In presenting this thesis in partial fulfillment of the requirement for an advanced degree at Idaho State University, I agree that the library shall make it freely available for inspection. I further state that permission for extensive copying of my thesis for scholarly purposes may be granted by the Dean of Graduate Studies and Research, Dean of my academic division, or by the University Librarian. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature _________________________________

Date _____________________________________
OLFACCTORV CONDITIONING ALTERS AMERICAN COCKROACH RESPONSE TO NARCOTIC SUBSTANCES

by

Kayla Victoria Pavlick

A thesis
submitted in partial fulfillment
of the requirements for the degree of
Master of Science in the Department of Biological Sciences
Idaho State University
May 2019
To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Kayla Victoria Pavlick find it satisfactory and recommend that it be accepted.

____________________________________
David J. Delehanty, PhD
Major Advisor

____________________________________
Jason Q. Pilarski, PhD
Committee Member

____________________________________
Michele R. Brumley, PhD
Graduate Faculty Representative
ACKNOWLEDGMENTS

This research was funded by an Idaho State University College of Science and Engineering Internal Grant. Narcotic licensing was obtained from the U.S. Department of Homeland Security Drug Enforcement Agency and the Idaho Board of Pharmacy.

Though my name may be listed as the author for this thesis, there were many who contributed both directly and indirectly. My fellow graduate students were an amazing support system throughout this entire journey, and I give my thanks to each and every one of them.

I wish to sincerely express my gratitude to David Delehanty for taking me under his wing and ensuring my growth and success. His knowledge and guidance provided me with the confidence in myself and in my work that I needed in order to develop into the scientist that I am. Without him, this would not have been possible.
OLFACTORY CONDITIONING ALTERS AMERICAN COCKROACH RESPONSE TO NARCOTIC SUBSTANCES

THESIS ABSTRACT—IDAHO STATE UNIVERSITY (2019)

I measured American cockroach behavioral plasticity in response to olfactory and gustatory stimuli with the overarching goal of assessing potential for trained cockroaches to aid law enforcement in detecting controlled substances. I simulated a real-world environment in which a location task was performed by individual cockroaches after cockroaches received behavioral conditioning. I conducted behavioral conditioning experiments using vanilla and peppermint odors with gustatory stimuli. My approach replicated and expanded the work of Watanabe et al. (2003). By making minor modifications to published work, I demonstrated substantial memory retention period for American cockroaches for both odors and narcotics in a small-scale area. My results also demonstrated spatial locating ability by cockroaches following training. Overall, the capacity of cockroaches to learn likely can be exploited to train cockroaches to assist in detecting opioids and perhaps other illicit substances. Cockroach detection of illicit substances has potential to augment current law enforcement detection methods.

Keywords

American cockroach, Biological detection unit, classical conditioning, insect learning, narcotic detection, operant conditioning, Watanabe.
**TABLE OF CONTENTS**

**LIST OF FIGURES** ........................................................................................................... ix

**LIST OF TABLES** ............................................................................................................. xi

**CHAPTER 1 INTRODUCTION TO CONCEPTS** ................................................................. 1

- **BEHAVIORAL LEARNING** ............................................................................................ 1
- **TYPES OF BEHAVIORAL LEARNING** ........................................................................ 1
  - **CLASSICAL CONDITIONING** ..................................................................................... 2
  - **OPERANT CONDITIONING** ....................................................................................... 4
- **PLASTICITY** ................................................................................................................ 6
- **INSECT LEARNING** ..................................................................................................... 8
- **OLFACTORY AND GUSTATION IN INSECTS** ............................................................. 9
  - **THEORIES OF INSECT NEURAL PROCESSES** ....................................................... 10
- **CONDITIONED TASTE AVERSION** ............................................................................ 11
  - **CONDITIONED TASTE AVERSION IN INSECTS** ................................................ 12
- **CURRENT STUDY AIMS** ............................................................................................ 12
- **LITERATURE CITED** ................................................................................................. 14

**CHAPTER 2 COCKROACH CONDITIONING USING PAIRED STIMULI** ......................... 19

- **ABSTRACT** ................................................................................................................ 19
- **INTRODUCTION** ........................................................................................................ 20
- **METHODS** ................................................................................................................ 21
- **REPLICATION OF WATANABE ET AL. (2003) COCKROACH TRAINING** ........... 22
- **REPLICATION OF WATANABE ET AL. (2003) COCKROACH TESTING** ........... 23
- **TEST OF SIX-WEEK RETENTION ON PEPPERMINT AND VANILLA** ............... 23
- **TEST FOR PRE-EXISTING NARCOTIC PREFERENCE** ........................................ 24
- **TRAINING COCKROACHES TO MAKE ASSOCIATIONS WITH NARCOTICS** .... 25
- **TESTING COCKROACHES CONDITIONED TO NARCOTICS** ................................ 26
- **TEST OF FOUR-WEEK RETENTION OF NARCOTIC TRAINING** ....................... 26
- **COCKROACHES LOCATING NARCOTICS** .................................................................. 27
- **RESULTS** .................................................................................................................... 28
- **REPLICATION OF WATANABE ET AL. (2003)** ..................................................... 28
- **TESTING FOR PRE-EXISTING PREFERENCES** ....................................................... 28
LIST OF FIGURES

Figure 1. General housing containers for cockroaches maintained on the Idaho State University campus 2018-2019 for behavioral conditioning experiments. A limit of 15 cockroaches was maintained within each tank. Tank dimensions: (7.5" W × 11.25" L × 9.25" H) Naïve cockroaches were kept in separate tanks from conditioned cockroaches. All cockroaches kept on a 12:12 light cycle. Temperature was kept between 21-30 C° and humidity kept between 50-70%. Peeled banana and water were given ad libitum and changed every 96 h. Cardboard and egg cartons provided shelter................................. 35

Figure 2. Narcotics used in experiments of cockroach behavioral conditioning at Idaho State University campus 2018-2019 were a pre-manufactured 4% cocaine-hydrochloride solution and Adderall XR mixed to make a 1mg/mL solution. All narcotics were obtained from Cardinal Health (7000 Cardinal Pl. Dublin, OH 43017)........................................................... 36

Figure 3. Professional licensing for Kayla V. Pavlick allowing for possession and use of Schedule II narcotics for experimental cockroach training at Idaho State University campus during 2018. Licensing was renewed as needed. ............. 37

Figure 4. Illustration of experimental configuration for pairing odor stimulus with taste reward or punishment for cockroaches receiving conditioned training at Idaho State University campus during 2018. The filter paper was soaked in peppermint or vanilla odor, or cocaine-hydrochloride or Adderall XR solution. The syringe was filled with a corresponding sucrose (appetitive) or saline (aversive) solution. After antennae contacted scented filter paper, a gustatory stimulus was delivered from syringe to cockroach mouth parts. ............... 38

Figure 6. Stimulus pair presentation schedule for cockroaches receiving conditioning training at Idaho State University campus during 2018. Each cockroach experienced five rounds of conditioning in which each olfactory x gustatory pair was presented five minutes apart, five times for each stimulus pair, for a total of ten presentations. The stimulus pair presented first was always the target stimulus (narcotics or peppermint) with appetitive sucrose reward followed by the alternative stimulus in the given pair followed by aversive saline punishment.......................................................... 40

Figure 7. Cockroaches at Idaho State University campus during 2018 were tested for adverse effects from narcotics using two petri dishes. One small petri dish contained the cockroach and a square of filter paper soaked in the narcotic, while another larger petri dish was placed on top to ensure containment of the cockroach. Cockroaches were monitored for 60 min. No adverse effects were observed. ......................................................................................... 41

Figure 8. Diagram of large-scale gridded enclosed area at Idaho State University used to test cockroach capacity to locate substances for which they had been conditioned to associate with a reward. Each square is 15.2 cm x 15.2 cm and
labeled using an alphanumeric coordinate system in which each column is identified by a number and each row is identified by a letter. Depicted is a hypothetical scenario in which a target narcotic was planted in box F7 indicated by “X”, and the cockroach release point (RP) at box J2.

**Figure 9.** Mean and standard error time difference between time spent at each stimulus by cockroaches presented with free choice between two alternative stimuli within experimental testing arrays at Idaho State University campus in 2018 and prior to any conditioned training. Ten naive cockroaches were used for each paired stimulus free choice test and no cockroaches were used more than once or in more than one paired stimulus test. W = Water, V = Vanilla, P = Peppermint, C = Cocaine-hydrochloride, A = Adderall XR. Non-zero positive value indicates that cockroaches spent more time at the stimulus listed first in each pairing listed on the x-axis. Statistical significance was calculated using paired t-tests for each stimulus pair. * Indicates a significant (paired t-test, d.f. = 9, p< 0.05) preference for the substance listed first in the stimulus pair.

**Figure 10.** Mean and standard error time difference spent at each stimulus by cockroaches presented with free choice between two alternative stimuli within experimental testing arrays at Idaho State University campus in 2018 after conditioning training. The ten previously trained cockroaches were used for each paired stimulus free choice test and no cockroaches were used more than once or in more than one paired stimulus test. W = Water, V = Vanilla, P = Peppermint, C = Cocaine-hydrochloride, A = Adderall XR. Non-zero positive value indicates that cockroaches spent more time at the stimulus listed first in each pairing listed on the x-axis. Statistical significance was calculated using paired t-tests for each stimulus pair. * Indicates a significant (paired t-test, d.f. = 9, p< 0.05) preference for the substance listed first in the stimulus pair.
LIST OF TABLES

Table 1. Four-week learning retention testing at Idaho State University campus during 2018 on cockroaches previously rewarded with sucrose solution when affiliating with cocaine-hydrochloride. Cockroaches (n=15) exhibited a non-random frequency of stimulus choice after four weeks in which there was no additional exposure to vanilla or cocaine-hydrochloride odors (chi-square=5.63, p=.017). ................................................................. 45

Table 2. Four-week learning retention testing at Idaho State University campus during 2018 on cockroaches previously rewarded with sucrose solution when affiliating with Adderall XR. Cockroaches (n=15) exhibited a non-random frequency of stimulus choice after four weeks in which there was no additional exposure to vanilla or Adderall XR odors (chi-square=7.5, p=.006). ................. 46

Table 3. Individual travel pathways and success or failure of cockroaches tested at Idaho State University campus during 2018 for a location task for the cocaine-hydrochloride group. Successful trials are defined as the cockroach traveling to the narcotic and spending ten consecutive seconds at the substance well. All cockroaches were released at J2. Path of travel is reported using the alphanumeric coordinate system in conjunction with Figure 8. ......................... 47

Table 4. Individual travel pathways and success or failure of cockroaches tested at Idaho State University campus during 2018 for a location task for the Adderall XR group. Successful trials are defined as the cockroach traveling to the narcotic and spending ten consecutive seconds at the substance well. All cockroaches were released at J2. Path of travel is reported using the alphanumeric coordinate system in conjunction with Figure 8. ...................................................... 49
CHAPTER 1 INTRODUCTION TO CONCEPTS

BEHAVIORAL LEARNING

Animals interact with their environment via their phenotype. One component of phenotype is behavior. When an animal is capable of learning, the animal can have an experience, and then adjust its behavior based on that experience. This adjusted behavior is a demonstration of behavioral plasticity and is an outcome of learning. Humans take advantage of animals’ abilities to learn by training animals in various ways. Humans have capitalized on the trainability of animals to do many tasks, such as training dogs to track scents for hunting and training horses to be ridden. Humans utilize animals to assist in a vast array of human endeavors. I addressed the question of whether trainability to assist human agendas includes an invertebrate animal, specifically the American cockroach (*Periplaneta americana*).

TYPES OF BEHAVIORAL LEARNING

There are two major categories of learning: individual learning and social learning. Social learning occurs between individuals through many pathways including observational learning and imitation (Bandura, 1977), but does not apply well to cockroaches insofar as the literature provides no evidence that cockroaches engage in learning by observing or imitating conspecifics. Social learning occurs frequently in eusocial insects, defined as insects having caste systems such as drones, workers, and queens (Yadav et al., 2017). The divisions of labor can be learned from other group members and these roles are subject to change. For example, honeybees (*Apis mellifera*) transition through different labor positions corresponding to their age and development (Yadav et al., 2017). Bees that are older than 21 days of age become foragers. Foraging tends to damage the forager and is a terminal caste position (Yadav et al., 2017).
Individual learning differs from social learning in that an individual forms associations without the need for conspecifics to be present. Individual learning includes, but is not limited to, observational learning, habituation, sensitization, and play. One form of individual learning is associative learning, which occurs when an individual forms an association between two stimuli that have occurred together (Hawkins & Byrne, 2015). The two stimuli with which the individual forms a paired association need not be intrinsically related in their properties. For example, associations can be formed between light, food, sound, movement, and pain. Associative learning may aid behavioral adaptation and survival of individuals. Two main types of associative learning currently are recognized: classical conditioning and operant conditioning (Hawkins & Byrne, 2015). These conditioning types can occur separately or in conjunction with one another.

**Classical Conditioning**

Classical conditioning occurs when an individual forms an association between two stimuli to elicit a physiological or involuntary response (Hawkins & Byrne, 2015). The components of classical conditioning are classified as the conditioned stimulus (CS), unconditioned stimulus (UCS), conditioned response (CR), and unconditioned response (UCR) (Hawkins & Byrne, 2015). The CS begins as a neutral environmental feature meaning that it does not elicit the target physiological response. The UCS naturally elicits the target physiological response or UCR. The first step in the animal associating a neutral stimulus with the UCS in order to elicit the target physiological response is presenting these two stimuli simultaneously. Through one or more presentations of the stimuli, an association can be formed between the UCS and neutral stimulus. This association sometimes is made after one presentation provided the stimuli are potent enough (section 1.5). Once this association has been made, learning has occurred. The
neutral stimulus has become the CS because it elicits the target physiological response and the UCR has become the CR relative to the CS.

Ivan Pavlov’s famous demonstration of inducing dog salivation with a bell was a pioneering example of classical conditioning (Pavlov, 1902). Pavlov was investigating the purpose of saliva and began to test potential triggers for salivation. He presented dogs with a sound, followed by food. At first, the dog only exhibited salivation in response to the food (UCS). The sound, at this point was a neutral stimulus that did not elicit a salivation (UCR) response. During conditioning, food (UCS) and bell (neutral) were presented together. After repeated exposures, the dog began to salivate (CR) in response to the sound alone in anticipation of receiving food meaning that the formerly neutral bell had become a conditioned stimulus (CS). Associations had been made between the UCS and the neutral stimulus to the point where the neutral stimulus became a CS and elicited a CR of salivation. In this experiment, the dogs learned to associate sound with food (Pavlov, 1902). Classical conditioning had occurred.

A similar experiment demonstrated conditioned salivation in American cockroaches (Watanabe & Mizunami, 2007). Olfactory stimuli such as apple, vanilla, or peppermint odors were presented to immobilized cockroach’s antennae along with gustatory (taste) stimulation such as sucrose (appetitive) or sodium chloride (aversive) solution and the salivation levels were recorded (Watanabe & Mizunami, 2007). Sucrose is naturally appetitive to cockroaches and thus it was thought it would increase salivation rates. After the pairing of odors with sucrose solution, the salivation responses to odors paired with sucrose did increase. If peppermint was paired with sucrose, the salivation response to peppermint alone after conditioning was significantly higher than before
conditioning. Similar results occurred for vanilla paired with sucrose. Odors that were not paired with sucrose did not elicit a heightened salivation response (Watanabe & Mizunami, 2007). The UCS of sucrose was successfully paired with odors to illicit salivation. This experiment, titled “Pavlov’s Cockroaches,” was the first time that an odor and salivation paradigm for classical conditioning had been demonstrated in insects. Clearly insects were capable of forming associations via classical conditioning methods.

Carpenter ants (Camponotus aethiops) also respond to classical conditioning (Desmedt et al., 2017). Here, a behavioral response called a mandible opening response (MOR) was paired with high temperature (aversive). The MOR is an aggressive reaction in ants. After determining what temperature (75°C) to apply and where on the ant (hind legs), the team paired the heat administration with two floral scents. Before conditioning, the ants only exhibited the MOR after receiving the 75°C heat on their hind legs. Floral scents were presented multiple times to ants in multiple rounds, with one scent always accompanied by the heat stimulus for each ant. After conditioning, ants exhibited significantly higher MOR frequency when presented with the specific floral scent that had been accompanied by the heat stimulus during conditioning. This response continued to occur following a retention time test of ten minutes after initial conditioning (Desmedt et al., 2017). Again, classical conditioning had occurred.

**OPERANT CONDITIONING**

Another type of associative learning is operant conditioning. Operant conditioning occurs when a behavior is reinforced by the effect it elicits. In general, rewards increase likelihood of a behavior being performed again whereas punishments decrease likelihood of being performed again (Ferster & Skinner, 1957). Operant conditioning differs from
classical conditioning in that the reinforcement is dependent upon an operational behavior of the organism. Operant conditioning was first used by B.F. Skinner in the early 20th century. Skinner tested Thorndike’s Law of Effect which states: *Responses that produce a satisfying effect in a particular situation become more likely to occur again in that situation, and responses that produce a discomforting effect become less likely to occur again in that situation.* (Thorndike, 1898). In other words, behaviors that receive pleasant rewards are more likely to be performed than behaviors that receive unpleasant punishment. This was demonstrated empirically across several taxa and was revolutionary in the field of psychology.

Skinner expanded Thorndike’s work into the area of reinforcement, also called rewards (Ferster & Skinner, 1957). Behavior that is favorably reinforced tends to be repeated whereas behavior that is punished tends to be avoided. Operant conditioning entails an individual’s behavioral response and the reinforcement or punishment that the particular behavioral response elicits. Reinforcement and punishments both can be either positive or negative. A positive reinforcement means an element was added to increase likelihood of the behavior occurring, and a positive punishment means an element was added to decrease likelihood of that behavior occurring. For example, if a rat in a maze enters a certain path and finds a rewarding treat at the end of the path, the likelihood that the rat will choose that path again increases. This demonstrates positive reinforcement. If that same rat were to enter a path and receive a non-lethal electric shock at the end of the path, then the likelihood that the rat will choose that path again will decrease and thus demonstrates positive punishment.
Negative reinforcements and punishments follow a similar pattern, except an element is removed in order to increase or decrease likelihood of the behavior occurring. For example, a rat in a maze could have an electric shock be constantly administered and the shock only ceases when the rat chooses the correct path. Removal of shock constitutes a negative reinforcement. An example of negative punishment would be to place the rat in solitary confinement after choosing the incorrect path. The element of social interaction for a social animal has been removed in order to decrease likelihood of the wrong path being chosen again. Operant conditioning is a powerful mechanism for promoting and extinguishing behaviors.

Operant conditioning is used to train dogs for law enforcement detection of illicit substances (Jezierski et al., 2010). Dogs initially are operantly trained to find a favorite toy and play with it. Next, the toy is washed of its scent and tagged with a scent of a target substance such as marijuana. The dog associates a target scent with its toy and playtime, forming a classical conditioning association. Following this association, the toy is hidden in training areas so the dog must use the target scent to find it. When the dog finds the source of the scent, the toy, the dog is rewarded with the toy and play time as a positive reinforcement of operant conditioning. Over time, the association becomes strong and the dog will search for the target scent and does not require reinforcement of play time after every successful identification of the target scent.

Plasticity

Plasticity is defined as the quality of being easily shaped or molded (Erhlich et al., 1986). When applied to neurons, plasticity describes the ability of neurons to be capable of changing their input and output properties based on stimuli received. This
phenomenon is common in mammals (Tsukahara, 1981). When a neural pathway is productive and increasingly used, the density of neurons within that area increases (Iglesias & Villa, 2006). This is due to axonal and dendritic growth and the formation of new synapses (Iglesias & Villa, 2006). If a neural pathway is not productive, it is reduced through a process called synaptic pruning. Fluorescently tagged neurons in mice activated during the learning phase of a fear response were also activated when the mice were tasked with retrieving the memory of the response, demonstrating correlation between neural activity and behavioral expression (Garner et al., 2012).

Additionally, when more neurons fire simultaneously, the links between neuronal synapses are strengthened and the relationship between stimulus and response is also strengthened (Hebb, 1949). This is known as Hebbian learning, after Donald Hebb, who hypothesized it in 1949. Conceptually, the strength of a connection between two neurons could be increased or decreased depending on whether the neurons are firing concurrently (Hebb, 1949).

Neural plasticity is also an important characteristic of developing brains. An example in humans is the learning of new language patterns. When an individual is young, the brain more readily creates new synapses in response to stimuli and eliminates underused synapses. At approximately 12 years of age, it becomes difficult to adopt new patterns and motor skills for speaking a language (Purves et al., 2001). A decline in language fluency may begin as early as age seven (Johnson & Newport, 1989).

Neural plasticity has been demonstrated in bees (Faber et al., 1999). Before conditioning, the cortical map showed low levels of activation. Odors were then paired with a sucrose solution as an appetitive reward or the odor was presented alone. After
training, cortical maps showed a significantly higher activity in odor centers when the bees were presented with both odors, either paired or unpaired.

**Insect Learning**

Learning may be defined as a relatively permanent change in behavior as a result of experiences. Insects are capable of learning from an array of stimuli. Insects possess multiple structures homologous to the mammalian central nervous system. Mushroom bodies (*corpora pedunculata*) act as an insect brain, and nerve-like projections from these bodies contribute to receiving and responding to stimuli from the environment (Mizunami et al., 1993). The mechanisms by which they operate and communicate are poorly understood.

Interspecific variation of the neuronal population within the mushroom bodies exists. Houseflies (*Musca domestica*) have approximately 42,000 neurons while fruit flies (*Drosophila melanogaster*) possess approximately 5,000. Honeybees possess approximately 340,000 neurons. American cockroaches possess approximately 4,000,000 neurons (David & Kyung-An, 1996).

Different species have different priorities for their neuronal structures (Erber, 1976, Heisenberg, et al. 1985). Lesion surgeries are used to verify the primary use of the mushroom bodies. Fruit flies rely on the mushroom bodies for olfactory learning while cockroaches rely on mushroom bodies for spatial memory. Honeybees use mushroom bodies for appetitive learning (Erber, 1976). Strains of flies with deformed or underdeveloped mushroom bodies were not able to perform odor-learning tests (Heisenberg et al., 1985). Cockroaches that had undergone lesion surgeries were not capable of learning the location of a hidden target (Mizunami et al., 1993).
OLFACTION AND GUSTATION IN INSECTS

Gustation is the chemical sense or chemosense that allows for reception and cortical perception of taste. Gustation can provide information for many stimuli, ranging from potential mates to lethal food. Gustation is best understood in mammals, where the tongue is the primary gustatory organ (Lindemann, 2001). The tongue is covered in receptors sensitive to different molecules resulting in the sensation of taste. Generally, different classes of receptors will only bind with certain classes of molecules (Simon et al., 2006). This allows for perceptions of different tastes. When receptors are stimulated, cells transduce the signal to neural regions where its information can be encoded. This information may entail what the substance is, how much there is, how strong it is, and whether it is potentially dangerous or toxic. Gustatory stimuli can be pleasant, unpleasant, or neutral to the individual.

Olfaction is the chemosense that allows for reception and cortical perception of odors. Being able to recognize odors facilitates social interactions, foraging, reproduction and anti-predator or defense behavior (Yoon et al., 2005). This chemosense follows the same general perception pathway as gustation. Odorants are received by receptors, and the information is transduced into a neural signal that the nervous system then interprets. Olfaction is crucial for pheromone detection and reproduction in many species.

Olfaction and gustation act as important sensory systems within animals. These two senses allow animals to determine whether a substance or material is pleasant or unpleasant, lethal or non-lethal, and the two senses interact very closely. Dogs can smell a wide variety of substances and do so at low substance concentrations. A “typical” dog may have 1.2 billion olfactory receptor neurons (ORNs), and a bloodhound artificially
selected for olfactory discrimination, possesses over 4 billion ORNs (Quignon et al., 2003).

Olfaction and gustation can vary in insects due to the variability within the sensing organs. Antennae vary greatly in size and shape and even between sexes of some species. For example, fan-like antennae on pheromone-sensitive male moths are significantly larger compared to female’s antennae. Basic antenna structure is shared across all insect species. The external anatomy of the antenna from proximal to distal is as follows: scape, pedicel, and flagellum. The scape and pedicel act as the primary stabilizers for the antennae. Each segment after the pedicel is called a flagellomere, and collectively a flagellum. antennae are used to sense smell, vibration, and taste. The neural pathways and mechanisms for antennal sensing, including olfaction, are poorly understood in insects. The basic neural anatomy from distal to proximal is as follows: antennal nerve axon containing different types of receptors converging to terminate in an antennal glomerulus that further projects into the mushroom bodies. The exact mechanisms by which an insect processes an olfactory stimulus is poorly understood.

**THEORIES OF INSECT NEURAL PROCESSES**

Prevailing evidence indicates that sensory neurons within insect antennae operate using acetylcholine, and therefore are described as cholinergic (Sanes & Hildebrand, 1976). Several species of moths and bees have been discovered to have cholinergic neuron activity. In moth antennae, neurons not only store acetylcholine, but also synthesize acetylcholine (Sanes & Hildebrand, 1976). In honey-bee antennae, olfactory and mechanosensory neurons project into areas that contain high densities of acetylcholine receptors, including nicotinic acetylcholine receptors (Baker et al., 2008). In cockroaches, injection of alpha-bungarotoxin or another acetylcholine antagonist
eliminated salivation induced by odors (Baker et al., 2008). This led researchers to accept that antennal neurons in insects use acetylcholine as a neurotransmitter. Acetylcholine has been documented to show increased synaptic pruning as well as attenuate strength of signals transmitted (Hasselmo, 2006).

Serotonin also has been located within the cockroach, but few studies have been able to isolate serotonergic neurons (Watanabe et al., 2013). Serotonin is known to be a key factor in motor activity; therefore, it is logical to find serotonin within the antennae since they are used for proprioception and are rarely immobile. In mammals, serotonin has been linked to memory, specifically aversive learning (Zhang & Lu, 2005).

**CONDITIONED TASTE AVERSION**

Within associative learning involving gustatory stimuli, there is a phenomenon known as “conditioned taste aversion” (Garcia & Kimeldorf, 1955). Conditioned taste aversion occurs when an organism associates an unpleasant experience with a gustatory or olfactory stimulus (Welzl et al., 2001). A previously neutral stimulus, such as food or taste or smell, can become associated with an aversive stimulus. This can result in an unconditioned response of aversion when later presented with the previously neutral taste or smell. The food taste or smell has become a conditioned stimulus when it triggers a conditioned response of avoiding the food item.

Conditioned taste aversions can occur even after one unpleasant experience. Many prey animals, such as insects and frogs, display aposematic coloration, brightly colored with yellows, red, and oranges (Prudic et al., 2007), described as “warning colors” and are associated with prey that does not taste pleasant or one that will cause sickness. For example, monarch butterflies (*Danaus plexippus*) display orange and black aposematic wings, and the butterfly’s tissue contains cardiac glycosides that deter
predators (Fink & Brower, 1981). The bright coloring of the butterfly may be a neutral stimulus to a naïve predator but may become an aversive CS to an experienced predator.

CONDITIONED TASTE AVERSION IN INSECTS
American Cockroaches exhibit conditioned taste aversions under controlled experimental conditions (Watanabe et al., 2003). Cockroaches naturally preferred vanilla over peppermint odors but this pattern was reversed when investigators paired vanilla with aversive saline solution and peppermint with appetitive sucrose solution. Results indicated that repeated presentations of the stimulus pairs increased the strength of the associations formed (Watanabe et al., 2003). Based on these results, cockroaches were tested four days after the last conditioning session. Results showed training was retained with an 80-100% accuracy (Watanabe et al., 2003). This work demonstrates cockroach behavioral plasticity with classical conditioning using conditioned taste aversions.

CURRENT STUDY AIMS
I investigated conditioned learning in cockroaches relative to olfactory stimuli and narcotics. I used classical and operant conditioning methods to test if American cockroaches could learn to locate narcotics. My overarching goal was to test the plausibility that cockroaches could be deployed in law enforcement drug search settings.

First, I replicated Watanabe et al.’s (2003) conditioning trials but increased the exposure to each stimulus pair from three to five to augment learning retention. Then, I tested cockroaches for any innate narcotic preference of aversion and the capacity for cockroaches to learn to seek narcotics. I tested cockroach learning retention and ability to locate randomly planted narcotic targets within a localized area. This included two extended memory retention tests, a six-week test on food odorants, and a four-week test on the narcotics. Lastly, I tested cockroach abilities to locate narcotics on a larger spatial
scale by releasing cockroaches into a small room in which a narcotic substance was
planted and monitored in order to analyze the robustness of training. This phase was the
closest simulation to real-world implementation that has been conducted for cockroaches.
I present my work in seven parts as three chapters: Introduction to Concepts, Cockroach
Conditioning Using Paired Stimuli, and Commercial Applications.
LITERATURE CITED


CHAPTER 2 COCKROACH CONDITIONING USING PAIRED STIMULI

ABSTRACT

Insects can learn via operant and classical conditioning methods. The American cockroach learns to alter preference for food-borne odors by pairing olfactory and gustatory stimuli (Watanabe et al., 2003). Cockroaches can be induced to reverse odor preferences after training using aversive saline or appetitive sucrose in association with odors. I replicated cockroach odor preference reversal, increased stimulus pair presentations, and demonstrated extended memory retention on both food and non-food borne odors i.e., narcotics. I also demonstrated the cockroach ability to apply training within a controlled setting by using a location task in which cockroaches found randomly planted narcotics within a large space. My results provide a foundation for employing cockroaches for drug detection in order to augment current detection methods.
INTRODUCTION

The American cockroach has been used as a model organism for behavioral studies because of its ability to demonstrate behavioral plasticity quickly in controlled settings (Watanabe et al., 2003). Cockroaches innately prefer dark and humid areas in addition to a diet of sugars (Bell et al., 2007). Cockroaches are predictable in that they are active, reproduce quickly, and are highly food motivated in seeking sugary foods. This allows humans to explore cockroach behavioral capacities by pairing different types of stimuli with sucrose solutions.

Cockroaches exhibit behavioral plasticity as evidenced by cockroaches reversing odor preferences after a naturally appetitive olfactory stimulus is paired with an aversive gustatory stimulus or pairing an aversive olfactory stimulus is paired with an appetitive gustatory stimulus (Watanabe et al., 2003). For example, after training, cockroaches choose naturally aversive peppermint odor over naturally preferred vanilla odor. This alteration of behavior was accomplished with only three presentations of each stimulus pair and cockroaches retained the conditioning four days after conditioning (Watanabe et al., 2003).

I employed Watanabe et al.’s (2003) paired stimulus paradigm to test aspects of cockroach learning. Cockroach learning potentially creates a new use of cockroaches for humans such as environmental detection of substances of interest. Because cockroaches also possess a set of traits that complement existing biological detection methods, investigation of cockroach ability to be conditioned on narcotic substances is valuable.
METHODS

I obtained 50 adult *P. americana* subjects from Carolina Biological Supply Company (Burlington, NC, USA) to use as brood stock and housed them in 30.5 x 16.5 cm plastic tanks with ventilation slits, also obtained from Carolina Biological. I provided food and water *ad libitum*. Diet consisted of 2 cm banana per tank and a petri dish of distilled water. Both food and water were changed every 96 h. *Ad libitum* food and water were intended to minimize hunger and thirst as factors influencing performance. Tank substrate consisted of compressed coconut fiber from Zoo Med Laboratories Inc. (San Luis Obispo, CA, USA). I provided cardboard poultry egg cartons and cardboard paper towel rolls for shelter (Fig. 1). I held temperature between 21-30°C using heat lamps. I held humidity between 50-70%. Housing configuration allowed cockroaches to socialize or to be isolated.

I used McCormick’s © (Baltimore, MD, USA) peppermint and vanilla essence as food odorants. I also used a 4% cocaine-hydrochloride solution and a 1mg/mL solution of Adderall XR (Fig. 2) obtained from Cardinal Health © (Dublin, OH, USA). The United States Department of Homeland Security, Drug Enforcement Agency and the Idaho Board of Pharmacy issued possession and scientific use licensing for Kayla Victoria Pavlick (Fig. 3).

For cockroach training phases, I used a 250mL glass beaker covered with Parafilm ® (Bemis Company Inc., Neenah, WI, USA) to hold cockroaches. I mounted stimulus-soaked filter paper on a 38mm syringe and filled the syringe with corresponding solutions of either saline or sucrose (Fig. 4).

For testing phases, I built an experimental chamber out of plexiglass that included a resting side for acclimation, moveable barrier of electrical tape wrapped cardboard, and
testing side which held petri dishes of filter paper soaked in stimuli (Fig. 5). I used a metal stirring rod to nudge cockroaches from the testing side to the resting side of the chamber when the testing time period had expired.

**Replication of Watanabe et al. (2003) Cockroach Training**

I conducted a modified replication of the Watanabe et al.’s (2003) experiment. Pre-existing preferences for vanilla over peppermint (V>P) were assumed based on published results (Watanabe et al., 2003) and I did not test these preferences further. I modified the training protocol to allow for the cockroaches to move freely within the 250 mL containment beaker (Fig. 4). Previously, Watanabe et al.’s (2003) study found that cockroaches restrained for training had a lower preference for peppermint one day after training compared to cockroaches that were unrestrained, potentially due to stress from being restrained.

I trained cockroaches singly and allowed 5 min of acclimation after being placed in a training beaker. I then presented cockroaches with peppermint and vanilla odors on filter paper squares mounted on a syringe. Within the syringe was the corresponding appetitive reward or aversive punishment (Fig. 4). I paired saline solution with vanilla, and I paired sucrose solution with peppermint. I lowered the stimulus pairs apparatus into the beaker for the cockroach to investigate. I define investigation as antennal touching for 0.5 seconds. After cockroach investigation, I administered saline or sucrose directly to the mouth of the cockroach. I presented vanilla attractant paired with saline solution first to each cockroach. Then, I transferred the cockroach into a clean beaker and allowed the cockroach five minutes to acclimate. I then exposed the cockroach to a peppermint attractant paired with sucrose solution. I repeated this process such that each cockroach experienced alternating vanilla with saline and peppermint with sucrose pairings five
times. This was an increase of two presentations each relative to Watanabe et al. (2003).

My pairings occurred five minutes apart (Fig. 6).

REPLICATION OF WATANABE ET AL. (2003) COCKROACH TESTING

Immediately after completing the last stimulus pair presentations to the cockroach, I transferred the cockroach into the resting side of the testing chamber (Fig. 5) for 5 min of acclimation. After the acclimation period, I lifted the barrier separating the cockroach from the testing chamber to allow the cockroach to move voluntarily to the testing chamber (Fig. 5). The testing chamber contained 4 wells. Two wells contained filter paper soaked in vanilla and the other two wells contained filter paper soaked in peppermint. Once the cockroach crossed into the testing chamber, it was allowed 5 min to choose a well. I defined choice as 1.5 seconds of antennal touching to the filter paper.

Once the cockroach made a choice, it was nudged back to the resting side of the chamber with a metal stirring rod. I then randomly re-ordered the testing well attractants and re-tested the cockroach. I scored results using the Peppermint Preference Index (PPI) adapted from Watanabe et al. (2003). The PPI is the frequency the target substance was chosen and is expressed as a percentage calculated as:

\[
100 \left( \frac{n_p}{n_p + n_v} \right)
\]

where \(n_p\) is the number of times peppermint was chosen and \(n_v\) is the number of times vanilla was chosen.

TEST OF SIX-WEEK RETENTION ON PEPPERMINT AND VANILLA

I performed a six-week retention test to analyze learning retention. After initial training, cockroaches were not exposed to vanilla, peppermint, sucrose solution, or saline solution during the following six weeks. To test retention, I placed each cockroach into
the testing chamber (Fig. 5) to repeat preference testing. I followed procedures identical to initial post-training testing.

**TEST FOR PRE-EXISTING NARCOTIC PREFERENCE**

To evaluate if cockroaches exhibited any strong innate preference aversion, or adverse effect to narcotics, I exposed ten cockroaches to the narcotic substances to observe cockroach responses. I soaked a 1 cm x 1 cm piece of filter paper in narcotic (cocaine-hydrochloride stimulus or Adderall XR stimulus) and placed the narcotic stimulus in a petri dish. I then placed a larger petri dish over the cockroach and smaller petri dish thereby causing cockroaches to occupy the area containing narcotic (Fig. 7). I observed cockroaches for any irregular behavior.

Because I did not observe deleterious effects of exposure to narcotic stimuli, I then tested cockroaches for pre-existing preferences by for narcotic stimuli relative to other stimuli. Some preference ranks for cockroaches were previously published (Watanabe et al. 2003). In particular, cockroaches prefer vanilla over water (V>W) and prefer both vanilla and water over peppermint (V>W>P). I tested narcotics within this paradigm. I assumed cockroaches possessed the capacity to detect the cocaine-hydrochloride and Adderall XR because octopaminergic pathways exist in invertebrates (Mizunami et al., 2009).

To measure preferences for each cockroach, I measured time the cockroach spent at each stimulus in free choice tests of paired stimuli. Then, I calculated the average across all cockroaches of difference in time spent at each stimulus per cockroach, within the stimulus pairs (Fig. 9). I used 15 naïve cockroaches for each set of paired stimuli.

I allowed each cockroach five minutes of acclimation time in the resting side of the chamber (Fig. 5) before the barrier was lifted. Once the cockroach crossed into the
testing side of the chamber, I lowered the barrier restricting the cockroach to the testing side of the chamber. The testing side of the chamber had two wells. Within each well was filter paper soaked in one of two different stimuli of the pairs (Fig. 9). I allowed a total of five minutes on the testing side before guiding the cockroach back to the resting side of the chamber.

**TRAINING COCKROACHES TO MAKE ASSOCIATIONS WITH NARCOTICS**

I placed the cockroaches in a 250mL beaker covered with Parafilm ® with an opening sufficient for insertion of odor and taste stimulus pairs while ensuring the unrestrained cockroach remain contained. This was a modification from Watanabe et al. (2003). To create odor and taste stimulus pairs, I mounted a small square of filter paper (1 cm x 1 cm) on a 38-mm hypodermic needle with corresponding solution in the syringe (Fig. 4). Odor stimulus and gustatory solution pairs were as follows: narcotic odor paired with sucrose solution (appetitive) or vanilla odor paired with sodium chloride solution (aversive). I sequentially presented stimulus pairs for the cockroach to investigate. I defined investigation as tactile touching of the odor-soaked filter paper with the antennae by the cockroach. After approximately 0.5 seconds of investigation, I dispensed one drop of the gustatory solution to the mouthparts of the cockroach. I then transferred the cockroach to a clean 250 mL beaker and performed the next sequential stimulus pair to that cockroach in an untainted environment five minutes later. I continued this for a total of five presentations for each stimulus pair, and ten total presentations per cockroach (Fig. 6). I trained fifteen cockroaches in this way pairing narcotic with sucrose and vanilla with saline for fifteen cockroaches exposed to cocaine hydrochloride, and fifteen cockroaches exposed to Adderall XR.
TESTING COCKROACHES CONDITIONED TO NARCOTICS
I tested cockroaches for choice of narcotic odor versus alternative odor immediately after the final training pair presentation. I used paired stimuli in a testing chamber as with previous testing (Fig. 5). I used two wells with stimulus-soaked filter paper inside each well. One well contained narcotic while the other contained vanilla. I allowed each cockroach five minutes to acclimate within the resting side of the chamber before I lifted the barrier. Once the barrier was lifted, the cockroach was allowed 5 min total time on the testing side of the chamber. Each time the cockroach investigated a well, defined as antennal touching of the filter paper within the well, I recorded the time the cockroach spent at that well. After 5 min, the cockroach was guided back to the resting side of the chamber. For each cockroach, I calculated the difference in time spent between the competing stimuli within the testing chamber (Fig. 10).

TEST OF FOUR-WEEK RETENTION OF NARCOTIC TRAINING
I performed a four-week retention test to analyze how permanent the training on narcotics had become. The fifteen cockroaches previously trained and tested on narcotics versus vanilla were not exposed to narcotics, vanilla, sucrose solution, or saline solution for four weeks. I then placed the cockroaches back into the testing chamber (Fig. 5) and repeated the preference testing conducted immediately after initial training. I tested each cockroach on the narcotic substance it was trained on (i.e. cocaine-hydrochloride or Adderall XR) versus vanilla 5 times for 5 min per test. I measured time spent at each stimulus and used the magnitude of mean value to score each cockroach as selecting narcotic (higher mean time) or vanilla (higher mean time). I used $\chi^2$ to test frequency of cockroach stimulus selection.
Cockroaches Locating Narcotics

To test cockroach ability to locate a stimulus in a large spatial area, I placed cockroaches in a 1.52 m x 2.74 m enclosed space that was gridded into 15.2 cm x 15.2 cm squares (Fig. 8). I identified each square using an alphanumeric coordinate system in which the rows were labeled by letters and columns by numbers so that each square could be identified by one letter followed by one number (i.e. A2, G8, L6, etc.). I used a video camera mounted above the grid to record each trial and individual cockroach movements continuously so that each square that the cockroach traveled through could be reported in the order in which the cockroach occupied it. A well with filter paper soaked in target narcotic was randomly placed within a square in the testing area. I randomly selected the square to plant the narcotic and re-randomized the location between trials. I released one cockroach into the test area at grid point J2 and allowed cockroaches 15 min to explore the test grid. I recorded the path taken by the cockroach in sequential order of squares occupied and the time taken for the cockroach to find the target substance.

I define a successful trial as the cockroach finding the narcotic well within a 15-minute trial period and spending ten consecutive seconds touching the filter paper with its antennae. Once the cockroach spent ten consecutive seconds at the well, I terminated the trial and removed the cockroach from the test grid. If the cockroach did not spend ten consecutive seconds at the target well, the trial was scored as the cockroach failing to locate the target.
**RESULTS**

Overall, I was able to rear, train, and test cockroaches for odor preferences. Prior to conditioning, cockroaches found narcotic odors to be aversive. After training, cockroach learned to prefer narcotic substances. Cockroaches also applied this training in context of a location task in which the cockroaches were able to locate the narcotics within 15 minutes of being released.

**REPLICATION OF WATANABE ET AL. (2003)**

With training, cockroaches were conditioned to select peppermint over vanilla, consistent with Watanabe et al. (2003). Fifteen cockroaches each subsampled 5 times generated a Peppermint Preference Index of 98.6 %. One cockroach chose peppermint four out of five times, and fourteen cockroaches chose peppermint five out of five times. This universal selection of peppermint relative to vanilla (15/15) was highly significant.

**TESTING FOR PRE-EXISTING PREFERENCES**

After exposing the cockroaches to narcotics, I monitored the cockroaches for 60 minutes and observed no behavioral changes or other adverse effects. Cockroaches investigated the narcotic stimuli but did not express notable responses to it.

In paired choice trials, cockroaches exhibited the same preference hierarchy as Watanabe et al. (2003). Cockroaches exhibited responses to narcotics within the hierarchy. Cockroaches treated narcotics as being similarly aversive as peppermint (N~P). Water paired with water as a control group resulted in no difference between water wells (W~W). In other paired comparisons, I observed differences between paired stimuli (Fig. 9). Paired t-tests comparing mean time spent at wells indicated significant differences within some pairs (Fig. 9) and revealed a distinct hierarchy of stimuli.
preference as follows: Vanilla > Water > Peppermint ~ Cocaine-hydrochloride ~ Adderall XR.

**TRAINING COCKROACHES ON NARCOTICS**

Using gustatory conditioning, I was able to train cockroaches to select narcotic odor over previously preferred odors. Water paired with water as a control group resulted in no difference between water wells (W~W). In other paired comparisons, I observed differences between paired stimuli (Fig. 10). Paired t-test comparing mean time spent at wells indicated significant differences within some pairs (Fig. 10) and revealed a distinct hierarchy of stimuli preference following conditioning as follows: Cocaine-hydrochloride ~ Adderall XR > Vanilla > Water > Peppermint.

**SIX-WEEK PEPPERMINT AND VANILLA RETENTION TESTING**

After modifying previously published methods to increase stimulus pair presentations to five repeated exposures, cockroaches retained the conditioned behavior training for six weeks. The same 15 cockroaches from the previous initial conditioning were tested and generated a Peppermint Preference Index of 96.0%. All cockroaches exhibited significant learning retention after 6 weeks. Three cockroaches chose peppermint four out of five times while the remaining 12 cockroaches chose peppermint five out of five times.

**FOUR-WEEK NARCOTIC RETENTION TESTING**

After modifying previously published methods to increase stimulus pair presentations to five repeated exposures, cockroaches retained the conditioned behavior training for four weeks on cocaine-hydrochloride and Adderall XR (Tables 1 and 2, respectively). One cockroach chose vanilla, and fourteen cockroaches chose cocaine (\(\chi^2 = \))
while the Adderall XR group did not have any cockroaches choose vanilla ($\chi^2 = 7.5, p = .006$).

**Cockroaches Locating Narcotics**

The cockroaches retained their training and successfully performed a location task. Eight out of ten cockroaches located Adderall XR within the 1.52 m x 2.74 m test area during the 15-minute test ($\bar{x} = 7.87$ min, SE = 2.40 min) while seven out of ten cockroaches located cocaine-hydrochloride ($\bar{x} = 6.28$ min, SE = 3.00 min). Individual paths taken by cockroaches for Adderall XR and cocaine-hydrochloride (Tables 3 and 4, respectively) indicate cockroaches did not travel directly to the narcotic, suggesting that the cockroaches were relying on olfaction. If the cockroach traveled further away from the narcotic, where presumably the olfactory signal became weaker, the cockroach would change its trajectory until arriving at the target.

**Discussion**

Cockroaches have a natural preference for vanilla, and a natural aversion to peppermint. Watanabe et al. (2003) demonstrated the behavioral plasticity of cockroaches by altering cockroach selection with a conditioning training paradigm in order to have peppermint selected over vanilla. This was accomplished by employing classical conditioning through pairing vanilla odor with aversive saline and peppermint odor with appetitive sucrose. I modified and replicated their approach to classically condition cockroaches. I found that cockroaches learned and retained their learning. Following conditioning, cockroaches consistently chose peppermint over vanilla six weeks after receiving training. This piece of my work contributes to the existing knowledge of learning in insects, as well as alludes to potential clinical purposes for studying cockroaches in regard to human health. Formation of long-term memories reorganizes the
insect brain on a synaptic level (Hourcade et al., 2010). Many diseases affecting memory could be further studied in the insect model, now that the model has been shown to perform an extensive memory task. This could be accomplished by incorporating molecular staining and imaging, as well as neurotransmitter inhibition or receptor blocking (Umezaki et al. 2011).

Because cockroaches naturally prefer vanilla, my strongest demonstration of preference alteration for cockroaches was my demonstration that classical conditioning caused cockroaches to select narcotic odor over vanilla odor. My initial preference testing indicated that narcotics were similarly aversive to the cockroaches as peppermint. Prior to training, the cockroaches were not seeking nor did they have a prior attraction to the narcotics. By reinforcing the narcotic odor with appetitive gustatory sucrose and appealing to the cockroach drive for sugary foods, I showed that training was effective enough to overcome narcotic aversion as well as the strong natural preference for vanilla. In this context, it was straight-forward to overcome aversion for peppermint with preference for narcotics paired with sucrose. The significant p-values given by the statistical tests confirm the strength of the relationships formed was not due to chance. This demonstrates the behavioral plasticity and learning ability of the cockroaches as well as the efficacy of my methods.

The six-week retention test demonstrated how robust the training was for cockroaches. Training has potential to last even longer than six weeks, and development of this training method to further investigate the extinction rate of learned behaviors would be valuable. My results also indicate that the cockroaches continued to choose peppermint over vanilla even six weeks after initial conditioning. This is the most
extensive memory task that has been performed with a cockroach to date. Expanding
upon this work to include possibilities of engrams (Hebb, 1949), association cortex
storage (Lashley, 1950), and the “switch-point” between short-term and long-term
memory could provide a memory trace (Young, 1964). This may be valuable in clinical
applications to humans regarding memory formation, storage, and degradation.

The four-week retention test similarly demonstrated how robust the training was
for the cockroaches. Though one cockroach preferred vanilla over cocaine-hydrochloride,
the remaining fourteen cockroaches, statistically significant evidence suggests that the
cockroaches were continuing to choose the narcotic substances over their naturally
preferred vanilla four weeks after initial conditioning. This is the first time that retention
for non-food borne odors has been demonstrated to this degree in cockroaches.

The purpose of giving cockroaches a locating task in a large space was to analyze
to what extent the cockroaches could locate the source of a target substance in terms of
distance and time. This location testing provided insight into the retention capacity of
cockroaches, and the behavioral plasticity in regard to application of training. This was
the first real-world simulation of insect detection for narcotics. I demonstrated the short-
term retention of training in the cockroaches and a capacity for cockroaches to perform at
a large spatial scale. Using the definition of behavioral learning as a relatively permanent
change in behavior, my results demonstrated learning, a relatively permanent change. The
environment in which the cockroaches performed was large and I avoided location bias
by randomly generating the target locations for each trial. Cockroaches appeared to apply
learning to locate.
Overall, cockroach behavioral plasticity was demonstrated with two different classes of stimuli: food-borne gustatory and non-food-borne odors. Previous work had demonstrated the cockroach ability to learn quickly in order to have an altered preference for food borne odors and speculated on the long-term retention of the training. Retention of odor preference alterations had been documented for up to four days (Watanabe et al., 2003). I showed six weeks retention. Furthermore, retention on non-food borne odors has not been documented until my experiment. The results from my work suggest that this conditioning paradigm is robust enough to have cockroaches learn and retain that learning for an extended period. Additionally, the location phase of my work highlights cockroach ability to express their learning in large environments. I demonstrated that cockroaches can learn, retain, and apply training in a short period of time within a controlled setting.
Literature Cited
https://doi.org/10.1093/icb/icn074.


https://doi.org/10.1523/JNEUROSCI.0841-10.2010.


Figure 1. General housing containers for cockroaches maintained on the Idaho State University campus 2018-2019 for behavioral conditioning experiments. A limit of 15 cockroaches was maintained within each tank. Tank dimensions: (7.5" W × 11.25" L × 9.25" H) Naïve cockroaches were kept in separate tanks from conditioned cockroaches. All cockroaches kept on a 12:12 light cycle. Temperature was kept between 21-30 C° and humidity kept between 50-70%. Peeled banana and water were given ad libitum and changed every 96 h. Cardboard and egg cartons provided shelter.
Figure 2. Narcotics used in experiments of cockroach behavioral conditioning at Idaho State University campus 2018-2019 were a pre-manufactured 4% cocaine-hydrochloride solution and Adderall XR mixed to make a 1mg/mL solution. All narcotics were obtained from Cardinal Health (7000 Cardinal Pl. Dublin, OH 43017).
Figure 3. Professional licensing for Kayla V. Pavlick allowing for possession and use of Schedule II narcotics for experimental cockroach training at Idaho State University campus during 2018. Licensing was renewed as needed.
Figure 4. Illustration of experimental configuration for pairing odor stimulus with taste reward or punishment for cockroaches receiving conditioned training at Idaho State University campus during 2018. The filter paper was soaked in peppermint or vanilla odor, or cocaine-hydrochloride or Adderall XR solution. The syringe was filled with a corresponding sucrose (appetitive) or saline (aversive) solution. After antennae contacted scented filter paper, a gustatory stimulus was delivered from syringe to cockroach mouth parts.
Figure 5. Diagram of testing chamber used to test cockroach learning at Idaho State University campus during 2018. Two wells on the testing side contained filter paper soaked in olfactory stimuli. A moveable barrier was constructed to keep the resting (acclimation) side and testing side separate. The chamber was sterilized after each trial with 90% isopropyl alcohol.
Figure 6. Stimulus pair presentation schedule for cockroaches receiving conditioning training at Idaho State University campus during 2018. Each cockroach experienced five rounds of conditioning in which each olfactory x gustatory pair was presented five minutes apart, five times for each stimulus pair, for a total of ten presentations. The stimulus pair presented first was always the target stimulus (narcotics or peppermint) with appetitive sucrose reward followed by the alternative stimulus in the given pair followed by aversive saline punishment.
Figure 7. Cockroaches at Idaho State University campus during 2018 were tested for adverse effects from narcotics using two petri dishes. One small petri dish contained the cockroach and a square of filter paper soaked in the narcotic, while another larger petri dish was placed on top to ensure containment of the cockroach. Cockroaches were monitored for 60 min. No adverse effects were observed.
Figure 8. Diagram of large-scale gridded enclosed area at Idaho State University used to test cockroach capacity to locate substances for which they had been conditioned to associate with a reward. Each square is 15.2 cm x 15.2 cm and labeled using an alphanumeric coordinate system in which each column is identified by a number and each row is identified by a letter. Depicted is a hypothetical scenario in which a target narcotic was planted in box F7 indicated by “X”, and the cockroach release point (RP) at box J2.
Figure 9. Mean and standard error time difference between time spent at each stimulus by cockroaches presented with free choice between two alternative stimuli within experimental testing arrays at Idaho State University campus in 2018 and prior to any conditioned training. Ten naive cockroaches were used for each paired stimulus free choice test and no cockroaches were used more than once or in more than one paired stimulus test. W = Water, V = Vanilla, P = Peppermint, C = Cocaine-hydrochloride, A = Adderall XR. Non-zero positive value indicates that cockroaches spent more time at the stimulus listed first in each pairing listed on the x-axis. Statistical significance was calculated using paired t-tests for each stimulus pair. * Indicates a significant (paired t-test, d.f. = 9, p< 0.05) preference for the substance listed first in the stimulus pair.
Figure 10. Mean and standard error time difference spent at each stimulus by cockroaches presented with free choice between two alternative stimuli within experimental testing arrays at Idaho State University campus in 2018 after conditioning training. The ten previously trained cockroaches were used for each paired stimulus free choice test and no cockroaches were used more than once or in more than one paired stimulus test. W = Water, V = Vanilla, P = Peppermint, C = Cocaine-hydrochloride, A = Adderall XR. Non-zero positive value indicates that cockroaches spent more time at the stimulus listed first in each pairing listed on the x-axis. Statistical significance was calculated using paired t-tests for each stimulus pair. * Indicates a significant (paired t-test, d.f. = 9, p < 0.05) preference for the substance listed first in the stimulus pair.
**Table 1.** Four-week learning retention testing at Idaho State University campus during 2018 on cockroaches previously rewarded with sucrose solution when affiliating with cocaine-hydrochloride. Cockroaches (n=15) exhibited a non-random frequency of stimulus choice after four weeks in which there was no additional exposure to vanilla or cocaine-hydrochloride odors (chi-square=5.63, p=.017).

<table>
<thead>
<tr>
<th></th>
<th>Vanilla</th>
<th>Cocaine-hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Observed</td>
<td>1</td>
<td>14</td>
</tr>
</tbody>
</table>
Table 2. Four-week learning retention testing at Idaho State University campus during 2018 on cockroaches previously rewarded with sucrose solution when affiliating with Adderall XR. Cockroaches (n=15) exhibited a non-random frequency of stimulus choice after four weeks in which there was no additional exposure to vanilla or Adderall XR odors (chi-square=7.5, p=.006).

<table>
<thead>
<tr>
<th></th>
<th>Vanilla</th>
<th>Adderall XR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Observed</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 3. Individual travel pathways and success or failure of cockroaches tested at Idaho State University campus during 2018 for a location task for the cocaine-hydrochloride group. Successful trials are defined as the cockroach traveling to the narcotic and spending ten consecutive seconds at the substance well. All cockroaches were released at J2. Path of travel is reported using the alphanumerical coordinate system in conjunction with Figure 8.

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>Success or Failure</th>
<th>Time to Narcotic</th>
<th>Path of Travel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Success</td>
<td>3:42</td>
<td>I2, H2, G2, G1, F1, E1, E2, F2, G2, G3, F3, E3, E4, D4, C4, B4, B5, B6, C6, D6, E6, F6, H6, H5, I5, I6, I7, J7, J8, I8, H8, H9, I9, J9, K9, K10, J10, I10, H10</td>
</tr>
<tr>
<td>2</td>
<td>Success</td>
<td>5:11</td>
<td>I2, H2, G2, F2, F1, E1, D1, C1, C2, C1, B1, B2, A2, A3, A4, B4, C4, D4, D5, E5, E4, E3, F3</td>
</tr>
<tr>
<td>3</td>
<td>Failure</td>
<td>–</td>
<td>J3, J4, I4, H4, G4, F4, E4, E3, E2, E1, D1, D2, E2, F2, G2, H2, H1, I1, J1, K1, L1, M1, N1, O1, P1, P2, Q2, R2, R1</td>
</tr>
<tr>
<td>4</td>
<td>Success</td>
<td>7:34</td>
<td>I2, I1, H1, I1, I2, J2, J3, I3, I4, I5, H5, G5, G6</td>
</tr>
<tr>
<td>5</td>
<td>Failure</td>
<td>–</td>
<td>I2, H2, H3, G3, F3, E3, E2, D2, C2, B2, A2, A1, A2, A3, A4, B4, C4, C5, D5, D6, D7, C7, B7, B8, C8, D8, E8, F8, F9, D10, C10, B10, A10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>Success</td>
<td>10:26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J1, K1, L1, L2, K2, J2, I2, H3, H4, I4, J4, J5, K5, L5, L6, K6, J6, J5, I5, H5, G5, F5, E4, D4, C4, C5, C6, D6, D7, D8, C8, B8, A8, A9, B9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Success</td>
<td>8:51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J1, K1, K2, L2, M2, N2, O2, Q3, N3, M3, M4, N4, N5, O5, P5, Q5, Q4, R4, R5, Q6, Q7, Q8, Q9, Q10, P10, O10, N10, N9, O9, N9, M9, N11, L10, L9, L8, L8, K8, K7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Success</td>
<td>8:04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K2, L2, L3, M3, M4, M5, N5, O5, N5, P5, Q5, R5, R4, R3, Q3, R3, R4, Q4, P4, P3, P2, Q2, Q1, P1, O1, N1, M1, M2, N2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Failure</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J3, I3, I4, I5, I6, J6, J7, I7, H7, H8, H9, G9, G10, F10, E10, D10, D9, D8, E8, E7, D7, C7, B7, A7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Success</td>
<td>6:29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K2, K3, K4, L4, L5, M5, N5, N6, M6, M6, O6, P6, Q6, Q5, Q4, P4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

48
Table 4. Individual travel pathways and success or failure of cockroaches tested at Idaho State University campus during 2018 for a location task for the Adderall XR group.

Successful trials are defined as the cockroach traveling to the narcotic and spending ten consecutive seconds at the substance well. All cockroaches were released at J2. Path of travel is reported using the alphanumeric coordinate system in conjunction with Figure 8.

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>Success or Failure</th>
<th>Time to Narcotic</th>
<th>Path of Travel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Success</td>
<td>5:06</td>
<td>I2, H2, G2, G3, H3, I3, I4, J4, J3, K3, L3, M3, N3, N4, M4, N4, O4, P4, P3, P2, P1, Q1, R1, Q1, Q2, R2, R3, R4, R5, R6, R7</td>
</tr>
<tr>
<td>2</td>
<td>Failure</td>
<td>–</td>
<td>J3, I3, H3, H2, G2, G1, F1, E1, D1, C1, B1, A1, B1, A1</td>
</tr>
<tr>
<td>3</td>
<td>Success</td>
<td>11:27</td>
<td>J1, K1, L1, L2, L1, M1, M2, N2, N3, O3, P3, Q3, Q2, P2, P3, O3, O2, O1, P1</td>
</tr>
<tr>
<td>4</td>
<td>Success</td>
<td>8:44</td>
<td>K2, L2, M2, M3, N3, O3, N3, N4, O4, P4, P5, P6, O6, N6, M6, M5</td>
</tr>
<tr>
<td>6</td>
<td>Success</td>
<td>8:16</td>
<td>J3, J4, K4, K5, L5, L6, L7, K7, J7, J6, I6, I5, J5, I5, H5, G5, G6, F6, E6, E5, D5, D6, C6, C5, C4, D4, E4</td>
</tr>
<tr>
<td>7</td>
<td>Failure</td>
<td>–</td>
<td>K2, L2, M2, N2, N1, O1, P1, Q1, R1, Q1, R1</td>
</tr>
<tr>
<td>8</td>
<td>Success</td>
<td>7:31</td>
<td>I2, H2, G2, G3, F3, E3, E4, F4, G4, H4,</td>
</tr>
<tr>
<td>9</td>
<td>Success</td>
<td>6:05</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10</th>
<th>Success</th>
<th>9:24</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| H5, G5, F5, F6, E6, E7, E8, E9, D9, C9, B9, A9, A8, B8, C8, C7, B7, A7, B7, B6 | I2, I1, H1, H2, G2, G3, F3, E3, E4, D4, C4, C5, D5, D6, C6, B6, A6, A7, A8, B8, B7, A7, A6, A5, B5, B4, A4, A3, B3, C3, C2, C3, D3, E3, F3, F2, E2, E1 |
| K2, K3, K4, J4, K4, L4, L5, L6, M6, N6, N7, M7, M8, L8, K8, K7, J7, I7, I6, H6, G6, G5, F5, E5, E6, F6, G6, G7, G8 |
CHAPTER 3 COMMERCIAL APPLICATION

ABSTRACT
Humans employ animals’ ability to learn in order to use animals’ abilities to assist humans in accomplishing a wide range of tasks. Humans do so with canines by employing canines as detection units for illicit substances. However, dogs are limited in several ways. Dogs are subject to handler bias, cannot fit into small spaces, are subject to mental stress, can harm humans, and are expensive to train and maintain. Many of these limitations could be overcome by co-deploying insects for detection, specifically American cockroaches (Periplaneta americana). Cockroaches are not subject to handler bias and can infiltrate smaller spaces than dogs. Cockroaches also do not express a complex stress response and are safe and affordable to train and maintain. Previously (Chapter 2), I demonstrated that cockroaches can be trained to detect narcotics. Here, I review the limitations of canine detection units and ways in which cockroach detection units might compensate for these limitations.
INTRODUCTION
The illegal drug market has been a consistent problem within the United States affecting the economy, healthcare, and culture (United Nations, 2018). Cocaine is a prime example. The United Nations Office on Drugs and Crime estimated the number of cocaine users globally increased from approximately 14 million in 1998 to 18.8 million in 2014 (United Nations, 2018). Cocaine has been documented to cause vascular diseases and be associated with blood-borne pathogens such as HIV (Bachi et al., 2017, Karila et al., 2012). The cultivation of cocaine contributes to deforestation, and the subsequent effects of deforestation (United Nations, 2018). Cocaine also is associated with heightened crime rates through abuse, violence, and trafficking (U.S. Dept. of Justice, 1994). Local, state, and federal law enforcement agencies seek to detect illegal possession of illicit substances via a range of legal search procedures.

Search methods currently are categorized as robotic or biological. The most common biological method for drug detection for law enforcement purposes is the use of drug-sniffing dogs (Canis lupus familiaris). A drug-sniffing canine, detection dog, or canine detection unit (CDU) is a domestic dog that has been trained to detect illicit substances within a localized area and signal the detection to a handler. CDUs are used because dogs have a heightened sense of smell (olfaction) and humans are able to train dogs in order to exploit the dog’s capacity for detections. Canine detection for substances has been used since the fifteenth century and was first introduced in England (Sloane, 1955). CDUs are trained on a range of targets such as narcotics and cannabis as well as for cadaver recovery, weapons recovery, explosives detection and a range of other scent-based search endeavors.
Legal limitations to employing CDUs vary among legal jurisdictions. These legal limitations are based on the CDUs’ susceptibility to handler bias and the reliability of the canine itself. For example, a Florida, USA court decision reflects potential CDU unreliability when ruling against searches that discover substances for which the CDU was not certifiably trained even if discovered substances were illegal (Florida v. Harris, 2013). A significant issue in the use of CDUs for law enforcement searches is whether the dog’s alert signal represents legal probable cause for further searching, or if the dog simply was responding to its handler’s cues. If the dog was responding to the handler’s cues, then the alert represents a form of false positive and any subsequent search activity by law enforcement may be a violation of citizens’ rights.

Regardless of the CDUs performance, certifications, or relationship to its handler, other limitations can prevent CDUs from aiding law enforcement fully. These limitations may be overcome by deploying other biological detection methods that circumvent inherent limitations of CDUs. One biological search method that is being explored is detection by insects, specifically cockroaches (*Periplaneta americana*). Cockroaches have demonstrated learning capabilities in response to both classical and operant conditioning methods. If cockroaches can be taught via conditioning to detect illicit substances, then the natural capacities of cockroaches may be of use to compensate for natural limitations of CDUs.
POTENTIAL FOR COMMERCIAL APPLICATION OF COCKROACH DETECTION

The fundamental concept for deploying cockroaches as biological detection units is to overcome limitations to using canines as biological detection units. Clever Hans effect, body size, chronic stress response, emotional bonding, and expense are all limitations of CDUs. These canine limitations result in law enforcement limitations. Cockroaches have the ability to overcome these limitations (Chapter 2).

Canine detection units are susceptible to the Clever Hans effect, in which an animal’s actions are a response to its handler’s cues, either intentional or unintentional (Lit et al., 2011). This phenomenon is named after Hans, a horse reported to be able to count by tapping his hoof in accordance with numerical values but merely responding to his handler’s body language (Pfungst, 1911).

Similarly, CDUs are susceptible to influence by cues of their handlers as demonstrated by Lit et al. (2001) in which a high rate of false positive detections was elicited when, experimentally, CDU handlers were led to believe detection targets were present. In other words, CDUs are subject to handler bias. These errors can diminish law enforcement effectiveness and can be described as probabilities (Lit et al., 2001). When a CDU is deployed for drug detection, four outcomes are possible:

1. Target is present and CDU signals to handler.
2. Target is present and CDU fails to signal to handler.
3. Target is absent and CDU fails to signal to handler.
4. Target is absent and CDU signals to handler.

Outcomes 1 and 3 are successes insofar as the CDU’s signal corresponds to reality. Outcomes 2 and 4 are failures insofar as the CDU’s signal does not correspond to reality.
However, all four outcomes, both successes and failures are subject to cueing error by handlers. Furthermore, the error rate due to cueing is difficult to estimate because in all cases CDUs are deployed with handlers. My work (Chapter 2) demonstrated that cockroaches address this limitation by performing a location task in the absence of a handler. By removing the handler from the environment, this eliminates the Clever Hans effect.

In addition to being susceptible to the Clever Hans effect, CDUs have a size limitation. CDUs typically are large breeds of dog and cannot occupy small spaces such as inner walls. Large size of dogs limits the spaces they can infiltrate and may limit the CDUs approach distance to a target. Where detection at close distances in enclosed spaces would be beneficial, trained cockroaches may be an effective biological detection unit. Cockroaches are thigmotactic and they fit into spaces as small as 4mm. Employing cockroaches as biological detection units could augment CDUs in a spatial sense.

Mental capacity and stress also limit performance of CDUs. It is well known that mammals exhibit a physiological stress response to stressful environments which can affect physical function and behavior via cortisol mediation. This can lead to endocrine disruption and dysfunctional behavior (Haase et al., 2015). CDUs can exhibit cortisol stress-responses (Horvath et al., 2007). Stress in a CDU is associated with behavioral symptoms ranging from depression to episodes of rage. In some incidents CDUs have attack people as a result of stress (Sweeney, 2018; Phillips, 2019).

Trained cockroaches may provide a partial solution to this problem. Cockroaches are not known to express a complex behavioral stress response. This is not to say that cockroaches do not experience stress or that stress cannot affect their behavior. Rather,
using the sentience quotient (Freitas, 1984), cockroaches are judged to have a lower sentient state than dogs and, as such, less likely to exhibit complex emotional responses to stress. We assume cockroaches do not experience the same mental stress that a mammal would.

There are also ethical aspects to using biological detection units such as CDUs. Humans are more inclined to send CDUs into hostile environments because canine life, in this formulation, is viewed as more expendable than human life. Dogs are complex and highly social animal and social bonds form between dogs and their handlers (Newsome, 2018). In this context, many experts debate how to value a dog’s life. Cockroach detection units may offer a partial solution to this ethical quandary. Because cockroach life is brief and cockroaches are judged to be less sentient, cockroaches are generally viewed as more expendable than dogs. There likely are circumstances in which cockroaches could be deployed in high risk environments rather than dogs.

Because of the limitations with CDUs, cockroaches may be beneficial to deploy as biological detection units in conjunction with dogs. Other invertebrates, beyond the scope of my study, may also merit consideration such as moths or eusocial insects. By integrating insects into the detection process, some of the limitations of CDUs could be circumvented. This is not to argue that the solution is to transition completely to using insects and eliminate CDU use. Rather, both biological methods could be used such that they complement one another.

Cockroaches are thigmotactic, but they have not yet been trained to return to a location on command. This problem and others remain to be solved if cockroaches are to be developed in drug detection. One possibility is implementation of miniature GPS/VHF
tracking technology to allow investigators to monitor cockroach location as the cockroach explores environments for presence of target substances. In a hypothetical situation with an unknown substance, the CDU could be deployed to condense a large area. Then, cockroaches could be deployed within the reduced search areas to increase accuracy in finding the target substance.

Canines trained for drug detection are trained via classical and operant conditioning. Associations are formed between playtime and target scents and these associations are strengthened via repeated reinforcement (Jezierski et al., 2010). In order to address these limitations of CDUs with cockroaches, it is imperative to demonstrate that the learning process that occurs in CDUs can also occur in cockroaches. My work demonstrated that learning ability and cockroach behavioral plasticity occurs on both food-borne and non-food-borne odors. I demonstrated cockroach retention of training, and I was able to apply that training such that cockroaches located narcotics within a controlled setting. These were the first steps toward the goal of cockroaches as detectors in order to complement canine detectors.

My work supports the concept that cockroaches address several of the limitations that arise from employing CDUs. For instance, the location task that the cockroaches performed highlighted that cockroaches do not rely on a handler to perform, and thus the Clever Hans Effect is eliminated. Additionally, cockroaches do not exhibit a complex response to chronic stress. This significantly lowers the potential for harm caused by aggressive behavior sometimes exhibited by dogs under stress. Lastly, cockroaches are thigmotactic. They readily, even preferentially, enter tightly enclosed spaces. Humans could exploit this behavior in order to address the infiltration limitation of CDUs. Under
this scenario, the accuracy of a search could be improved by overcoming barriers, such as cracked walls, that CDUs cannot enter but cockroaches can. This would aid investigators in gathering accurate information. This also allows close identification of a substance source, potentially aiding searches. Overall, a complementary deployment of CDUs and cockroach-based detection could aid the detection of illicit substances for the betterment of society.
LITERATURE CITED
Bachi, K., Mani, V., Jeyachandran, D., Fayad, Z., Goldstein, R., & Alia-Klein, N.
https://doi.org/10.1016/j.atherosclerosis.2017.03.019.


cortisol levels to metabolic rate in mammals. *Biology Letters*, 12, 20150867.
https://doi.org/10.1098/rsbl.2015.0867.


styles in police dogs exposed to a short-term challenge. *Hormones and Behavior*,

Karila, L., Petit, A., Lowenstein, W., & Reynaud, M. (2012). Diagnosis and
consequences of cocaine addiction. *Current Medicinal Chemistry*, 19, 5612–
5618.

https://doi.org/10.1007/s10071-010-0373-2.

https://doi.org/10.1093/biosci/biy017.


CONCLUSION

An organism’s ability to learn serves as an adaptation to aid survival and reproduction. Learning occurs through several mechanisms and in different environments. An individual’s ability to learn via classical conditioning aids in physiological defense such as forming conditioned taste aversions while the ability to learn via operant conditioning aids in behavioral adaptations that further increase likelihood of survival. Humans have capitalized on this by conditioning animals to perform various tasks that aid in human goals. This exploitation comes in many forms such as obedience, tracking, entertainment, companionship, and animal traction.

A familiar example of animal use is the implementation of canines for law enforcement. Law enforcement personnel condition canines to locate various targets such as narcotics. The implementation of canines in this environment highlights the advantages of canine olfaction and trainability, but also the limitations that naturally occur. These limitations can be detrimental in a legal sense, in that an alert from a canine is not necessarily because of the canine’s detection of a target substance. These limitations are also detrimental in that there is potential to cause harm to the handler, or civilians involved. One potential solution to address these limitations is to employ insects as biological detectors.

Insects, specifically the American cockroach, naturally address the handler bias limitation of canines. In chapter 2, I demonstrated the cockroaches’ ability to locate narcotics within a controlled setting in the absence of a handler. Narcotics were randomly planted within the room, and the majority of both narcotic-trained groups were able to locate the target sources. Because there was no handler presence, and the cockroaches
still performed the task of location, this demonstrated that the cockroaches do not need a handler and thus eliminates the handler bias limitation.

Secondly, the cockroaches demonstrated behavioral plasticity on both food and non-food borne odors. This provides foundation for further research to extend to other non-food borne odors of law enforcement interest such as explosives. Implementing insect detectors would also allow for safer, more accurate locating of target substances. This would benefit law enforcement in situations where the target substance is unknown, or harmful. For example, an explosive substance would require a specialized team and equipment to handle safely but until the target is located and identified, those specializations cannot be put in place cockroaches could find specific locations.

Lastly, cockroaches address the negative consequences of mental health liabilities of canines. During my experiments, cockroaches did not exhibit agitated or aggressive behavior at any point, including the real-world simulation of a location task. This implies that cockroaches do not react to the stresses of narcotic conditioning and locating in a negative manner. This highlights the cockroaches as a safe biological detection tool.

Overall, cockroaches demonstrated behavioral plasticity, extended memory retention, and location of target substances within a controlled setting. This is the first demonstration of a location task on non-food borne substances by an insect. The novelty of my experiments provide foundation for development of insects into biological detection units that could be employed to complement existing biological detectors. Ultimately, this would allow for law enforcement to adhere more efficiently and safely to citizen legal rights, while continuing to combat drug trafficking.