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Influences of TNT-Food Pairings on the Performance of Mine Detection Rats in Early Training Stages

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by

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Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO), a Belgian nongovernmental organization headquartered in Tanzania, trains giant African pouched rats (*Cricetomys gambianus*) to detect land mines and deploys the rats for operational use in countries afflicted with mines and explosive remnants of war. In the present study an evaluation of the influence of ongoing scent-food pairings on the performance of the rats in early mine detection training was conducted. Twenty young rats in APOPO's mine detection rat program were divided into two groups and exposed to five daily stimulus-food pairing sessions each week. For the experimental group the stimulus was the scent of TNT, an explosive commonly found in land mines. For the control group the stimulus was the scent of sugar. The influence of the respondent conditioning procedure on performance during early training was evaluated by examining key performance indicators throughout the early training process. Measures included latency to arrival at the TNT target, duration of the detection response, number of false indications, and number of days required to complete early training. Only one of the measures was associated with a statistically significant outcome. However, the group exposed to TNT-food pairings consistently outperformed the control group throughout early training, suggesting that

such pairings may have a small positive influence on performance in early scent discrimination training. Because of the small effect sizes and the predominance of statistically non-significant findings, the pairings have not been incorporated into APOPO's standard operating procedures for training of mine detection rats.

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CHAPTER I

INTRODUCTION

Land Mines

Land mines (mines) are victim-activated explosive devices used in military operations. Anti-personnel (AP) mines are designed to injure or kill one or more persons. Anti-tank (AT) mines are designed to disable or destroy tanks and other large vehicles. Although 160 countries have signed the Ottawa Treaty, which prohibits using, stockpiling, producing, and transferring AP mines, they are still being manufactured and deployed (Landmine and Cluster Munition Monitor, November 2012).

Anatomy

Mines are generally composed of an outer casing, a firing mechanism, a detonator, and a main charge. The outer casing is often made of plastic but can also be made of wood, metal, glass, or other materials. Fragmentation mines are also encased in steel fragments that are projected outward upon detonation. The firing mechanism, which typically comprises a firing pin and a pressure plate or trip wire, transfers energy from the victim's movement to the detonator. The detonator, when

activated, releases the energy required to activate the main charge, either directly or through the detonation of a booster charge. The main charge in mines is most commonly 2,4,6-trinitrotoluene (TNT), but other explosives such as cyclotrimethylenetrinitramine (RDX) and 2,4,6-trinitrophenylmethylnitramine (tetryl) are also used.

Mines are commonly designed to withstand erosion and can remain active for decades after their initial placement. Manufacturers of mines also take into consideration detection and removal of mines and many take measures to make mines difficult to detect, remove, or inactivate. Some of these measures include using non-metallic casing materials, using a minimal amount of metal in the firing mechanism, and installing anti-handling devices – alternate firing mechanisms that can also trigger the detonation of the main charge.

Impact

In 2011, 4,286 mine and explosive remnant of war (ERW) casualties were reported; 42% of the victims were children, 10% were female, 72% were civilians, and 31% were killed in the accident. An estimated 1000 additional casualties were not reported (Landmine Cluster and Munition Monitor, November 2012). AP mine injuries are characteristically lower limb injuries, often including traumatic amputation of a foot or leg (Trimble, Adams, & Adams, 2006). Many survivors of mine accidents do not receive appropriate rehabilitation services and are unable to

obtain gainful employment or care for themselves after the accident (Walsh & Walsh, 2003).

The presence of mines or suspicion of their presence renders the mined or suspected area unusable until the threat is removed. Restricted access to water, farmable land, and hunting and gathering ground can result in the displacement of human populations to less suitable land and exacerbate the influences of poverty in developing countries. Moreover, livestock and wildlife are frequently injured or killed by landmines, resulting in economical loss and a reduction in biodiversity (Berhe, 2007).

Mine Detection Animals

Animals have been used successfully in demining operations to detect the presence of vapors associated with mines and ERW, usually vapor from the main explosive charge. They are trained to emit an identifiable response in the presence of the targeted vapor. When an animal emits the response, the location at which the response was emitted can be investigated further, typically using manual demining procedures, which involve the use of metal detectors and manual excavation tools to find and remove, disable, or destroy mines and ERW.

Giant African Pouched Rats

Cricetomys gambianus (giant African pouched rats) are nocturnal, burrowing omnivores found throughout much of sub-Saharan Africa with a typical body length between 240 and 450 mm and a tail length between 354 and 460 mm. Body weight in the wild is usually between 1 and 2.8 kg for males and between 0.96 and 1.39 kg for females (Smithers, 1983). Pouched rats have poor vision, but they have sensitive hearing and olfaction. Pouched rats that are raised in captivity are easy to train and can live up to 14 years (Cooper, 2008).

Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling

Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO), a Belgian non-governmental organization with headquarters in Morogoro, Tanzania, has used *Cricetomys gambianus* in demining operations in Mozambique since 2007 and has been conducting research on the capacity of the rats to function as mine detection animals since 1997. The rats are bred and trained at APOPO's headquarters and transported to operational demining sites when they have completed training.

Mine Detection Rat Training

Early Training

While the young rats are being weaned, they are handled extensively and exposed to a wide variety of stimuli. The rats are then exposed to clicker training, in which a click is consistently followed by food. After the click reliably produces orientation toward the food source, the rats are placed in a chamber with a single open hole in the center of the floor and a food hole at the side. A TNT solution is placed below the hole, and each time the rat approaches the hole, the clicker is sounded and food delivered at the food hole. The trainer then requires the rat to remain above the TNT for progressively longer periods of time until it reliably approaches the TNT hole and remains above it for at least three seconds. Two additional holes in the floor of the chamber are then opened, and discrimination training is conducted by placing a TNT solution, or *positive sample*, beneath one hole and containers with no TNT, or *negative samples*, beneath the other two. Each time the rat holds its nose over the hole containing a positive sample for three or more seconds, the trainer clicks and delivers food but never does so when the rat holds its nose over a hole that contains a negative sample. The rats are tested under blind conditions in which they must identify 8 of 8 positive samples without incorrectly identifying over more than 1 of 22 negative samples. The trainer does not know the location of the positive samples but, when a rat holds its nose over a sample for three or more seconds, the trainer calls out the

sample number to a supervisor who then instructs the trainer to reinforce the response or to withhold the reinforcer, depending on the status of the sample.

Soil Floor

After the rats have passed the blind test, they are trained on a soil floor in which perforated steel balls are placed, some with and some without TNT inside. The rat is placed in a harness attached to a rope that runs the length of a steel wheel and axle system that spans the soil floor. After the rat walks from one side of the floor to the other, the trainers advance the axle forward in .5-m increments, enabling the rat to systematically search .5-m lanes. When a ball containing TNT is encountered, if the rat scratches at the ball for three or more seconds, the trainer sounds the click and delivers food at the edge of the soil floor. If the rat scratches at a ball that does not contain TNT, the trainer takes no action. Soil floor training continues until the rat scratches over each ball containing TNT and does not scratch over any other ball in one session.

Field Training

Subsequent stages of training take place in a 28-ha simulated minefield containing 1,533 deactivated mines buried between 0 and 10 cm below the surface. In the first stage of training on the simulated minefield, the wheel and axle system is employed, and the rats search for perforated steel balls containing TNT in three-

meter-wide areas of land, or *boxes*, prepared for the safe deployment of mine detection animals. Once the rats have reached the same criteria required for successful soil floor training, they are trained in boxes that contain deactivated mines at or near the surface. The rats then progress to 5-m boxes that also contain mines at or near the surface. The wheel and axle system is eventually replaced by a rope system in which the ends of a rope are stretched over the box, each end looped around one leg of each of the handlers. Training progresses in 200-m^2 boxes with mines buried up to 10 cm deep until the rats reliably *indicate*, or scratch for three or more seconds, in areas within .5 m of a mine and do not indicate in areas that do not contain mines. In a final series of blind tests, rats must locate 85% of targets in the first two blind tests and 100% of targets in the final blind test with no more than one false indication in any of the tests. Rats that have passed the final series of blind tests are internally accredited and considered to be ready for delivery to an operational site.

Mine Detection Rats at Operational Sites

Upon arrival at an operational site, rats continue training at a simulated minefield near the site. At the operational site, weather conditions and soil conditions may differ from those at the original training site and influence performance at the new location. New handlers, housing, and transportation containers, may also influence the rats' performance. When a rat is reliably indicating over mines at the new site, an external evaluation is conducted by the local national mine action

authority in accordance with International Mine Action Standard (IMAS) 09.42 (2008), which specifies that each animal must search a 400-m^2 area with a minimum of five targets, indicate within one meter each target, and emit no more than two false indications. If the rat passes, it can be deployed operationally.

Research on Mine Detection Rats

In the first formal evaluation of the rats' ability to locate mines under operational conditions, overall detection accuracy exceeded 95% (Verhagen, Weetjens, Cox, Weetjens, & Billet, 2006). In a more extensive operational evaluation, pairs of rats searched 93,400 m^2 of land and indicated within one meter of 100% of the 41 mines discovered subsequently with manual demining methods (Poling, Weetjens, Cox, Beyene, Bach, & Sully, 2011). As specified in IMAS 09.42 (2008), an indication within one meter of a mine or ERW is referred to as a *hit*. An indication greater than one meter from a mine or ERW is referred to as a *false indication*. In the Poling et al. study, the rats' false indication rate was .33 per 100 m^2, which is below the maximum allowable false indication rate of 2 per 400 m^2 specified in IMAS 09.42.

Under operational conditions the locations of mines and ERW are unknown, therefore the rats operate under extinction conditions in the minefield. That is, if a rat correctly indicates over a mine, the trainer does not reinforce the response. Mahoney et al. (2012) examined the influence of extinction on the mine detection accuracy of

five rats and found that accuracy began to diminish after three days and required an average of 4.4 days to return to baseline levels. Training is, therefore, conducted once after every two operational days, or twice a week under normal circumstances.

Mahoney et al. (in press) examined a method of providing opportunities for reinforcement in the operational minefield in which small plastic bags containing TNT are extended into the field with a fiberglass rod and allowed to rest on the ground, contaminating the spot with TNT. After a 1-hr soak period, rats indicated within .5 m of the contaminated spot on 22% of opportunities to do so, and after a 16-hr soak period, rats indicated on 95% of opportunities to do so. After the bag containing TNT had been removed for 24, 72, and 144 hours, rats indicated the spot on 62%, 50%, and 22% of the opportunities to do so, respectively.

Whereas most research on APOPO's mine detection rats has been conducted with rats in advanced stages of training or rats that have already been accredited for demining, the present study examined the influence of respondent conditioning on the performance of the rats in early training. Improvements in early training procedures could reduce the time required for APOPO to train a mine detection rat and could result in improved performance throughout the rats' careers as mine detection animals.

CHAPTER II

BACKGROUND RESEARCH

Stimulus-Reinforcer Pairings and Operant Discrimination Training

Previous research suggests that discrimination training is facilitated by respondent conditioning in which the discriminative stimulus (S^D) to be established later is first paired with a reinforcing stimulus in such a way that the S^D-to-be is positively correlated with, and therefore predictive of, presentation of the reinforcing stimulus.

Bower and Grusec (1964) trained eight rats to lever press under a variable interval (VI) 30-s schedule of water reinforcement in Phase 1. In Phase 2, they removed the lever and conducted respondent pairing sessions in which two stimuli were alternately presented for 30-s intervals. While one of the stimuli (the S^D, or S+) was presented, water was delivered at irregular times. While the other stimulus was presented (the S-), no water was delivered. In Phase 3, the lever was replaced and, in the presence of one of the stimuli, lever pressing was reinforced under a VI 30-s schedule of water reinforcement. No consequences were scheduled for lever presses in the presence of the other stimulus. For four of the rats, the S+ in Phase 2 was the S^D during operant discrimination training in Phase 3. For the other four rats, the S+ in

Phase 2 was predictive of extinction during operant discrimination training in Phase 3 (i.e., it was the S^{Δ}).

The rate of learning, displayed as the mean percentage of responses to the S^{D} across training days during operant discrimination training in Phase 3, was much faster for the group that learned to press in the presence of the S^{D} that had previously served as S+ during respondent conditioning in Phase 2. For this group, after 10 days of training, the percentage of responses to the S^{D} reached approximately 80% and continued near that level until day 27 of training, when the study was terminated. For the other group, the percentage of responding in the presence of the S^{D} was approximately 60% after 10 days of training and did not reach 80% until day 27 of training. Additionally, for the first 23 days of training, the mean number of responses emitted in the presence of the S^{Δ} was lower when the S^{Δ} was the S- in Phase 2.

Trapold and Fairlie (1965) trained 16 rats to lever press on a horizontal lever in some sessions and a vertical lever in other sessions under a fixed-ratio (FR) 1 schedule of food reinforcement in Phase 1. In Phase 2, rats in Group 1 received operant discrimination training of a horizontal lever press, and each rat in Group 2 was yoked to a rat in Group 1 with respect to the S^{D} and reinforcer presentation schedule independent of the rat's behavior. For Group 1, during 320-s S^{D} periods lever pressing was reinforced under a VI 1-min schedule of food reinforcement and never reinforced during S^{Δ} periods of at least 320 s. In Phase 3, each group was divided into subgroups that were exposed to operant discrimination training of a vertical lever press with the S^{D} (or S+) from Phase 2 serving as either the S^{D} or the S^{Δ}.

The rats in Groups 1 and 2 did not differ with respect to their performance on discrimination training in Phase 3, indicating that operant discrimination training and respondent discrimination training were equally effective at facilitating later operant discrimination training. Within both groups, rats that were trained to lever press in the presence of the S^D from Phase 2 performed better on average than the rats that were trained to lever press in the presence of the S^Δ (i.e., a higher proportion of lever presses were emitted in the presence of the S^D). This difference was statistically significant.

Hyde, Trapold, and Gross (1968) trained 20 rats to lever press under a FR 1 schedule of food reinforcement in Phase 1. In Phase 2, they removed the lever and exposed 10 rats to two different stimuli, one predictive of the delivery of one food pellet, the other predictive of the delivery of 10 pellets. The stimuli, a tone and a clicker, were presented for 3 s with a variable 1-min inter-stimulus interval. The other 10 rats were exposed to the same stimulus and food presentations, but the stimuli were not predictive of food delivery. In Phase 3, the lever was re-introduced and a discrete-trial lever-pressing task was programmed in which, each time the houselight was illuminated, a lever press was followed by delivery of one food pellet and termination of the houselight. After nine sessions with 24 trials each, generalization sessions were programmed in which 4 of the trials were replaced with one of the stimuli from respondent conditioning in Phase 2.

For the group of rats exposed to respondent conditioning in which the tone and clicker were predictive of food delivery, the mean latency from tone or clicker

onset to the first lever press was lower than the mean latency of the control group across the seven test sessions. This difference was statistically significant. However, no difference was found between the latency in the presence of the stimulus predictive of 1 food pellet and the stimulus predictive of 10 food pellets.

Capaldi and Hovancik (1974) trained 24 rats to lever press under a FR 1 schedule of food reinforcement in Phase 1. In Phase 2, they immobilized the lever and exposed the rats to two different stimuli, a tone and a buzzer, presented at variable 30-s inter-stimulus intervals with 3-s stimulus duration. For the six rats in Group 1, both stimuli were presented 12 times and followed by food delivery 50% of the time. For Group 2, one stimulus was presented 12 times, followed by food delivery 50% of the time, and the other was presented 6 times, followed by food delivery 100% of the time. For Group 3, both stimuli were presented 6 times and followed by food delivery 100% of the time. For Group 4, both stimuli were presented 12 times but were not predictive of food delivery. In Phase 3, a discrete-trial procedure was programmed in which the first response following onset of a light situated above the lever resulted in the delivery of a food pellet and termination of the light. After 10 sessions of 12 trials each, generalization sessions were programmed in which 2 of the trials were replaced with the two stimuli from respondent conditioning in Phase 2.

Groups 1 through 3, which were exposed to respondent conditioning procedures in which the two stimuli were predictive of food delivery, responded with lower latency on generalization tests with the two stimuli than Group 4, which was exposed to the stimuli in a manner that was not predictive of food delivery. The

difference in performance between Group 4 and each of the other groups was statistically significant. Groups 1 and 3 responded with approximately equal latency to both stimuli, but Group 2 responded with lower latency to the stimulus that was followed by food 100% of the time during respondent conditioning compared to the stimulus that was followed by food 50% of the time but presented twice as often.

Hovancik (1978) trained 36 rats to lever press under a FR 1 schedule of food delivery in Phase 1 of Experiment 2. In Phase 2, half of the rats (Group 1) were exposed to daily sessions of 15 3-s tones paired with food delivery and the other half (Group 2) to 15 tone presentations and 15 food presentations that were uncorrelated. Within each group half of the rats were maintained at 70% of their free-feeding weight and half maintained at 90% of their free-feeding weight. In Phase 3, all rats were maintained at 90% of their free-feeding weight, and a discrete-trial task was programmed in which the first lever press after tone onset was reinforced.

The mean latency from tone onset to the first lever press was lower for Group 1 than for Group 2 across the eight sessions in Phase 3. This difference was statistically significant. However, no difference was found between subgroups maintained at different weights during Phase 2.

Taken together, the results of several studies provide substantial evidence that arranging respondent conditioning (i.e., stimulus-stimulus pairings) to establish a predictive relation between a stimulus that will later be established as an S^D and the stimulus that will serve as a positive reinforcer in the presence of that S^D facilitates acquisition of the operant discrimination. These findings suggest that such a

manipulation might be of value in teaching pouched rats to discriminate the presence of land mines.

TNT-Food Pairings and Mine Detection Rat Training

Based on the findings presented in this body of literature, it was hypothesized that respondent conditioning in which the scent of TNT is correlated with food delivery would facilitate early scent discrimination training of mine detection rats at APOPO. The findings suggest that rats exposed to respondent TNT-food pairings will respond with lower latency in discrete trial training and reach criterion more rapidly. The findings also suggest that such pairings might reduce the number of false indications (i.e., pauses over TNT-negative samples) emitted during training.

Another potential advantage of respondent conditioning is that the scent of TNT may be established as a more effective conditioned reinforcer. An established S^D generally functions as a conditioned reinforcer in that behavior that is followed by the presentation of the S^D will become more probable under similar circumstances (Kelleher & Gollub, 1962). Once TNT has been established as an S^D, any behavior that has resulted in the presentation of the S^D, such as active searching, will become more probable. Directly pairing TNT with food may enhance the conditioned reinforcing properties of the stimulus, and young rats may more readily learn to orient toward and actively search for the source of the TNT.

The present research is also relevant to autoshaping or sign-tracking, a commonly observed phenomenon in which an animal engages with a stimulus that is predictive of reinforcement in a manner that is consistent with the way the animal engages with the reinforcer itself (Poling & Poling, 1978). For example, pigeons will peck a lighted key that is predictive of food reinforcement using the same movement and beak position that is used for food consumption, but pecks at a lighted key that is predictive of water reinforcement resemble the movement and position that is used for water consumption (Jenkins & Moore, 1973). Establishing the scent of TNT as a predictor of food should theoretically result in species-specific behavior related to food directed toward the source of the stimulus. *Cricetomys gambianus* frequently dig for food and, because scratching over landmines is targeted for reinforcement during later stages of training, establishing the scent of TNT as a strong predictor of food may improve the strength and reliability of the scratching response.

This prediction rests in part upon the assumption that scratching is species-specific behavior for *Cricetomys gambianus*, which has not been demonstrated experimentally. However, during training at APOPO, the transition from pausing over TNT-positive samples to scratching over TNT-positive samples often occurs without the scratching response being targeted for reinforcement. Because the rats are raised under conditions in which scratching is not required for food procurement, it may be reasonable to assume that the scratching response spontaneously occurs in the presence of stimuli that are predictive of food. It is possible, therefore, that these advantages may carry over into later stages of training, where active searching and

reliable, easily identifiable responding in the presence of the scent of TNT becomes increasingly important. However, the impact of respondent conditioning on later stages of training was not evaluated in the present study.

In sum, it was hypothesized that respondent conditioning procedures would have the following effects: (1) lower the latency to arrival at the target, (2) increase the time the rat would spend in proximity to the target, (3) decrease the training time required to reach criterion in discrimination training, and (4) reduce the number of false indications emitted during training.

CHAPTER III

METHOD

Subjects

Twenty rats (*Cricetomys gambianus*) were selected from APOPO's breeding facility for participation in the study. As soon as the rats were weaned, they were placed in individual cages in a room with 100 cages, a double row of cages in the center of the room, and a single row on each side of the room such that there were two walkways from which all of the cages could be accessed. Each 75-cm × 75-cm × 50-cm cage was constructed of concrete and a rigid steel grid with 3-cm^2 holes supplemented with additional wire mesh to prevent the small rats from escaping. The rats were randomly assigned to two groups of 10, a control group (Group 1) and an experimental group (Group 2), one group placed in cages on the left side of the room and the other in cages on the right side of the room with a double row of cages between the two groups. The rats had already begun habituation before they had been removed from their mothers and placed in individual cages. Habituation entailed extensive handling and exposure to a wide variety of stimuli. Three of the rats died from unknown causes during the course of the study. Of the remaining 17 rats, 8 were male (4 in the control group and 4 in the TNT group) and 9 were female (4 in the control group and 5 in the TNT group). This research protocol was approved by

APOPO's Institutional Animal Care and Use Committee (Appendix A). Because the research was carried out in Tanzania, approval from the author's institution in the U.S.A. was not required.

Apparatus

For scent presentation purposes, 20 glass bottles measuring 7 cm high and 3 cm in diameter with metal lids were cleaned thoroughly. Three holes approximately 1 mm in diameter were made in the metal lid of each bottle. For each of five TNT-positive (T+) bottles, approximately 2 g of TNT was placed into a small sealable bag and inserted into the bottle. For each of five control-positive (C+) bottles, approximately 2 g of sugar crystals were placed into a bag and inserted into the bottles. For each of ten control-negative (C-) bottles, an empty bag was inserted into the bottle. Care was taken when preparing the T+ and C+ bottles so that other bottles would not be contaminated with the scent. The perforated lid was placed on each of the bottles and each bottle was marked with a label indicating its status.

Four airtight containers with locking lids were cleaned thoroughly and marked and loaded as follows. One container was marked with a red "+" and loaded with the five C+ bottles numbered 1 through 5; one container was marked with a red "–" and loaded with five C- bottles numbered 1 through 5; one container was marked with a blue "+" and loaded with the five T+ bottles numbered 1 through 5; and one container was marked with a blue "–" and loaded with five C- bottles numbered 1 through 5.

The red containers were placed in a basket marked with red ribbon. The blue containers were placed in a basket marked with blue ribbon. The red basket was stored in the rat room but never taken to the side of the room where the blue group was housed. The blue basket was stored in a small chemistry laboratory to reduce the risk of cross contamination between the baskets or contamination of other objects with the scent of TNT.

Monday through Thursday prior to TNT-food pairing sessions, 40 small plastic canisters measuring 5-cm high and 3-cm in diameter with a sealable lid were each loaded with 5 chopped peanuts and 2 chopped dried anchovies. On Friday, in addition to the small plastic canisters, a large plastic canister for each rat was filled with the rat's weekend ration, which included chopped tomatoes, avocados, carrots, and other fruits and vegetables.

Pairing Procedure

A presentation schedule indicating the order of bottle presentation for each rat, which included two positive bottle presentations and three negative bottle presentations randomized across rats, was created (Appendix B). Five different presentation schedules were created, one for each weekday, and the schedules were reused each week for approximately nine weeks of pairings.

Each day from Monday through Friday at 14:00, three rat handlers rotated through the following duties. Handler 1 followed the presentation schedule and

operated a stopwatch, instructing the other two handlers on presentation order and timing. Handler 2 presented negative bottles to the rats with one hand and delivered food to the rats with the other hand. Handler 3 only presented positive bottles.

All rats in both groups were exposed to the first of the five daily bottle presentations before the second bottle presentations commenced after which the second bottle presentations were carried out and so on until all rats had been exposed to all bottle presentations. Handler 1 stated whether the presentation was positive or negative and kept track of the presentation number, which determined the bottle number that was to be used. Handlers 1 and 2, wearing latex gloves, loaded the red basket onto a small trolley with the requisite number of food canisters and entered the walkway where the red group was located. When all rats in the red group had received their first presentation, the handlers removed the red basket from the trolley, changed gloves, loaded the blue basket into the trolley, entered the walkway where the blue group was located, and proceeded with the first presentations for the blue group. This process was repeated, with handlers changing gloves between each group, until all presentations were completed.

Presentation of a negative bottle involved Handler 1 giving a start signal to Handler 2 and starting the timer, Handler 2 raising the negative bottle to the lower-right corner of the cage with the cap touching the wire mesh and, after 15 s had elapsed, Handler 1 instructing the bottle handler to stop the presentation, at which Handler 2 removed the bottle from the cage and replaced it into the negative airtight container and closed the lid.

Presentation of a positive bottle involved Handler 1 giving a start signal to Handler 3 and starting the timer, Handler 3 raising the positive bottle to the lower-right corner of the cage with the cap touching the wire mesh and, after 10 s had elapsed, Handler 1 giving Handler 2 the food delivery signal, at which Handler 2 dumped the contents of a food container into the top of the cage near the front right corner. After an additional 5 s had elapsed, Handler 1 gave Handler 3 the stop signal, at which Handler 3 removed the bottle and replaced it into the positive airtight container and closed the lid. On Fridays during the last positive presentation for each rat, in place of the normal food delivery, the cage door was quickly opened and the rat's weekend ration was placed inside the door. On positive presentations, all handlers attempted to minimize any signals that might reliably precede food delivery and thus compete with the target odor as a predictor of food delivery. For example, the food canister was picked up from the trolley quietly and the lid was not opened until Handler 1 gave the food delivery signal.

Daily rotation of the handlers' duties was done to prevent the scent of one handler from becoming a predictor of food delivery. Randomization of negative and positive bottle presentation was done to prevent bottle presentation itself or the order of presentation from becoming predictive of food delivery. Distribution of responsibilities between handlers for presenting positive and negative bottles and glove changes between each group were done to prevent contamination of negative bottles and cross-contamination between the bottles for each group. Due to the special storage and handling requirements of the TNT-positive containers, the handlers were

not blind to the status of the TNT and control groups. Procedural integrity data were collected once every two weeks. Collection of procedural integrity data involved observation of the TNT-food pairing session and checking each step listed on the TNT-food pairing checklist (Appendix C) as correct or incorrect.

Training and Evaluation

Clicker Training

Two pretests and two evaluations were conducted during the early training process to evaluate the influence of the ongoing TNT-food pairings on key performance indicators throughout early training. Standard operating procedures for early training of mine detection rats were followed closely, and supervisors observed training sessions daily. Rats in both groups were randomly assigned to four handlers who worked in pairs throughout the training process. The handlers continued habituating the rats during the first week of pairings, after which they began clicker training. During clicker training, the rats were placed in a training chamber measuring 65 cm × 65 cm × 45 cm with Plexiglas walls and metal flooring with a feeding hole in the right side of the chamber. The handlers activated a hand-held clicker and immediately inserted a syringe filled with a mixture of mashed peanut, banana, and infant formula into the feeding hole, squeezing a small quantity of food into the rat's mouth when it approached. After sufficient click-food pairings the rats reliably

oriented toward and approached the feeding hole regardless of their location in the cage.

One-Hole Pretest

Following clicker training, after 14 pairing sessions for all rats, a pretest designed to determine latency to approach and duration of contact with TNT and controls was administered. A small plastic container with a solution of water and TNT (1 g of TNT per 100 ml of water), a solution of water and sugar (1 g of sugar per 100 ml of water), or water only was placed below a single open hole in the floor of the training chamber. An aqueous solution was used in training and evaluation because vapor from explosives in minefields is carried to the surface through processes of advection and diffusion with water, which is more accurately simulated with an aqueous solution than direct presentation of TNT crystals. On each trial the rat was placed in the center of the chamber and timed from the moment of release to the moment its nose crossed the edge of the open hole. If the rat did not reach the scent hole within 30 s, the latency was recorded as 31 s. The cumulative duration of contact with the stimulus within each 30-s trial was timed starting when the rat's nose first crossed the edge of the scent hole and ending when the rat's nose was no longer crossing the edge of the open hole. The timer was restarted each time the rat's nose crossed the edge of the scent hole during the 30-s interval. Each stimulus was presented twice in a randomized order. The rat was removed from the chamber

following each trial. A second observer simultaneously recorded the latency and duration measures on 10% of the trials so that interobserver agreement (IOA) could be calculated.

One-Hole Training and Evaluation

After the pretest, regular one-hole training commenced in which a TNT solution was placed below the single open hole in the training chamber and the clicker and food used to reinforce successive approximations to the rats approaching the hole and holding their noses above the hole for three seconds. During the second week of one-hole training, after 20 pairing sessions, an evaluation of the rats' performance was conducted during normal training sessions. For 10 trials randomly interspersed throughout normal training trials, the handlers were instructed not to reinforce pausing over the hole. For each of these trials, the observer indicated whether the time required for the rat to travel from the food hole to the scent hole was less than three seconds, then recorded the duration of contact with the target scent as defined for the pretest. This evaluation was conducted twice for all rats on consecutive days. A second observer simultaneously recorded data on 13% of the trials so that IOA could be calculated.

Discrimination Pretest

Following one-hole training, after 24 TNT-food pairing sessions for all rats, a three-hole pretest was administered to determine approach latency and duration of contact with TNT when two other controls were available simultaneously. A TNT solution was placed beneath one hole, while water alone was placed beneath two additional open holes in the floor of the cage. The location of the TNT solution was randomized across trials. On each 30-s trial, the rat was released in the center of the cage and timed from the moment it was released to the moment its nose crossed the edge of the hole with TNT. If the rat did not reach the hole within 30 s, the latency was recorded as 31 s. The cumulative duration of contact with TNT was timed starting when the rat's nose crossed the edge of the hole and ending when the rat's nose was no longer crossing the edge of the hole. If the rat's nose crossed the edge of the hole again during the 30-s trial, the timer was restarted. TNT was placed in each location twice. A second observer simultaneously recorded the latency and duration measures on 25% of the trials so that IOA could be calculated.

Discrimination Training and Evaluation

Discrimination training was conducted as normal, which involved reinforcing pausing of three or more seconds over TNT-positive solutions and never reinforcing pausing over TNT-negative solutions. Throughout training, three solutions were presented beneath three open holes in the floor of the training chamber, and the

location of TNT-positive solutions was randomized. At the beginning of the third week of discrimination training an evaluation was conducted in which the duration of pausing over TNT-positive solutions was measured and the number of false indications, or pauses of 1 or more seconds over TNT-negative solutions, was tallied. During 10 trials interspersed throughout regular training, the handlers were instructed not to reinforce pausing over the TNT solution. When the rat's nose crossed the edge of the hole containing the TNT solution, the timer was started, and the timer was stopped as soon as the rat's nose was no longer crossing the edge of the hole. If the rat's nose crossed and remained across the edge of any other hole for more than one second, a false indication was tallied for that trial. The location of the TNT solution was randomized across trials. This evaluation was conducted twice for all rats on consecutive days. A second observer recorded data on 24% of the trials so that IOA could be calculated.

Blind Test

Based on the handler's appraisal of the performance of the rat, they could request a blind test at any time after three weeks of discrimination training. In accordance with APOPO policy, handlers of rats that passed a discrimination training blind test were given a financial incentive. Blind tests involved the preparation of 30 numbered containers, 8 of which contained a TNT solution. The handlers were blind to the status of the containers and presented the containers, three at a time, to the rats.

If the handler saw the rat pausing over a container for three or more seconds, they called out the number on the container to a supervisor who indicated whether it was positive or negative. If the container was positive, the handler reinforced the response. To pass a blind test, the rat and handler had to correctly identify all TNT-positive containers and were only allowed a single false indication. If a rat failed a blind test, they could retake the test after an additional two days of training. The number of training days before the each rat passed the blind test was recorded for comparison between the groups.

CHAPTER IV

RESULTS

Procedural Integrity

Procedural integrity was calculated for each TNT-food pairing session as the number of steps performed correctly divided by the total number of steps on the standard operating procedure checklist. The average procedural integrity score was .97, ranging from .95 to 1, indicating that procedural integrity was very good.

One-Hole Pretest

The mean latency to arrival at the scent hole in the one-hole pretest was 20.85 s (*SD* = 8.65) for the control group and 13.33 s (*SD* = 7.43) for the TNT group when TNT was present, which is a notable difference. However, the mean latency to arrival at the scent hole was higher for the control group when the two control scents were present as well (see Figure 1).

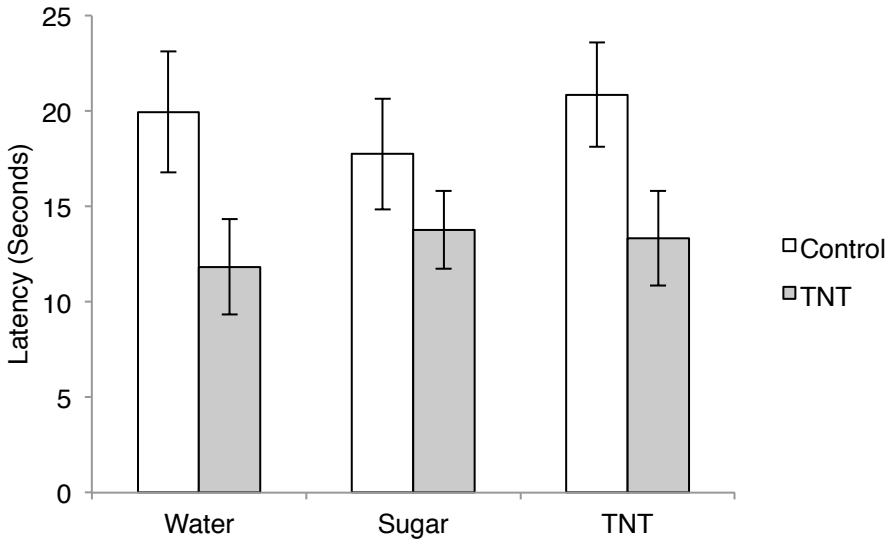

Figure 1. Mean latency and standard error from release time to arrival at scent hole for each group with each type of stimulus.

The mean latency measures from each rat for each sample type were subjected to a two-way ANOVA with group membership as a random factor and sample type as a fixed, repeated-measures factor. The alpha level for all subsequent statistical tests was set at .05. Mauchly's test for sphericity suggested that the assumption of sphericity was not violated. The main effect for group membership was found to be statistically significant ($F[1,17] = 5.62, p = .03$), the main effect for sample type was not found to be statistically significant ($F[2,34) = .55, p = .58$), and the interaction between the two factors was not found to be statistically significant ($F[2,34] = .4763, p = .63$). These results suggest that latency from release to arrival at the scent hole is influenced by the TNT-food pairings but the influence of sample type and the dependence of that influence upon group membership is unclear.

The mean cumulative duration over the scent hole in the one-hole pretest was 1.86 s (*SD* = 1.81) for the control group and 2.46 s (*SD* = 1.31) for the TNT group when TNT was present, which is also a notable difference. However, the mean duration over the scent hole was lower for the control group when the two control scents were present as well (see Figure 2).

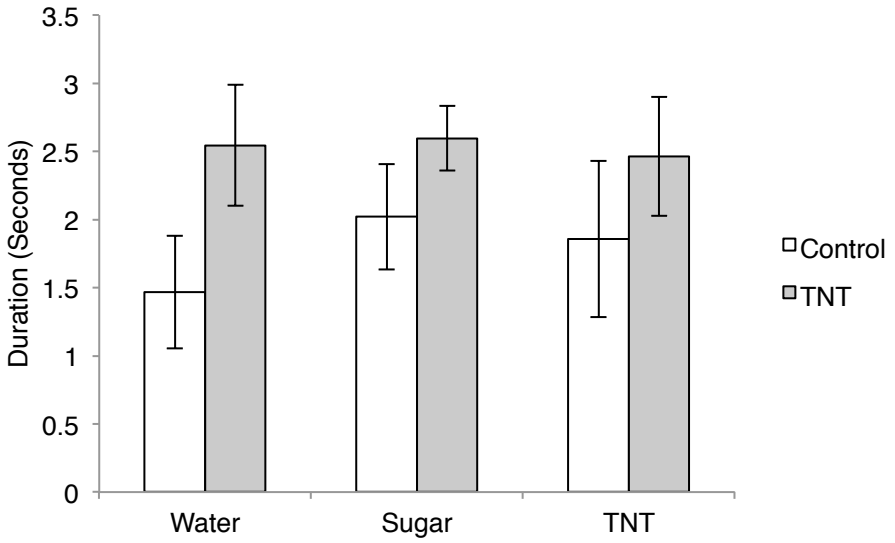

Figure 2. Mean duration and standard error over the scent hole for each group with each type of stimulus.

The mean duration measures were analyzed in the same manner as the mean latency measures. Mauchly's test for sphericity indicated that the assumption of sphericity was violated ($W = .65, p = .03$). Therefore degrees of freedom were corrected using Greenhouse-Geisser estimates ($\varepsilon = .74$). The main effect for group membership was not found to be statistically significant ($F[1,17] = 2.48, p = .13$). The main effect of sample type was not found to be statistically significant ($F[2,34] = .60$,

$p = .51$). The interaction between group membership and sample type was also not found to be statistically significant ($F[2,34] = .36, p = .70$).

IOA was evaluated by calculating an intraclass correlation coefficient (ICC) using the 10% of the data that were collected by two observers. The obtained ICC was .98 ($p < .01$) for latency and .97 ($p < .01$) for duration, indicating that IOA for the one-hole pretest was very good.

One-Hole Evaluation

The proportion of trials in which each rat arrived at the TNT scent hole within three seconds of leaving the food hole was determined by calculating the mean of the binary response data across the two evaluation days. The mean proportion of trials in which the rat arrived at the TNT scent hole within three seconds of leaving the food hole was .85 ($SD = .13$) for the control group and .86 ($SD = .17$) for the TNT group. Because the group means were approximately equal, a statistical analysis of the data was not conducted.

The mean duration of each rat's first pause over the scent hole in the one-hole evaluation was calculated for each of the two evaluation days. The group mean was 1.65 s ($SD = 1.0$) for the control group and 1.73 s ($SD = .80$) for the TNT group. Levene's test indicated that the assumption of homogeneous variances was satisfied ($F = .61, p = .44$). An independent-samples t-test was conducted on the mean

durations, and the difference between group means was not found to be statistically significant ($t[28] = -0.25, p = .81$).

IOA was evaluated by calculating an ICC on the 13% of the data that were collected by both observers. The obtained ICC was .90 ($p < .01$) for latency and .96 ($p < .01$) for duration, indicating that IOA for the one-hole evaluation was good.

Discrimination Pretest

The mean latency across discrimination (three-hole) training pretest trials from the release time to arrival at the TNT scent hole was calculated for each rat. The group mean latency was 14.36 s ($SD = 8.19$) for the control group and 10.39 s ($SD = 5.82$) for the TNT group. Levene's test indicated that the assumption of homogeneous variances was satisfied ($F = .22, p = .64$). An independent-samples t-test was conducted on the mean latency scores, and the difference between group means was not found to be statistically significant ($t[14] = 1.14, p = .28$).

The mean cumulative duration over the TNT scent hole was also calculated for each rat. The mean duration was 1.99 s ($SD = .63$) for the control group and 2.73 s ($SD = .96$) for the TNT group. Levene's test indicated that the assumption of homogeneous variances was satisfied ($F = .33, p = .57$). An independent-samples t-test was conducted on the mean latency scores, and the difference between group means was not found to be statistically significant ($t[14] = 1.77, p = .10$).

IOA was evaluated by calculating an ICC on the 25% of the data that were collected by both observers. The obtained ICC was .99 ($p < .01$) for latency and .97 for duration ($p < .01$), indicating that IOA for the discrimination pretest was very good.

Discrimination Evaluation

The mean number of false indications across trials for each rat on each of the two discrimination (three-hole) evaluation days was calculated. The mean number of false indications per trial was 0.17 ($SD = .24$) for the control group and 0.09 ($SD = 0.18$) for the TNT group. Levene's test indicated that the assumption of homogeneous variances was satisfied ($F = .60, p = .44$). An independent-samples t-test was conducted on the mean false indication scores, and the difference between the group means was not found to be statistically significant ($t[32] = .65, p = .52$).

The mean duration of each rat's first pause over the scent hole in the discrimination evaluation was calculated for each of the two evaluation days. The mean duration was 1.83 ($SD = .55$) for the control group and 1.83 ($SD = .68$) for the TNT group. The lack of a difference between the two group means made further statistical analysis unnecessary.

IOA was evaluated by calculating and ICC on the 24% of the data that were collected by both observers. The obtained ICC was .85 ($p = .02$) for the false

indication data and .92 ($p < .01$) for the duration data, indicating that IOA for the discrimination evaluation was good.

Duration of Training

The number of training days required for each rat to successfully complete the discrimination blind test was calculated for each rat. The mean number of training days was 44.5 ($SD = 1.69$) for the control group and 43.67 ($SD = 1.58$) for the TNT group. Levene's test indicated that the assumption of homogeneous variances was satisfied ($F = .21$, $p = .66$). The difference between the means was not found to be statistically significant ($t[15] = 1.05$, $p = .31$).

Overall Findings

It is noteworthy that, although most tests for statistical significance of the difference between group means on the data obtained throughout the evaluation process did not result in rejection of the null hypothesis, the descriptive statistics show that the TNT group performed better than the control group in all but one evaluation, the duration measure in the discrimination training evaluation, in which the means from both groups were the same. One method of assessing the probability of such an outcome under the condition that there is no difference between groups is to conduct a sign test on the number of measures on which the TNT group performed better than the control group and the number of all remaining measures taken

throughout early training, based on the descriptive statistics. On eight of the nine measures taken throughout early training, the TNT group performed better (i.e., lower latency, longer duration, fewer false indications, and fewer days of training before passing the blind test) on average than the control group. The probability of such an occurrence under the condition that the groups were equally likely to perform better on any one measure is .04. If the measure on which both groups performed equally is not considered in the calculation, the probability drops below .01. Therefore, we may conclude that TNT-food pairings have a positive impact on this collection of early training performance indicators. Figure 3 displays the standardized effect size for each measure, positive values indicating that the TNT group performed better than the control group for the associated measure.

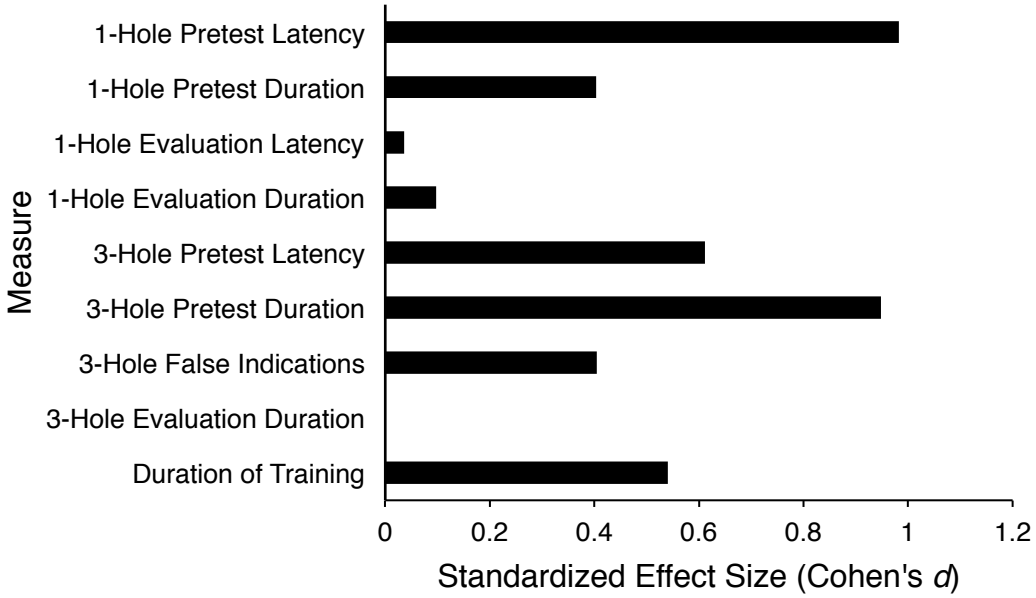

Figure 3. Standardized effect size (Cohen's *d*) for each measure taken throughout early training.

CHAPTER V

DISCUSSION

Findings

Twenty young rats in APOPO's mine detection rat program were divided into two groups and exposed to five daily stimulus-food pairing sessions each week. For the experimental group the stimulus was TNT, an explosive commonly used in landmines, and for the control group the stimulus was sugar. The influence of the respondent conditioning procedure on performance during early training was evaluated by examining key performance indicators throughout the early training process.

Prior to training the rats to approach and pause over a scent hole with TNT, the rats' latency to approach the scent hole and cumulative duration over the scent hole was measured with TNT and two controls. The rats in the TNT group approached the TNT with lower latency than the rats in the control group. However, the rats in the TNT group also approached the two control stimuli with lower latency than the rats in the control group and did not approach TNT with lower latency than the two controls. There was no statistically significant difference between or within group means with respect to the cumulative duration rats spent over the scent hole across stimuli.

While training the rats to approach and pause over TNT, an evaluation was conducted on the proportion of trials in which the rats arrived at the scent hole within three seconds and the duration of their first contact with the scent hole. No statistically significant difference between the group means was found for either of these measures. Prior to conducting discrimination training, the rats' latency to approach the scent hole with TNT and the cumulative duration over the scent hole with TNT was measured. No statistically significant difference between the group means was found for either of these measures. After three weeks of discrimination training, an evaluation was conducted to determine the number of false indications before the rats reached the TNT scent hole and the duration of their first contact with the TNT scent hole. No statistically significant difference between the group means was found for either of these measures. The difference between the mean number of training days required for rats in each group to pass the final discrimination blind test was not statistically significant. However, the TNT group consistently outperformed the control group on all but one of the measures taken. A sign test conducted on this outcome resulted in a statistically significant result, indicating that the pairings had a positive influence on this collection of performance indicators.

Shortcomings

Several shortcomings of the present research should be considered when interpreting the results. Perhaps the most critical shortcoming is that the rat handlers

were not blind to the status of the groups and may have treated the rats in each group differently. Although the trainers were paid an incentive for participating in the experiment, they were told that the incentive did not depend upon the outcome of the experiment. They were also closely monitored for adherence to the standard operating procedures and were never responsible for recording data on any of the measures reported herein.

An additional shortcoming of the present research is that no direct measures of respondent conditioning were recorded. Respondent conditioning procedures were arranged in such a way that the scent of TNT or the control scent alone should have come to elicit food-associated respondent behavior, such as increased salivation, but the instrumentation required to confirm such an effect was not available. Similarly, the research informing the present study did not include direct measures of respondent conditioning. Handlers reported that the rats became more active and that some rats emitted vocalizations when the food-paired stimulus was presented, but no data were recorded to confirm such an increase in activity.

Practical constraints precluded a long period of respondent conditioning prior to the start of operant conditioning. This may not be a shortcoming of the research itself, but the majority of experiments to which the present experiment can be compared examined operant conditioning outcomes following the termination of respondent conditioning. In the present experiment 88 pairings were presented over 44 pairing sessions, on average. In the literature reviewed herein, 353 pairings (range: 165–600) were presented over 19 pairing sessions (range: 10–38), on average. The

results obtained from the present experiment may have differed from those reported in the reviewed literature because of these methodological inconsistencies.

Small group sizes in the present experiment also resulted in low power for inferential statistical analysis. However, a large effect size would be required to justify a modification of this complexity to APOPO's standard operating procedures. Therefore, the small sample selected for this experiment was adequate for evaluating the impact of respondent conditioning on early scent discrimination training for the present purpose.

The results from the first measure of latency in the one-hole pretest indicate that the groups differed with respect to the latency from release time to arrival at the scent hole regardless of the stimulus present in the scent hole. This finding could be indicative of preexisting group differences despite random assignment, differential treatment of the groups because the handlers were not blind to the status of the groups, an overall activating influence of the TNT-food pairings, or another unidentified cause. Therefore, the finding that the TNT group performed better than the control group on nearly all subsequent evaluations should be interpreted with caution.

Conclusion

Together, these findings suggest that TNT-food pairings improve early scent discrimination training but that the effects are small at best. As such, a significant

investment in an automatic or manual feeding apparatus arranging for TNT-food pairings as devised in the present experiment is not justified. If pairings could be arranged with little cost and effort, implementation may provide a small advantage in early training of mine detection rats or other scent detection animals.

The findings also serve to emphasize the importance of developing and adhering to high quality standard operating procedures for operant discrimination training, as operant training was influenced little by supplemental respondent conditioning, and rats in the control group were trained to accurately identify TNT by the conclusion of the study.

Future Research

A pilot study investigating the influence of chronic exposure to a target scent in the rat's living cage is currently underway. Some evidence that nonassociative early exposure to target scents improves subsequent discrimination training and preference for the target scent has been reported (Rodríguez Echandía, Fóscolo, & Broitman, 1982; Sandoz, Loloi, Odoux, & Pham-Delègue, 2000). One practical advantage of this type of conditioning is that it would require very little cost or effort to implement if it were found to have a positive effect.

Future research may also examine the influence of exposing young rats to a wider variety of scents, particularly those that are similar to the target scent. Research demonstrating the positive influence of early enrichment with similar scents on later

scent discrimination suggests that this may be a fruitful avenue of research (Mandairon, Stack, Kiselycznyk, & Linster, 2006).

Respondent-operant relationships are complex, and the degree and nature of their interrelatedness remains a controversial topic. However, existing research findings suggest that respondent conditioning can and frequently does impact operant learning, although the results are often inconsistent (Holmes, Marchand, & Coutureau, 2010). Potentially beneficial applications of laboratory findings to practical problems should, therefore, be evaluated and the results of such evaluations considered when formulating future research questions.

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Appendix A

Institutional Animal Care and Use Approval Letter

Western Michigan University
Graduate College
Kalamazoo, MI

15 May 2013

RE: IACUC APPROVAL OF APOPO'S RAT INSTITUTIONAL PROTOCOL

This letter affirms that APOPO's 'TNT-Food Pairings & MDR Training' project has been approved by the organization's Institutional Animal Care and Use Committee. The Institutional Animal Use Protocol Number is 2013-05. Specifically, the IACUC has reviewed the relevant Animal Research Protocol for technical, scientific, ethical, and legal merit and recommends the project for commencement.

The care and use of animals, specifically 20 giant African pouched Rats (*Cricetomys gambianus*) will be conducted in accordance with the US National Research Council's 1996 *Guide for the Care and Use of Laboratory Animals* and applicable Federal regulations.

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Appendix B

TNT-Food Presentation Schedule

MONDAY DATE: _____ TRAINERS: _____, _____, and _____

On Left - Group 1 [RED]

Time	A1	A2	A3	A4	A5	A6	A7	A10
1	+	–	–	–	+	–	+	–
2	–	–	+	+	–	+	–	+
3	–	+	–	–	–	+	–	–
4	+	+	–	+	–	–	–	–
5	–	–	+	–	+	–	+	+

On Right - Group 2 [BLUE]

Time	B1	B2	B3	B4	B5	B6	B7	B8	B9
1	+	–	–	–	+	–	+	+	–
2	–	–	+	+	–	+	–	–	–
3	–	+	–	–	–	+	–	+	+
4	+	+	–	+	–	–	–	–	–
5	–	–	+	–	+	–	+	–	+

When finished: ☐ Remove all used gloves from the kennels
☐ Take blue basket to laboratory

Instructions:

– Each time there is a "–" in the box for a rat, put a "–" jar in the holder, start the timer, and remove the jar after 15 sec.

\+ Each time there is a "+" in the box for a rat, put a "+" jar in the holder, start the timer, deliver food after 10 seconds, then remove the jar after another 5 seconds

✔ As soon as the jar is removed, place a check in the box.

Note: Check to make sure that colors match.

Appendix C

TNT-Food Pairing Checklist

TNT-Food Pairing Checklist

Preparation

- ☐ Ensure that 40 pots are loaded with food
- ☐ Place correct number of food pots in the basket for each line of presentations
- ☐ Check the presentation schedule for the correct day of the week
- ☐ Make sure Handler 1 has a stopwatch, clipboard, and pen
- ☐ Handlers rotate responsibilities each day

Procedure

- ☐ Handlers 2 and 3 put on clean gloves before handling any equipment
- ☐ Handler 2 takes the basket to the correct group for each line of presentations
- ☐ Handlers 2 and 3 follow presentation instructions of Handler 1 and present bottles and food according to the presentation program (see "bottle presentation" below)
- ☐ Handler 2 replaces the basket between each line of presentations for each group
- ☐ Handlers 2 and 3 change gloves between each line of presentations for each group
- ☐ Handler 2 places the correct number of food pots in the basket for each line of presentations
- ☐ Gloves are removed from kennel and baskets are placed in the correct locations at the conclusion of the pairing session

Bottle Presentation

- ☐ For each rat, Handler 2 presents the negative bottle if there is a "–" and Handler 3 presents the positive bottle if there is a "+"
- ☐ Only "+" handler (Handler 3) makes contact with "+" bottles and makes contact with nothing else
- ☐ Handler 1 gives start signal and immediately starts the timer on each trial
- ☐ Handler 1 gives stop signal after 15 seconds and food signal after 10 seconds on "+" trials
- ☐ If it is a "+" trial, after hearing food signal, Handler 2 opens a canister and dumps food into the top of the cage near the bottle, Handler 3 removes the bottle after hearing the stop signal
- ☐ If it is a "–" trial, after hearing stop signal, Handler 2 removes the bottle (and presents no food)
- ☐ After the trial is completed, Handler 1 puts a check in the box and moves to the next rat