of the assumptions necessary for the traditional parametric test (i.e., using a Kruskal-Wallis test in the event of a violation of one of the assumptions necessary for an ANOVA). Post-hoc comparisons for ANOVAs and Kruskal-Wallis tests were performed using Tukey's Honestly Significant Difference (HSD).

CHAPTER IV

RESULTS

The overall findings can be seen in Figure 3. Master control bees consistently restricted their movement to the correct compartment (correct compartment restriction: CCR) at a higher rate than non-preference responding (150 seconds) and yoked control bees. Additionally, in two-phase groups, master control bees had greater CCR than learned helplessness and immunization group bees, but this was not the case in three-phase groups. This may be due to a wide variance in CCR, as is evident in Figure 3. For learned helplessness bees, previous experience with unavoidable shock only partially inhibited acquisition of avoidance behavior; however, variance in individual CCR scores was highest of all groups and CCR time was no different from yoked control bees. For immunization bees, previous experience with avoidable shock created a preference for the correct compartment that was conserved when the shock later became unavoidable in three-phase groups but not two-phase groups. In the following sections, these results are discussed in detail.

General Activity Test and Context Control. We found general activity decreased significantly between the first and third phases for context neutral bees, *t* (31) = -4.55, $p < 0.001$, but no significant decrease in activity was observed between the first and third phases of master controls, *t* (31) = -1.32, *ns*, yoked controls, *t* (31) = -0.84, *ns*, learned helplessness, *t* (31) = -1.82, *ns*, and immunization bees, *t* (31) = -1.63, *ns*. Furthermore, post-hoc comparisons using Tukey's honest significant difference (HSD) indicated no differences in general activity between master control, yoked control, learned helplessness, and immunization bees in both two-phase and three-phase groups.

Compartment preference indicated no side preference in the absence of color cues in the two-phase, *t* (31) = -1.56, *ns*, and three-phase group, *t* (15) = -1.41, *ns*. However, a preference for the blue compartment was observed when color cues were available in the two-phase group, *t* (31) = -3.33, $p = 0.002$, but no preference was observed in the three-phase group, *t* (15) = -0.24, *ns*.

General Between-Group Analysis. Master control, yoked control, learned helplessness, and immunization groups were compared for the final analysis. The descriptive statistics for each of the groups are presented in Table 3, and a summary of the results of the OOM ordinal analysis are presented in Table 4. Variances in the four groups were significantly different in two-phase groups, Bartlett's K^2 (3)=8.566, p=0.04, so a Kruskal-Wallis test was used to analyze differences in CCR between the four groups, X^2(3)=33.852, p<0.001. Three-phase groups had no significant violations of statistical assumptions so a one-way ANOVA was used to compare CCR times among four groups; there was a significant difference between the four groups, *F* (3,124)=4.346, p=0.006.

Master and Yoked Controls. When presented with avoidable shock, master control bees were able to restrict their movement to the correct compartment to avoid shock at a rate greater than non-preference responding (150 seconds) in the two-phase group, t (95) = 7.21, $p < 0.001$, and three-phase group, t (31) = 6.32, $p < 0.001$. Post-hoc comparisons using Tukey's HSD indicated the mean CCR times of master control bees to be consistently greater than that of yoked control bees in both the two-phase group ($p < 0.001$), and the three-phase group ($p = 0.005$). An ordinal analysis using OOM indicated similar results for both the two-phase group (PCC = 72.6, $c < 0.001$), and the three phase group (PCC = 76.1, $c < 0.001$).

In contrast, when presented with unavoidable shock, yoked control bees did not restrict their movement to either compartment at a rate significantly different from non-preference responding in the two-phase group, t (95) = 1.33, *ns*, and three-phase group, t (31) = 0.77, *ns*. Since color preference was not observed in yoked control bees, the color preference observed in context neutral bees is likely due to context acclimation or varying exploration strategies.

Learned Helplessness. Previous experience with unavoidable shock did not appear to completely inhibit acquisition of avoidance behavior in subsequent avoidance tasks, but a degree of interference was observed. Bees in the learned helplessness group, trained with unavoidable shock and tested with avoidable shock, were able to restrict their movement to the correct compartment to avoid shock at a rate greater than non-preference responding in the two-phase group, t (95) = 2.77, $p = 0.007$, and three-phase group, t (31) = 2.05, $p = 0.049$. However, learned helplessness bees did not avoid shock at a rate significantly greater than yoked control bees; thus, experience with unavoidable shock creates a non-preference pattern of response that is partially conserved when shock is subsequently made avoidable. Furthermore, in the two-phase group, post-hoc comparisons using Tukey's HSD indicated master control bees avoided shock at a significantly greater rate than learned helplessness bees ($p < 0.001$). However, this was not the case for the three-phase groups; there were no statistically significant differences found between CCR times of master control and learned helplessness bees in the three-phase groups. This is likely due to the variance in CCR observed in the learned helplessness bees shown in Figure 3. These results suggest experience with unavoidable shock interferes with, but does not prevent, acquisition of avoidance behavior in honey bees.

An ordinal analysis using OOM indicated that master control bees avoided shock at a significantly greater rate than learned helplessness bees in both the two-phase groups (PCC = 67.1, $c < 0.001$), and three-phase groups (PCC = 66.6, $c = 0.01$). This result conflicts with the post hoc comparisons using Tukey's HSD. This is likely due to the variance observed in the three-phase groups.

Immunization. Previous experience with avoidable shock created a preference for the correct compartment that was conserved when the shock later became unavoidable. Bees in the immunization group restricted their movement to the previously correct compartment at a rate greater than non-preference responding in two-phase groups, t (95) = 3.33, $p = 0.001$, and three-phase groups, t (31) = 4.10, $p < 0.001$. However, immunization bees did not avoid shock at a rate significantly greater than yoked control bees; thus, experience with avoidable shock creates a pattern of response that is only partially conserved when shock is subsequently made unavoidable.

Furthermore, in two-phase groups, a post-hoc comparisons using Tukey's HSD indicated master control bees avoided shock at a significantly greater rate than immunization bees in two-phase groups ($p < 0.001$), but not three-phase groups; thus, time in the training role is an important factor in the conservation of trained response patterns. An ordinal analysis using OOM indicated similar results; master control bees avoided shock at a significantly greater rate than immunization bees in two-phase groups (PCC = 67.4, $c < 0.001$), but not three-phase groups. Immunization bees had less CCR variance and slightly, though not significantly, greater overall CCRs than learned helplessness and yoked control bees, as is visually evident in Figure 3. These results suggest experience with avoidable shock creates a preference that is maintained in the absence of avoidable shock; however, this preference is less pronounced than master controls.

CHAPTER V

DISCUSSION

Our results support the general predictions of learned helplessness literature; master control and immunization bees restrict their movement to a compartment while yoked control bees do not, and learned helplessness bees do not restrict their movement to avoid shock as well as master control bees. Although our experiment produced effects in the expected directions, some findings were less pronounced than predicted. This is likely due to the unique behavior of our subject species, which is an advantage of our experiment.

The behavior of honey bees in shuttle boxes is substantially different than that of other species. While a rat or a roach freezes in response to shock, honey bees do not. Instead, bees maintain the same level of general activity, but restrict activity to an area not associated with shock. Experience with unavoidable shock that interferes with subsequent avoidance and has been described as a learned decrease in escape or avoidance behavior. This decrease in behavior is often characterized by “passivity” in response to traumatic events that typically elicit an escape response (Eisenstein & Carlson, 1997). The passivity of subjects in learned helplessness experiments is proposed to be a reduced incentive for initiating voluntary responses which supports cognitive interpretations including depression, lack of motivation, and helplessness (Alloy & Seligman, 1979). Unlike other species, honey bees do not display passivity in response to unavoidable or inescapable shock (Abramson, 1986; Dinges *et al*., 2013), and therefore these cognitive interpretations may not be appropriate. Another interpretation is that a decrease in avoidance behavior is the product of an incompatible response, such as freezing, and not a product of cognitive or motivational deficits in associative ability (Glazer & Weiss, 1976). If an aversive stimulus elicits freezing, an animal would simply not be able actively avoid shock and freeze simultaneously. Again, bees respond distinctly; they do not exhibit an incompatible freezing response.

The honey bee's distinct response to unavoidable shock may explain how our results differ slightly from other experiments, but it also marks honey bees as a unique and potentially valuable model organism. The proposed biological mechanism of learned helplessness is tonic immobility, the freezing response to stress (Eisenstein & Carlson, 1997). Tonic immobility, also called thanatosis or conservative withdrawal, has been investigated in a wide variety of organisms over hundreds of years (Gallup, 1974). However, bees and wasps are distinctly absent in the body of learned helplessness and tonic immobility research until recently, and social bee and social wasp investigations are still absent (King & Leaich, 2006). It may be that social bees do not have the biological defense mechanism (tonic immobility) that is basis of learned helplessness. Breed, Guzman-Novoa, and Hunt, (2004) discuss many bee defensive responses to threat, such as the sting response, but none include passivity, immobility, or withdrawal. Honey bees therefore provide a unique opportunity to investigate the biological foundation of learned helplessness. Our experiment is an important contribution to the literature as it is the first learned helplessness experiment with a species that does not display tonic immobility. Further investigation of learned helplessness behavior in honey bees may elucidate important findings that would not be possible in species with defensive responses characterized by inactivity.

Another important consideration of our experiment is the similarity between our learned helplessness experiment and other aversive conditioning experimental designs. Learned helplessness is often considered an operant conditioning paradigm. The avoidance behavior of master subjects can be considered operant behavior maintained because it prevents an aversive stimulus. However, the behavior of learned helplessness subjects can be considered a respondent behavior. Lubow and Moore (1959) describe several respondent conditioning (classical conditioning) preexposure effects that may explain learned helplessness. One effect, latent inhibition, occurs when a subject is preexposed to a conditioned stimulus (CS) before it is associated with an unconditioned stimulus (US). The result is reduced acquisition of a conditioned response (CR). This effect has been demonstrated in free flying honey bees (Abramson & Bitterman, 1986a). Another effect, the US-preexposure effect, describes how previous exposure to the US prior to CS-US association can also reduce acquisition of a CR. This effect has also been demonstrated in free flying honey bees (Abramson & Bitterman, 1986b). A third process, learned irrelevance, describes how random (unpaired) exposure to both the CS and US delays acquisition of the CR when the CS and US are later associated. Our learned helplessness experiment contains aspects of all three respondent processes, but is best described as learned irrelevance. Future research is needed to determine the magnitude of the learned irrelevance effect, or if latent inhibition or US-preexposure alone are adequate to produce learned helplessness behavior. These interpretations suggest that respondent mechanisms should be considered in addition to operant mechanisms, and that, again, many parsimonious explanations can be made that do not require cognition.

Another major benefit of our research is the demonstration of a practical, well-understood, invertebrate species that can be used as a model organism to study a pervasive and important behavioral phenomenon such as learned helplessness. Honey bees have been established as a prime model organism for the study of the neural components and how they relate to behavior (Menzel & Müller, 1996). Recent work with neuromodulators, such as dopamine and octopamine, in honey bees has revealed fascinating interactions between bioamines and behavior in decision making tasks (Giray *et al*., 2015), punishment tasks (Agarwal *et al*., 2011), and appetitive and aversive respondent tasks (Vergoz, Roussel, Sandoz, & Giurfa, 2007). Honey bees serve as an ideal model to study the impact of such neuromodulators on behavior because bioamines, such as dopamine, can be injected directly into the brain allowing a measure of control unrivaled by vertebrate studies (Vergoz *et al*., 2007).

We hope that our experiment will stimulate renewed interests in learned helplessness. We have already described the sharp decline in learned helplessness research across recent years (see Figure 1) that is occurring despite the impact that studying a wide-spread phenomena, such as learned helplessness, would provide. Our results show that learned helplessness can be studied in honey bees, and that, because of their unique lack of a tonic immobility response and the advancement of invertebrate neurobiological research methods, honey bees are uniquely suited to advancing our understanding of learned helplessness. We also provide operant and respondent explanations for our results, and we believe that the usual cognitive explanations of learned helplessness in terms of "expectancies" or "cognitive sets" are not only unwarranted at this time, but may hinder our understanding of more fundamental mechanisms (Abramson, 2013). We hope that future research will explore the molecular and physiological mechanisms of learned helplessness, and other related behavior phenomena, while maintaining a parsimonious approach that only advocates cognitive explanations when other explanations have been exhausted.

REFERENCES

Abbott, C. E. (1926). Death feigning in *Anax junius* and *Aeschna sp*. Psyche, 33, 8–10.

Abramson, C. I. (1986). Aversive conditioning in honey bees (*Apis mellifera*). *Journal of Comparative Psychology*, *100*, 108-116. doi.org/10.1037/0735-7036.100.2.108

Abramson, C. I. (2013). Problems of teaching the behaviorist perspective in the cognitive revolution. *Behavioral Sciences*, *3*, 55-71. doi:10.3390/bs3010055

Abramson, C. I., & Bitterman, M. E. (1986a). Latent inhibition in honeybees. *Animal Learning & Behavior*, *14*(2*)*, 184-189. doi:10.3758/BF03200054

Abramson, C. I., & Bitterman, M. E. (1986b). The US-preexposure effect in honeybees. *Animal Learning & Behavior*, *14*, 374-379. doi:10.3758/BF03200081

Abramson, C. I., & Boyd, B. J. (2001). An automated apparatus for conditioning proboscis extension in honey bees (*Apis mellifera L.*). *Journal of Entomological Science*, *36*, 78-92.

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

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

Abramson, C. I., Kandolf, A., Sheridan, A., Donohue, D., Bozic, J., Meyers, J. E., *et al*. (2004). Development of an ethanol model using social insects: III. Preferences for ethanol solutions. *Psychological Reports*, *94*, 227–239.

Abramson, C. I., Place, A. J., Aquino, I. S., & Fernandez, A. (2004). Development of an ethanol model using social insects: IV. Influence of ethanol on the aggression of

Africanized honey bees (*Apis mellifera L.*). *Psychological Reports, 94*, 1107–1115.

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

Abramson, C. I., Stone, S. M., Ortez, R. A., Luccardi, A., Vann, K. L., Hanig, K. D., *et al*. (2000). The development of an ethanol model using social insects I: behavior studies of the honey bee (*Apis mellifera* L.). *Alcoholism: Clinical and Experimental Research, 24*, 1153–1166.

Acheampong, A. & Mitchell, B. K. (1997). Quiescence in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Entomologia Experimentalis et Applicata, 82*(1), 83-89.

Agarwal, M., Guzman, M. G., Morales-Matos, C., Del Valle Diaz, R., Abramson, C. I., & Giray, T. (2011). Dopamine and octopamine influence passive avoidance learning of honey bees in a place preference assay. *PLoS ONE, 6*(9), e25371. doi:10.1371/journal.pone.0025371

Allen, A. (1990). Death-feigning in *Exochomus quadripustulatus* L. (Col: Coccinellidae). *Bulletin of Entomological Research, 102*, 23.

Alloy, L. B., & Seligman, M. E. P. (1979). On the cognitive component of learned helplessness and depression. *The Psychology of Learning and Motivation, 13,* 219-276. doi:10.1016/S0079-7421(08)60084-5Braud, W., Wepman B., & Russo, D., (1969). Task and species generality of the 'helplessness' phenomenon. *Psychonomic Science, 16*, 154–155. doi:10.3758/BF03336349

Bertoluci, J., Brassaloti, R. A., Sawakuchi, H. O., Ribeiro Jr., J. W., & Woehl, G. (2007). Defensive behaviour with stiff-legged posture in the Brazilian tree toads *Dendrophryniscus brevipollicatus* and *D. leucomystax* (Anura, Bufonidae). *Alytes 25*(1-2), 38-44.

Bitterman, M. E., Menzel, R., Fietz, A., & Schäfer, S. (1983). Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 97*, 107- 119.

Bozic, J., Abramson, C. I., & Bedencic, M. (2006). Reduced ability of ethanol drinkers for social communication in honeybees (*Apis mellifera carnica Poll*.). *Alcohol, 38*, 179–183.

Braud, W., Wepman B., & Russo, D., (1969). Task and species generality of the 'helplessness' phenomenon. *Psychonomic Science*, *16,* 154–155.

Breed, M. D., Guzman-Novoa, E. & Hunt, G. J. (2004). Defensive Behavior of Honeybees: Organization, Genetics, and Comparisons with Other Bees. Annual Review of Entomology, 49, 271–98. doi:10.1146/annurev.ento.49.061802.123155

Breed, M. D., Robinson, G. E., & Page, R. E. (1990). Division of labor during honey bee colony defense. *Behavioral Ecology and Sociobiology*, *27,* 395–401.

Brown, G. E., Anderson, C. L., & Scruggs, J. L. (1994). Shock-induced analgesia in the cockroach, *Periplaneta americana*. *Psychological Reports, 74,* 1051–1057.

Brown, G. E., Busby, P. L., & Klopfenstein, M. K. (1992). Decay and reversibility of learned helplessness in the cockroach, *Periplaneta americana*. *Psychological Reports, 71*, 1107–1113. doi:10.2466/pr0.1992.71.3f.1107

Brown, G. E., Davenport, D. A., & Howe, A. R. (1994). Escape deficits induced by a biologically relevant stressor in the slug, *Limax maximus*. *Psychological Reports*, *75*, 1187–1192. doi:10.2466/pr0.1994.75.3.1187

Brown, G. E., Davenport, D. A., & Howe, A. R. (1995). Naloxone blocks learned helplessness in the slug (*Limax maximus*). *Psychological Reports*, *74*, 14. doi:10.2466/pr0.1995.76.1.14

Brown, G. E., & Dixon, P. A. (1983). Learned helplessness in the gerbil. *Journal of Comparative Psychology*, *97,* 90-92. doi:10.2466/pr0.1994.75.3.1187

Brown, G. E., Howe, A. R., & Jones, T. E., (1990). Immunization against learned helplessness in the cockroach, *Periplaneta americana*. *Psychological Reports*, *67*, 635–640. doi:10.2466/pr0.1990.67.2.635

Brown, G. E., Hughes, G. D., & Jones, A. A. (1988). Effects of shock controllability on subsequent aggressive and defensive behaviors in the cockroach (*Periplaneta americana*). *Psychological Reports*, *63,* 563-569. doi:10.2466/pr0.1988.63.2.563

Brown, G. E., Smith, P. J., & Peters, R. B. (1985). Effects of escapable versus inescapable shock on avoidance behavior in the goldfish (*Carassius auratus*). *Psychological Reports, 57*, 1027 1030. doi:10.2466/pr0.1985.57.3f.1027

Brown, G. E., & Stroup, K., (1988). Learned helplessness in the cockroach, *Periplaneta americana*. *Behavioral and Neural Biology, 50,* 246–250. doi:10.1016/S0163-1047(88)90921-1

Buerger, A. A. & Chopin, S. F., (1976). Instrumental avoidance conditioning in the Spinal rat. *Brain Research Bulletin*, *1*(2), 177-183. doi:10.1016/0361-9230(76)90067-8

Burghardt, G. M., & Greene, H. W. (1988). Predator stimulation and duration of death feigning in neonate hognose snakes. *Animal Behaviour*, *36*, 1842–1844.

Cakmak, I., Song, D. S., Mixson, T. A., Serrano, E., Clement, M. L., Savitski, A. Johnson, G., Giray, T., Abramson, C. I., Barthell, J. F., & Wells, H. (2010). Foraging response of honeybee subspecies to flower color choices and reward consistency. *Insect Behavior*, *23,*100-116. doi:10.1007/s10905-009-9199-7

Carlberg, U. (1986). Thanatosis and autotomy as defense in *Baculum sp.* 1 (Insecta: Phasmida). *Zoologischer Anzeiger, 217,* 39–53.

Chemsak, J., & Linsley, E. (1970). Death-feigning among North American Cerambycidae (Coleoptera). *The Pan-Pacific Entomologist*, *46*, 305–307.

Chourbaji, S., Zacher, C., Sanchis-Segura, C., Dormann, C., Vollmayr, B., & Gass, P. (2005). Learned helplessness: Validity and reliability of depressive-like states in mice. *Brain Research Protocols, 16,* 70-78. doi:10.1016/j.brainresprot.2005.09.002

Cloudsley-Thompson, J. L. (1995). A review of anti-predator devices of spiders. *British Arachnological Society*, *10*, 81–96.

Craig, D. P. A., Varnon, C. A., Sokolowski, M. B. C., Wells, H., & Abramson, C. I. (2014). An assessment of fixed interval timing in free-flying honey bees (*Apis mellifera ligustica*): An analysis of individual performance. *PloS One, 9,* e101262. doi:10.1371/journal.pone.0101262

Crane, E. (1999). *The world history of beekeeping and honey hunting.* New York: Routledge

Cunard, S. J. & Breed, M. D. (1998). Post stinging behavior of worker honey bees (Hymenoptera:Apidae). *Annals of the Entomological Society of America*, *91*, 754–757.

Dinges, C. W., Avalos, A., Abramson, C. I., Craig, D. P. A., Austin, Z. M., Varnon, C. A., Dal, F. N., Giray, T., & Wells, H. (2013). Aversive conditioning in honey bees (*Apis mellifera anatolica*): A comparison of drones and workers. *Journal of Experimental Biology*, *216*, 4124-4134. doi:10.1242/jeb.090100

Dudley, R. (1989). Thanatosis in the neotropical butterfly *Caligo ilioneus* (Nymphalidae: Brassolinae). *The Journal of Research on the Lepidoptera*, *28*, 125–126.

Edmunds, M. (1972). Defensive behaviour in Ghanaian praying mantids. *Zoological Journal of the Linnean Society*, 51, 1–32.

Eisenstein, E. M., & Cohen, M. J., (1965). Learning in an isolated prothoracic insect ganglion. *Animal Behavior*, *13*, 104–108. doi:10.1016/0003-3472(65)90079-5

Eisenstein, E. M., Carlson, A. D. (1997). A comparative approach to the behavior called 'learned helplessness.' *Behavioural Brain Research*, *86*, 149–160. doi:10.1016/S0166-4328(96)02260-7

Eisenstein, E. M., Carlson, A. D., & Harris, J. T. (1997). A ganglionic model of "learned helplessness." *Integrative Physiological and Behavioral Science*, *32*(3), 265-271. doi:10.1007/BF02688624

Engel, G. L., Schmale, A. H. (1972). Conservation-withdrawal: A primary regulatory process for organismic homeostasis. In: D.Hill (Ed.), *Physiology, Emotion and Psychosomatic Illness*. Elsevier, Amsterdam, 57–85.

Fosco, F., & Geer, J. H. (1971). Effects of gaining control over aversive stimuli after differing amounts of no control. *Psychological Reports*, *29*, 1153-1154.

Francq E. N. (1969). Behavioral aspects of feigned death in the opossum *Didelphis marsupialis*. *The American Midland Naturalist Journal*, *81*, 556–567.

Gallup, G. G. (1974). Animal hypnosis: Factual status of a fictional concept. *Psychological Bulletin*, *81*(11), 836-853. doi: 10.1037/h0037227

Gallup, G. G. (1977). Tonic immobility: The role of fear and predation. *The Psychological Record*, *27*(Spec Issue), 41-61.

Garber, J., & Seligman, M. E. P. (1980). *Human helplessness: Theory and applications*. New York: Academic Press.

Gargaglioni, L. H., Pereira, A. S., & Hoffmann, A. (2001). Basal midbrain modulation of tonic immobility in the toad *Bufo paracnemis*. *Physiology & Behavior*, 72(3), 297-303.

Gibran, F. Z. (2004). Dying or illness feigning: An unreported feeding tactic of the comb grouper *Mycteroperca acutirostris* (Serranidae) from the southwest Atlantic. *Copeia*, 2, 403–405.

Giray, T., & Robinson, G. E. (1996). Endocrine-mediated behavioral development in male honey bees and the evolution of division of labor. *Proceedings of the National Academy of Sciences, USA*, 93, 11718-11722.

Glass, D. C., & Singer, J. E. (1972). *Urban stress: Experiments on noise and social stressors.* New York: Academic Press.

Glazer, H. I., & Weiss, J. M. (1976). Long-Term Interference Effect: An Alternative to "Learned Helplessness." *Journal of Experimental Psychology, 2*(3), 202-213. doi:10.1037/0097 7403.2.3.202

Godden, D. H. (1972). The motor innervation of the leg musculature and motor output during thanatosis in the stick insect *Carausius morosus Br*. *Journal of Comparative Physiology*, *80*, 201–225.

Grice, J. W. (2011). *Observation oriented modeling: analysis of cause in the behavioral sciences*. San Diego, CA: Academic Press.

Grice, J. W., Barrett, P. T., Schlimgen, L. A., & Abramson, C. I. (2012). Toward a brighter future for psychology as an observation oriented science. *Behavioral Science,* 2, 1- 22.

Greene, H. W. (1988). Antipredator mechanisms in reptiles. In *Biology of the reptilia* (C. Gans & R.B. Huey, eds.). Alan R. Liss, New York, *16*, 1-152.

Harding, J. H. (1997). *Amphibians and Reptiles of the Great Lakes Region*, University of Michigan Press, Ann Arbor, Michigan.

Hiroto, D. S. (1974) Locus of control and learned helplessness. *Journal of Experimental Psychology*, *102*, 187-193. doi:10.1037/h0035910

Hiroto, D. S., & Seligman, M. E. P. (1975). Generality of learned helplessness in man. *Journal of Personality and Social Psychology*, *31*, 311- 327.

Hokanson, J. E., DeGood, D. E., Forrest, M. S., & Brittain, T. M. (1971). Availability of avoidance behaviors in modulating vascular-stress responses. *Journal of Personality and Social Psychology*, *19*, 60-68.

Holmes, J. S. (1906). Death-feigning in Ranatra. *Journal of Comparative Neurology*, *16*, 200–216.

Holmes, S. J. (1908). The instinct of feigning death. *Popular Science Monthly*, *72*, 179.

Horridge, G.A., (1962). Learning of leg position by the ventral nerve cord in headless insects. *Proceedings of the Royal Society B.*, *157,* 38–52. doi:10.1098/rspb.1962.0061

Howe, J. C. (1991). Field observations of death feigning in the convict tang, *Acanthurus triostegus* (Linnaeus), with comments on the nocturnal color pattern in juvenile specimens. *Journal of Aquaculture and Aquatic Sciences*, *6*(4), 13-15.

Kimble, D. (1997). Didelphid behavior. *Neuroscience & Biobehavioral Reviews*, *21*, 361–369.

King, B. H., & Leaich, H. R. (2006). Variation in propensity to exhibit thanatosis in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior, 19*(2), 241-249. doi:10.1007/s10905-006-9022-7

Klein, D. C., Fencil-Morse, E., & Seligman, M. E. P. (1976). Learned helplessness, depression, and the attribution of failure. *Journal of Personality and Social Psychology*, *33*(5), 508-516. doi:10.1037/0022-3514.33.5.508

Krantz, D. S., Glass, D. C., & Snyder, M. L. (1974). Helplessness, stress level, and the coronary prone behavior pattern. *Journal of Experimental Social Psychology*, *10,* 284-300.

Larsen, T. (1991). The art of feigning death—thanatosis in Euploea (Danainae) and other aposematic butterflies. *Bulletin of Entomological Research, 103,* 263–266.

Maier, S. F., Albin, R. W., & Testa, T. J. (1973). Failure to learn to escape in rats previously exposed to inescapable shock depends on nature of escape response. *Journal of Comparative and Physiological Psychology, 85*, 581-592.

Maier, S. F., & Seligman, M. E. P. (1976). Learned helplessness: theory and evidence. *Journal of Experimental Psychology: General*, *105*, 3-46.

Maier, S. F., Seligman, M. E. P., & Solomon, R .L. (1969). Pavlovian fear conditioning and learned helplessness: effects on escape and avoidance behavior of: (a) the CS-US contingency; and (b) the independence of the US and voluntary responding. In: B.A. Campbell & R.M. Church (Eds.), *Punishment and Aversive Behavior*, Appleton-Century-Crofts, New York, pp. 299–342.

Menahem, S. (1994). Conservation-withdrawal reaction in infancy? An underdescribed entity. *Child: Care, Health and Development*, *20*(1), 15-26.

Menzel, R., & Müller, U. (1996). Learning and memory in honeybees: From behavior to neural substrates. *Annual Review of Neuroscience, 19,* 379-404. doi:10.1146/annurev.ne.19.030196.002115

Miller, W. R., & Seligman, M. E. (1975). Depression and learned helplessness in man. *Journal of Abnormal Psychology, 84,* 228–238. doi:10.1037/h0076720

Miyatake, T. (2001a). Diurnal periodicity of death-feigning in *Cylas formicarius* (Coleoptera: Brentidae). *Journal of Insect Behavior*, *14*, 421–431.

Miyatake, T. (2001b). Effects of starvation on death-feigning in adults of *Cylas formicarius* (Coleoptera: Brentidae). *Annals of the Entomological Society of America*, *94*, 612–616.

Miyatake, T., Katayama, K., Takeda, Y., Nakashima, A., Sugita, A., and Mizumoto,M. (2004). Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. *Proceedings of the Royal Society of London B, 271*, 2293–2296.

Nash, S. M., Martinez, S. L., Dudeck, M., & Davis, S. F. (1983). Learned helplessness in goldfish under conditions of low shock intensity. *The Journal of General Psychology*, *108*, 97-101.

Nelsen, D. R., Kelln, W., Hayes, W. K. (2014). Poke but don't pinch: risk assessment and venom metering in the western black widow spider, *Latrodectus hesperus*. *Animal Behaviour, 89*, 107–114.

Nishino, H., & Sakai, M. (1996). Behaviorally significant immobile state of so called thanatosis in the cricket *Gryllus bimaculatus degeer*—its characterization, sensory mechanism and function. *Journal of Comparative Physiology A*, *179,* 613–624.

Oliver, M. (1996). Death-feigning observed in *Hippopsis lemniscata* (Fabricius) (Coleoptera: Cerambycidae). *Coleoptera Bulletin*, *50*, 160–161.

Overmier, J. B. (1968). Interference with avoidance behavior: failure to avoid traumatic shock. *Journal of Experimental Psychology*, *78*, 340–343.

Overmier, J. B., & Seligman, M. E. P. (1967). Effects of inescapable shock upon subsequent escape and avoidance learning. *Journal of Comparative and Physiological Psychology*, *63,* 23-33. doi:10.1037/h0024166

Padilla, A. M., Padilia, C., Ketterer, T., & Giacalone, D. (1970). Inescapable shocks and subsequent escape: avoidance conditioning in goldfish, *C. auratus*. *Psychonomic Science*, *20*, 295–296.

Prohammer, L., & Wade, M. (1981). Geographic and genetic variation in death-feigning behavior in the flour beetle, *Tribolium castaneum*. *Behavior Genetics*, *11,* 395–401.

Rodd, Z. A., Rosellini, R. A., Stock, H. S., & Gallup, G. G. (1997). Learned helplessness in chickens (*Gallus gallus*): Evidence for attentional bias. *Learning and Motivation*, *28*, 43-55. doi:10.1006/lmot.1997.0952

Roth, S., & Bootzin, R. R. (1974). Effects of experimentally induced expectancies of external control: An investigation of learned helplessness. *Journal of Personality and Social Psychology*, 29, 253-264.

Roth, S., & Kubal, L. (1975). The effects of noncontingent reinforcement on tasks of differing importance: Facilitation and learned helplessness effects. *Journal of Personality and Social Psychology, 32*, 680-691

Rovee, C. K, Kaufman, L. W., & Collier, G. H. (1976). Periodicity of death feigning by domestic fowl in response to simulated predation. *Physiology & Behavior*, *17*, 891–895.

Santos, M. B., Oliveria, M. C. M, Verrastro, L., & Tozetti, A. M. (2010). Playing dead to stay alive: death-feigning in *Liolaemus occipitalis* (Squamata: Liolaemidae). *Biota Neotropica*, *10*(4), 362-364.

Sargeant, A. B. & Eberhardt, L. E. (1975). Death feigning by ducks in response to predation by red foxes (*Vulpes fulva*). *The American Midland Naturalist Journal*, *94*, 108-119.

Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., ... Thenius, R. (2013). Standard methods for behavioral studies of *Apis mellifera*. In: Dietemann V, Ellis JD, Neumann P (Eds). The COLOSS BEEBOOK: standard methodologies for *Apis mellifera* research. *Journal of Apicultural Research*, *52*(4). doi: 10.3896/IBRA.1.52.4.04

Seeley, T. D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology*, *11*, 287-293.

Seeley, T. D. (1997). Honey bee colonies are group-level adaptive units. *The American Naturalist*, *150*(S1), S22-S41

Seeley, T. D. (1995). *The wisdom of the hive: The social physiology of honey bee colonies*. Cambridge, Massachusetts: Harvard University Press.

Seligman, M. E. P. (1992). *Helplessness: On depression, development, and death*. New York, NY: W. H. Freeman.

Seligman, M. E .P. & Beagley, G. (1975). Learned helplessness in the rat. *Journal of Comparative and Physiological Psychology*, *88*, 534–541.

Seligman, M. E. P., & Groves, D. (1970). Non-transient learned helplessness. *Psychonomic Science*, *19*,191-192.

Seligman, M. E. P., Maier, S. F. (1967). Failure to escape Traumatic shock. *Journal of Experimental Psychology, 74*(1), 1-9. doi:10.1037/h0024514

Seligman, M. E. P., Maier, S. F., & Geer, J. (1968). The alleviation of learned helplessness in the dog. *Journal of Abnormal and Social Psychology*, *73*, 256-262.

Seligman, M. E. P., Rosellini, R.A., & Kozak, M.J. (1975). Learned helplessness in the rat: time course, immunization, and reversibility. *Journal of Comparative and Physiological Psychology*, *88*, 542-547.

Seward, J., & Humphrey. L. (1967) Avoidance learning as a function of pretraining in the cat. *Journal of Comparative and Physiological Psychology*, *63*, 338-341. doi:10.1037/h0024381

Sokolowski, M. & Abramson, C. I. (2010). From foraging to operant conditioning: A new computer-controlled Skinner box to study free flying nectar gathering in bees. *Journal of Neuroscience Methods*, *188,* 235-242. doi:10.1016/j.jneumeth.2010.02.013.

Søvik, E., & Barron, A. B. (2013). Invertebrate models in addiction research. *Brain, Behavior and Evolution*, *82*, 153-165. doi:10.1159/000355506

Suarez, S. D. & Gallup, G. G. (1976). Tonic immobility as a response to rape in humans: a theoretical note. *The Psychological Record*, *29,* 315-20.

Thornton, J. W., & Jacobs, P. D. (1971) Learned helplessness in human subjects. *Journal of Experimental Psychology*, *87,* 369-372. doi:10.1037/h0030529

Tojo, S., Morita, M., & Hiruma, K. (1985). Effects of juvenile hormone on some phase characteristics in the cotton cutworm, *Spodoptera litura*. *Journal of Insect Physiology*, *31*. 243–249.

Varnon, C. A., & Abramson, C. I. (2013). The propeller experiment controller: Low-cost automation for classroom experiments in learning and behavior. *Innovative Teaching, 2,* 2. doi:10.2466/07.08.IT.2.2.

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. doi:10.1371/journal.pone.0000288

Villet, M. H. (1999). The cicada genus *Nyara n. gen*. (Homoptera Cicadidae): systematics, behavior and conservation status. *Tropical Zoology*, *12,* 157–163.

Wilson, E. O., & Hölldobler, B. (2005). "Eusociality: Origin and Consequences". PNAS, *102*(38), 13367–13371.

Winston, M. L., & Neilson-Punnett, E. (1982). Factors determining temporal division of labor in honeybees. *Canadian Journal of Zoology*, *60*(11), 2947-2952. doi:10.1139/z82-372

Yang, Z., Bertolucci, F., Wolf, R., Heisenberg, M. (2013). Flies cope with uncontrollable stress by learned helplessness. *Current Biology*, *23*, 799–803. doi:10.1016/j.cub.2013.03.054

APPENDICES

Table 1.

Brief Literature Review of Learned Helplessness and Immunization

Species	Demonstrated Learned Helplessness?	Demonstrated Immunization?	Representative Citation(s)
Cat	Yes		Seward & Humphrey (1967)
Chicken	Yes		Rodd, Rosellini, Stock, Gallup (1997)
Cockroach	Yes	Yes	Brown, Howe, & Jones (1990); Brown & Stroup (1988)
Dog	Yes	Yes	Overmier & Seligman (1967); Seligman & Maier (1967)
Fruit Fly	Yes		Yang, Bertolucci, Wolf, & Heisenberg (2013)
Gerbil	Yes		Brown & Dixon (1983)
Goldfish	Yes		Padilla, Paditla, Ketterer, & Giacalone (1970)
Humans	Yes	Yes	Hiroto, (1974); Thornton & Jacobs, (1971)
Locust	Yes		Horridge, (1962)
Mice	Yes		Braud, Wepman, & Russo, (1969); Chourbaji *et al*. (2005)
Rat	Yes	Yes	Seligman & Beagley, (1975); Seligman, Rosellini, & Kozak, (1975)
Slug	Yes		Brown, G.E., Davenport, D.A., & Howe, A.R. (1994)

Table 2.

Experimental Design

Two-phase	Name	Training	Testing	N/A
	Color Preference	No Shock	No Shock	-----------
	Side Preference	No Shock	No Shock	-----------
	Master Control	Master	Master	-----------
	Yoked Control	Yoked	Yoked	-----------
	Immunization	Master	Yoked	-----------
	Helplessness	Yoked	Master	-----------

Three-phase	Name	Training	Training	Testing
	Color Preference	No Shock	No Shock	No Shock
	Side Preference	No Shock	No Shock	No Shock
	Master Control	Master	Master	Master
	Yoked Control	Yoked	Yoked	Yoked
	Immunization	Master	Master	Yoked
	Helplessness	Yoked	Yoked	Master

Table 3.

Descriptive Statistics

Two-phase	Name	Mean	SD	N
	Color Preference	135.84	24.09	32
	Side Preference	142.27	28.07	32
	Master Control	183.70	45.79	96
	Yoked Control	154.93	36.28	96
	Immunization	161.88	34.97	96
	Helplessness	160.81	38.19	96

Three-phase	Name	Mean	SD	N
	Color Preference	147.67	38.52	16
	Side Preference	136.65	37.75	16
	Master Control	191.57	37.23	32
	Yoked Control	155.97	43.90	32
	Immunization	178.73	39.66	32
	Helplessness	166.53	45.58	32

Note. Two-phase groups include data from the second phase of matching three-phase groups.

Table 4.

Ordinal Analysis

Two-Phase

Group 1		Group 2	PCC	*c*
Master	>	Yoked	72.6	0
Master	>	Helplessness	67.1	0
Master	>	Immunization	67.4	0
Yoked	>	Helplessness	44.5	0.88
Immunization	>	Yoked	56.29	0.07
Immunization	>	Helplessness	50.6	0.43

Three-Phase

Group 1		Group 2	PCC	*c*
Master	>	Yoked	76.1	0
Master	>	Helplessness	66.6	0.01
Master	>	Immunization	60.2	0.09
Yoked	>	Helplessness	42	0.88
Immunization	>	Yoked	67	0.013
Immunization	>	Helplessness	56.3	0.2

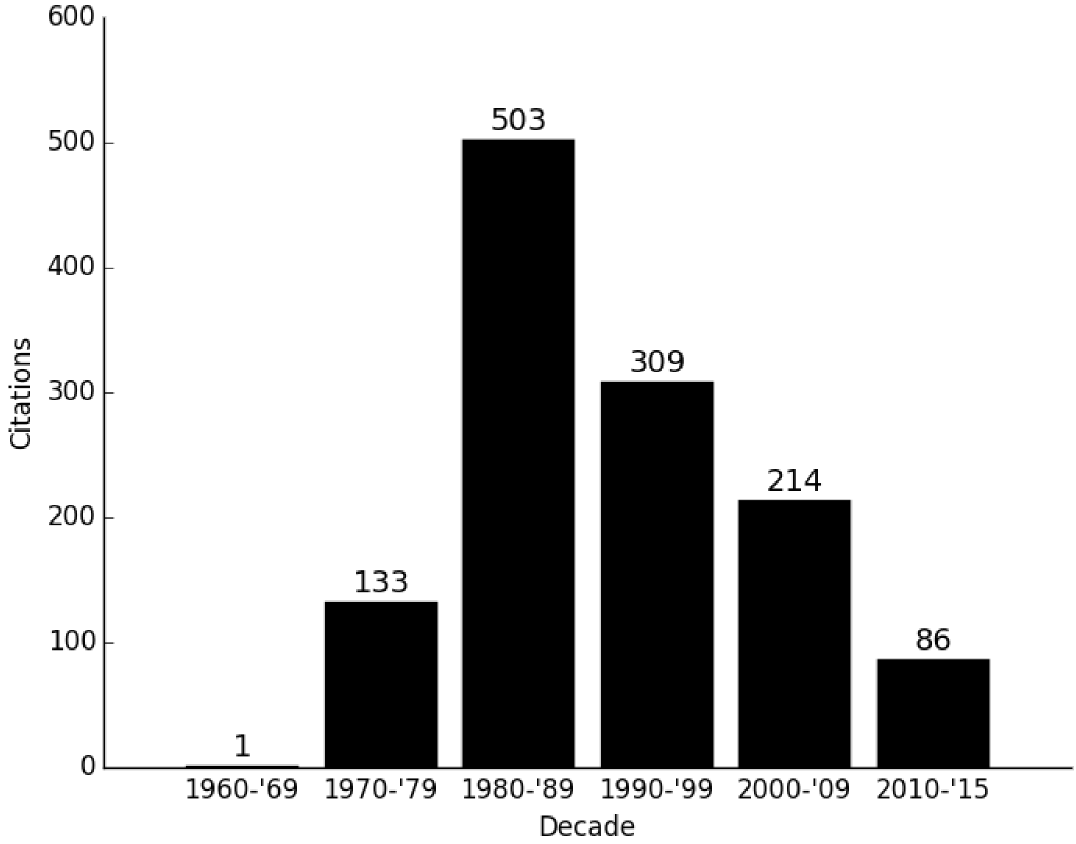

Figure 1. Number of articles, retrieved from EBSCO host database, with "learned helplessness" as a subject heading by decade.

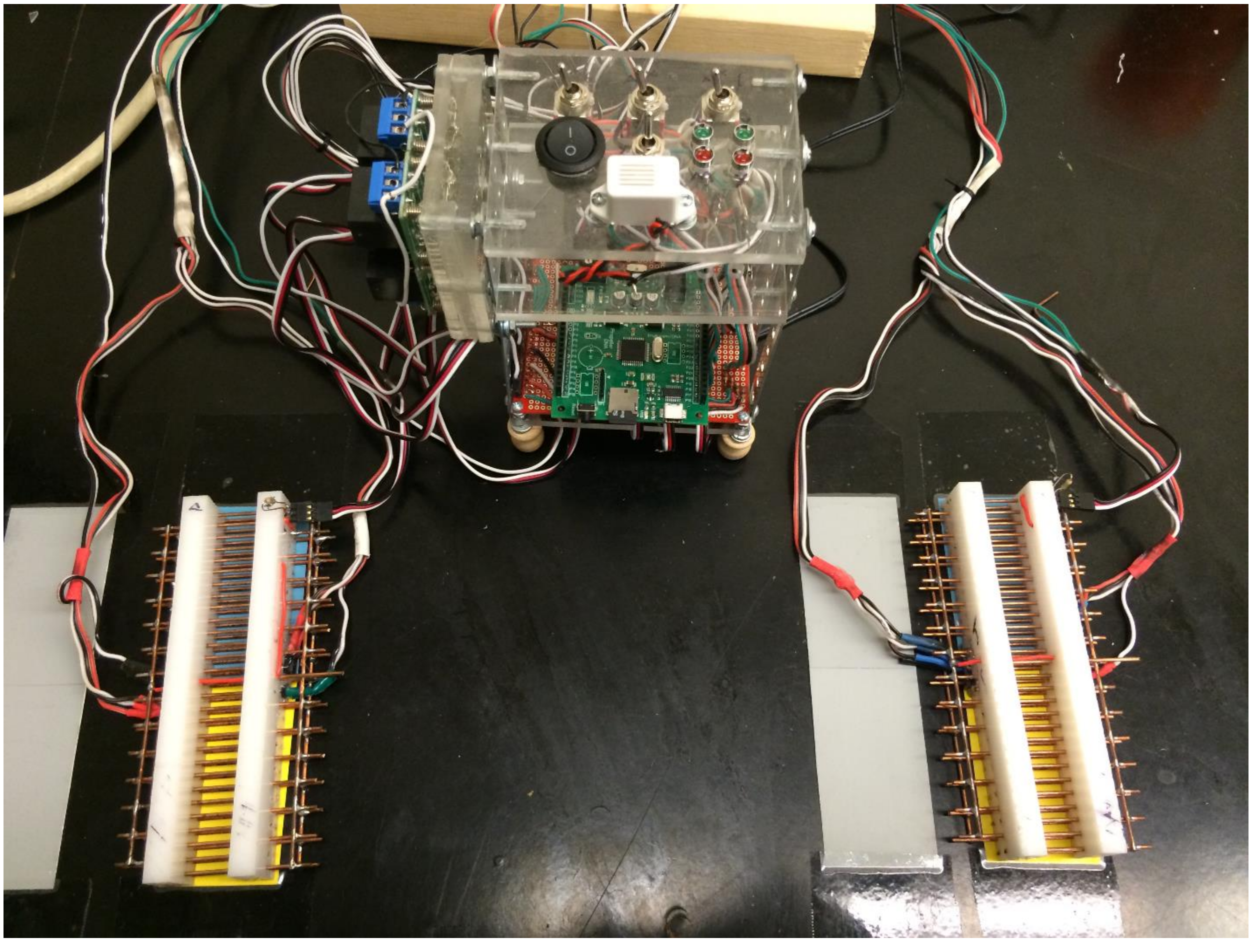

Figure 2. Photograph of the shuttle boxes and control unit.

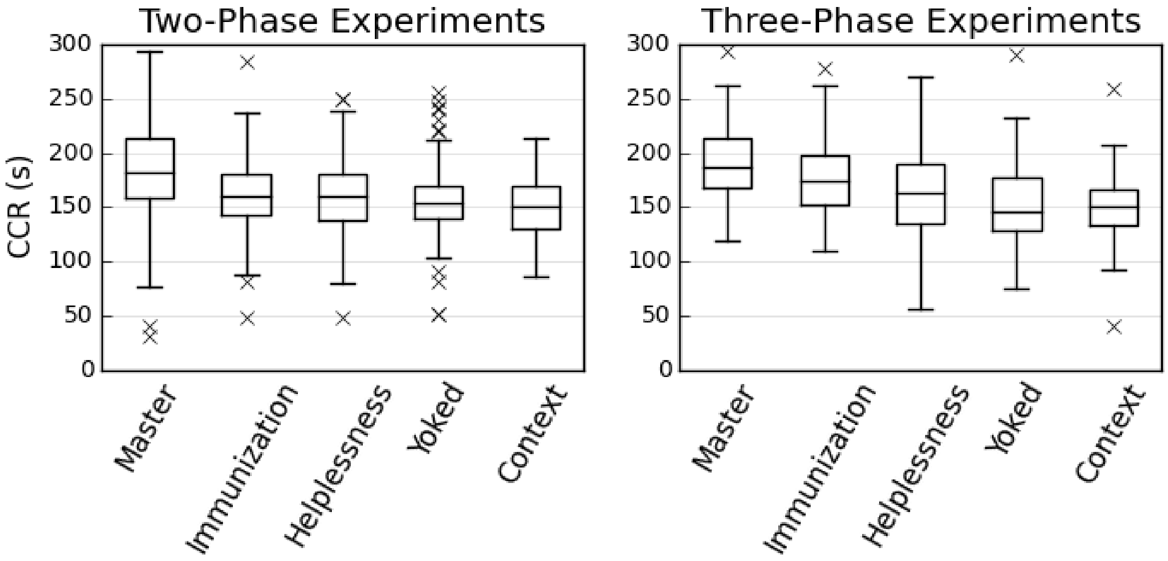

Figure 3. The box-plots display correct compartment restriction (CCR) for the experimental and control groups. The plots are divided by number of phases in the experiment (left graphs, two-phases; right graphs, three-phases). Context neutral color and context neutral black groups were combined for this figure.

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Dinges, C. W., Varnon, C. A., Abramson, C. I., Cota, L. D., & Slykerman, S. C. Studies of learned helplessness in the honey bee (*Apis mellifera ligustica*) – Manuscript submitted.

Dinges, C. W., Avalos, A., Abramson, C. I., & Giray, T. Influence of stimulus control on genetic expression in honey bee (*Apis mellifera l.*) Neural tissue: Hsp70, Syn, Dh44, Dlg1. – Manuscript in Preparation.

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