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The Influence of Training Procedures on Generalization Performance in Scent-Detection Rats

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THE INFLUENCE OF TRAINING PROCEDURES ON
GENERALIZATION PERFORMANCE IN SCENT-DETECTION RATS

by

Erin E. Watkins

A dissertation submitted to the Graduate College
in partial fulfillment of the requirements
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Erin E. Watkins, Ph.D.

Western Michigan University, 2017

The global illicit trade in tobacco products leads to an overall increase in the availability of tobacco, and this increase in tobacco availability and consumption undermines effective health, safety, and taxation policies in place to protect current and future populations. Dogs working at ports and customs have been trained to detect tobacco products and research has shown rats can detect tobacco-soaked filters (Mahoney et al., 2014). Cigarette smoking is the most common form of tobacco use, and cigarettes are the most commonly trafficked product in the illicit tobacco trade. In the current study, rats were trained to respond to filter samples of 21 cigarette brands and not to respond to filter samples of controls (e.g., coffee, tape). Training resulted in average hit rates ranging from 91% to 100% and false alarm rates ranging from 2% to 5%. A series of tests were then conducted with 15 untrained cigarette brands to measure generalization. Two tests conducted after concurrent training resulted in hits on 38% and 49% of generalization samples. These results indicate modest generalization from trained to untrained cigarette brands, with performance improving as the number of brands trained increased. After training cigarette brands in succession the hit rate on generalization samples reached 67%. The findings of this study suggest that preparing samples by pulling air from a container through a filter is an effective method for training cigarette scent-detection discrimination. Further research

is needed before pouched rats can be employed as illicit tobacco-detection animals in practical applications, as performance did not exceed a mean hit rate of 49% on novel brands of cigarettes.

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INTRODUCTION

Illicit Tobacco Trade

Nicotine, found in tobacco (*Nicotiana tabacum*), is one of the most commonly used drugs in the world. Cigarettes are the most popular tobacco product worldwide accounting for 92.3% of tobacco sales (NCI & WHO, 2016). Inhalation of tobacco smoke is a highly effective route of administration since nicotine has been shown to travel to the brain within 7 s after inhalation (Maisto, Galizio, & Connors, 2015). More than 1.1 billion people over the age of 15 are smokers (about 21% of the population) worldwide (NCI & WHO, 2016).

Cigarettes are known to contain at least 69 carcinogens and cause numerous health conditions (e.g., heart disease, hypertension, stroke, and cancer) (NCI & WHO, 2016). The *WHO Global Report: Mortality Attributable to Tobacco* estimates that smoking causes 71% of lung cancer, 42% of chronic respiratory disease and nearly 10% of cardiovascular disease (WHO, 2012). Tobacco use, principally cigarette smoking, is responsible for an estimated US \$ 1 trillion in health care costs and lost productivity each year (NCI & WHO, 2016). Tobacco use is the world's leading cause of preventable premature death and is responsible for almost 6 million lives lost, both from direct tobacco use and second-hand smoke, annually (NCI & WHO, 2016). If cigarette consumption continues to increase as expected tobacco use deaths are expected to rise from 6 to around 8 million annually by 2030 (NCI & WHO, 2016).

Unregulated, untaxed illicit cigarettes lead to an overall increase in tobacco consumption by entering the market as more accessible and affordable than legally sold cigarettes (WHO,

2015). Illicit trade is a global enterprise affecting both low and high-income countries and is perpetrated on small-scale by individuals and on large-scale by international criminal networks. The illicit trade of tobacco products is a transnational inherently illegal activity making it hard to collect accurate data. Despite the dearth of global data Joossens and Raw (2012) calculated 657 billion cigarettes (or one out of every ten cigarettes) is illicit, amounting to 11.6% of cigarette consumption in 2007.

The World Health Organization Framework Convention on Tobacco Control (WHO FCTC), which entered into power as international law in 2005, enforces policies to protect current and future generations from the consequences of tobacco consumption and exposure by providing actions to reduce the supply and demand of tobacco products. In 2012, as a response to the growing international illicit trade in tobacco, The Protocol to Eliminate Illicit Trade in Tobacco Products was developed. Illicit trade as defined in Article 1 of the Protocol is “Any practice prohibited by law which relates to production, shipment, receipt, possession, distribution, sale or purchase including any practice or conduct intended to facilitate such activity” (WHO FCTC, 2013, p. 6).

There are three components to illicit trade in tobacco products; smuggling, counterfeiting, and evasion of local taxes. Of the three components smuggling, defined as the illegal trade of products across borders, poses the most serious challenge. Smuggling often involves large quantities of tobacco products, one of the most highly trafficked goods being cigarettes, and has a greater impact on public health and global economies (Allen, 2011). Large-scale smuggling of well-known cigarette brands was the main type of illicit tobacco trade for decades (Joossens & Raw, 2012). Smugglers took advantage of trade laws that allowed products to leave one jurisdiction without paying taxes since taxes were due to be paid at the final market location.

Cigarette smuggling contributed to a 300 billion-item gap between reported cigarette exports and imports between 1995-2000 (Joossens & Raw, 2012).

Recently illegal manufacturing of cigarettes, counterfeiting, and the explicit creation of brands (termed ‘cheap whites’) for export has surpassed mass smuggling as the main type of illicit tobacco trade. The most common cheap whites brand Jin Ling, is produced in Russia, Ukraine, and Moldova and exported out of these countries for sale without domestic duty paid. Illegal manufacturers of these cigarettes do not abide by regulatory guidelines and the World Customs Organization (WCO) reports finding increased nicotine and tar levels, as well as arsenic and other hazardous contaminants (e.g., mites, chlorine gas), contained within or on the surface of these illicit cigarettes (*Illicit Trade Report 2012, 2013*). These cigarettes pose an increased risk to public health and well-being, especially to young, low-income, and other vulnerable populations who are more likely to purchase untaxed, unregulated cigarettes.

It is evident that the illicit cigarette trade undermines the effectiveness of: taxation policies leading to revenue loss for retailers and governments, health policies leading to further addiction and degradation of health, and safety policies leading to environmental damage and tobacco related deaths. In 2012 it was estimated that if illicit trade were eliminated more than 160,000 lives would be saved per year from 2030 onwards and governments would retain at least 31 billion dollars in revenue globally (Joossens & Raw, 2012). In addition to saving lives and increasing revenue, eliminating the illicit cigarette trade would also decrease additional criminal activity (e.g., corruption, arms trafficking, and terrorism) carried out by smuggling networks, which are supported by illicit revenue (Titeca, Joossens, & Raw, 2011).

The Protocol’s main objective is the elimination of all forms of illicit trade in tobacco products including smuggling and illicit cigarette manufacturing (WHO FCTC, 2013).

Recommendations to control cigarette smuggling highlighted the use of multiple detection technologies to track cigarettes from manufacturing to shipment (through digital stamps, GPS devices, Radio-Frequency IDentification (RFID) chips, etc.) and to trace illicit products, upon seizure, back to their point of diversion (through shipment records). Technologies recommended for customs agencies included high-risk profiling conducted by customs officers, x-ray scanners for cargo containers, and on site detector dogs. Many customs agencies identified a need for technological tools in order to support efforts to detect and seize contraband, and nonintrusive methods such as the use of detection dogs have proven efficient (NCI & WHO, 2016).

Currently, eight WCO Regional Dog Training Centers located in Azerbaijan, China, Czech Republic, Germany, Kazakhstan, Russian Federation, Saudi Arabia, and Uzbekistan train handlers and dogs for detection of narcotic substances and tobacco products. Dogs are trained to sniff along the outside and inside of containers (if opened). Scent-detection dogs contributed to 18 global container seizures in 2015, which resulted in the seizure of more than 100 million illicit cigarettes and more than 23 tons of tobacco (WCO, 2015).

Animal Scent-Detection

Olfaction, or the sense of smell, is phylogenetically the oldest sense and has been found in the most primitive single cell organisms (Philpott, Bennett, & Murty, 2008), however the olfactory system is far from primitive. Olfaction involves the detection and perception of chemicals in the environment, and all animals have an olfactory system evolved for the ability to utilize olfactory cues to enhance the probability of reproduction and survival. How well an organism can detect an odorant (i.e., the ability to detect an odorant at a low threshold), relates to

the biological relevance of that odorant to the organism. Rats are able to discriminate 2,4,5-trimethylthiazoline, an odor associated with fox feces, between 0.04 and 0.10 part per trillion (ppt) (Laska et al., 2005). In humans olfaction is often considered the least sensitive of the senses and a number of animals are superior to humans in their olfactory abilities (Purves et al., 2001). Dogs are known to possess highly developed olfactory abilities and humans have utilized these abilities for over thousands of years, training dogs for a large number of diverse detection tasks (reviewed in Browne, Stafford, & Fordham, 2006 and Johnen, Heuwieser, & Fischer-Tenhagen, 2013).

Dogs have been trained for scent-detection in a wide range of fields and scenarios including public safety, medical applications, pest control, and animal management. In public safety detection dogs have been used to detect disaster survivors (Fenton, 1992), cadavers (Oesterhelweg et al., 2008), explosives (Furton, 2001; Göth, McLean, & Trevelyan, 2003), and illicit drugs (Lorenzo et al., 2003). In the medical field dogs have been used for the detection of seizures (Strong, Brown, & Walker, 1999), and a number of cancers such as melanoma (Pickel, Manucy, Walker, Hall, & Walker, 2004), bladder (Willis et al., 2004), and prostate cancer (Gordon et al., 2008). Dogs have been trained and employed in pest control to detect the presence of invasive species, such as bed bugs (Cooper, Wang, & Singh, 2014) and snakes (Engeman, Vice, York, & Gruver, 2002). In the field of animal management dogs have been trained to detect wild animal scat (Smith et al., 2003), and to track the location of endangered animals, such as desert tortoises (Cablak & Heaton, 2006).

Dogs are an efficient, adaptable scent-detection technology currently used in a wide range of jobs including in customs operations. However, they are not the only species trained for scent-detection tasks. The rat olfactory system is comparable to the dog olfactory system in both

odor detection thresholds and the ability to discriminate odors (Goldblatt, Gazit, & Terkel, 2009), and rats have been trained for humanitarian scent-detection tasks.

Giant African Pouched Rats

APOPO is a Flemish acronym for Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling, or in English, Anti-Personnel Landmines Detection Product Development. APOPO, founded in 1998, is an organization that researches, develops and implements scent-detection rat technology for humanitarian purposes such as clearing landmines and detecting tuberculosis (TB) in humans. APOPO is a Belgian non-governmental organization (NGO), with headquarters in Tanzania and operations in Mozambique, Angola, Zimbabwe and Cambodia. The organization uses operant conditioning to train an indigenous species of African rat (*Cricetomys ansorgei*) that have a highly adapted sense of smell.

Cricetomys are burrowing, nocturnal scavengers ranging in size from 1075 to 1275 g for mature males and 957 to 1157 g for mature females in captivity. They have a similar nose anatomy to dogs, in that they have alar folds and turbine bones, which enhance their ability to absorb, heat, and detect chemicals in the environment (Harkema & Morgan, 1996). Their size, temperament, relatively short gestation period (up to one month), and long life span (averaging eight years in captivity) make them ideal animals for work with humans. At four weeks of age the rats are socialized through extensive handling, at five weeks the rats are removed from their mothers for increasing durations of time while being exposed to an array of stimuli (habituation), and around six weeks rats are exposed to a target stimulus (e.g., TNT for landmine detection rats, or TB positive sputum samples for TB detection rats) for detection training.

APOPO's scent-detection training procedures have been extensively described and published elsewhere, see Poling et al. (2010) and Poling et al. (2011) for a detailed description of procedures. In brief, discrimination training aims to establish stimulus control or reliable responding to a target scent. Differential reinforcement, a common method of operant conditioning, involves reinforcing an indication (an easily-detected response) in the presence of a target scent, but not indications in the presence of non-target scents (i.e., controls) at a young age. Over 111 landmine detection rats and over 50 TB detection rats have been trained at APOPO's center in Morogoro, Tanzania resulting in 104,984 located and destroyed landmines and unexploded ordnance (UXO) and 10,490 detected cases of TB (additional sputum samples missed by microscopists), to date (APOPO, 2017).

APOPO has been recognized for their innovation, impact and sustainability as an NGO and was ranked as #11 overall by *The Global Journal* in 2013 and #22 overall by *Global Geneva's Top 500 NGOs* rankings in 2015. Additionally, the organization has received several endorsements and international social entrepreneurship awards including an ASHOKA endorsement in 2006, The Schwab Foundation fellowship in 2007, and the Skoll Award in 2009.

As a successful and growing NGO, APOPO actively explores additional humanitarian applications of scent-detection rats. Locating common types of contraband found in shipping containers such as illicit cigarettes is one such application. In 2014, proof-of-principle research was conducted by APOPO on the rats' ability to detect various tobacco products and generalize responses to novel tobacco products (Mahoney et al., 2014).

Stimulus Generalization

Evolution has shaped both an organism's capacity to engage in unlearned environment-behavior relations, and an organism's capacity to respond to changes in the environment through selection by consequences (Michael, 2004). It is necessary for survival for an organism to quickly acquire behavior appropriate to complex and changing environments. Hence, all living organisms' capacity to learn through operant conditioning (i.e., behavior altered by its consequences) is adaptive.

One way to explicitly arrange operant conditioning is through discrimination training. Often a behavior (right lever press) is reinforced in the presence of one stimulus or one set of stimuli (drug) and another behavior (left lever press) is reinforced in the presence of another stimulus or set of stimuli (vehicle). This process establishes stimulus control when different stimuli (drug vs. vehicle) come to occasion different responses (right vs. left lever presses). A stimulus that comes to signal the availability of differential reinforcement is not likely to remain constant in a complex and changing environment highlighting the need for adaptive responses to untrained stimuli.

In 1939, Hull questioned how best to account for responses that occurred in the presence of stimuli that had not been directly trained. Hull proposed a process he described as primary stimulus generalization. Primary stimulus generalization is a process in which learned responses to particular stimuli can also occur in response to new stimuli, which are perceptually similar to those trained. A more contemporary description of this phenomenon by Cooper, Heron, and Heward (2014), describes this process as stimulus generalization and they define stimulus generalization in terms of stimuli that share similar physical properties evoking a similar response.

Further, multiple stimuli that evoke the same response are said to be members of a functional stimulus class (Michael, 2004). Similarly, Keller and Schoenfeld in 1950 suggested organisms exhibit conceptual behavior when they respond similarly to members (stimuli that share clusters of features): “Generalization within classes and discrimination between classes- this is the essence of concepts (Keller & Schoenfeld, 1950, p. 155).” In order to demonstrate conceptual behavior an organism must respond similarly to members of one stimulus class and respond differently to members of another stimulus class, and to generalize differential responses to novel members of a stimulus classes (Wasserman, 2016).

Research on Stimulus Generalization

In 1956, Guttman and Kalish published an article on stimulus generalization in pigeons, helping to establish stimulus generalization as a productive area of research. Guttman and Kalish trained four groups of pigeons to reliably peck a key illuminated at different intensities on a variable interval (VI) schedule. Next, stimulus generalization was tested by randomly presenting 11 different light intensities repeatedly in extinction. The test values included the training value and changed by 10 nm of light intensity in each direction from the training value. A graph of the results depicted orderly decremental gradients of generalization around each training value. As the training stimuli and the test stimuli became more dissimilar responding declined and the changes in responding allowed for a measure of stimulus control.

In 1964, Herrnstein and Loveland published an article reporting visual discrimination and stimulus generalization in pigeons. Counter to previous research in visual discrimination with nonhumans, Herrnstein and Loveland trained complex stimuli. Color photographs of two categories, images with a person or people and images without a person or people, were

displayed on a small screen. The pigeons readily learned to peck when an image containing at least one person was presented, and not to peck when an image was presented that did not contain at least one person. Pigeons learned this discrimination with a large number of diverse stimuli, and transferred this discrimination to novel photographs of both categories. Subsequent studies by Herrnstein involved pigeons learning to discriminate pictures of trees, bodies of water, or a particular person (Herrnstein, Loveland, & Cable, 1976).

Much of the empirical research inspired by Herrnstein's pivotal studies continued to utilize a single target category (e.g., 100 Hz tone) together with its corresponding category (e.g., no tone) in a go/no-go paradigm. This go/no-go task, also considered a response inhibition task, requires a performance of a target response (e.g., pecking a key) in the presence of a particular stimulus (e.g., 100 Hz tone) and inhibition of the target response (e.g., not pecking a key) when alternate stimuli are present (e.g., no tone). Training in this way results in the target response occurring only when the organism detects the presence of the reinforced stimulus. Using this method, both pigeons and primates have been shown to be able to learn several different concepts and to transfer their performance to novel instances of the target concept (e.g., Matsukawa, Inoue, & Jitsumori, 2004; Kirkpatrick-Steger, Wasserman, & Biederman, 1996; Vogels, 1999).

Research on Olfactory Stimulus Generalization

It has been well documented that pigeons and monkeys more readily show learning with complex visual stimuli, while rats more readily show learning with odor stimuli (Lu, Slotnick, & Silberberg, 1993; Prichard, Panoz-Brown, Bruce, & Galizio, 2015). This difference in learning is due to the sensitivity of the modality being used (Nigrosh, Slotnick, & Nevin, 1975). For rats,

odorants provide particularly salient cues as demonstrated by Thorne and O'Brien (1971) who found that rats trained on a visual go/no-go task utilized accompanying paint odor cues instead of the arranged visual cues for discrimination.

Studies focused on how to best maximize generalization in scent-detection research have focused on two common techniques: (a) blocking, and (b) concept training through multiple exemplar training. Blocking occurs when a competing stimulus, presented prior-to and during training, inhibits stimulus control of the target stimulus. For example, Johnson (1970) trained two groups of pigeons on a compound line discrimination consisting of colored vertical and horizontal lines. One group of pigeons was exposed to line orientation training before being trained to discriminate a compound line and color stimulus. Generalization tests showed control of responses by line-orientation for the pretraining group and by the color of the compound stimulus for the remaining group. Thus the pretraining decreased (blocked) control by color and increased control by orientation. In scent-detection training, isolating a common odorant found across all target bouquets (e.g., TNT) could block the development of stimulus control by irrelevant accompanying odors (e.g., plastic) (Goldblatt, Gazit, & Terkel, 2009). In this way, responding would generalize only to untrained complex bouquets containing the common odor.

Another common strategy for promoting strong generalization is concept formation training (Stokes & Baer, 1977). Concepts are trained by presenting large sets of stimuli and differentially reinforcing responses to the target stimuli. Training in this way would require a large set of both target and non-target stimuli as well as assessment of stimulus control (Jones, 2011). This requires presenting a sufficient number of exemplars, as well as the target stimulus, at different strengths and in combination with a range of irrelevant odors. However, it is generally unknown before the onset of training what constitutes a sufficient number of

exemplars. Training different strengths of the target stimulus and combinations of the target stimulus with irrelevant odors is needed to adequately reflect the range of the target stimulus in situ. Locating and training with a large number of odors in this way requires resources and time (Jones, 2011), and a wide range of non-target odors must also be used for training so that the animal does not respond to all novel odorants instead of the odor feature selected for training. After training such a large number of diverse yet similar stimuli, an organism is likely to respond to a similar novel stimulus, as they are more likely to have been exposed to that feature during training. Unlike blocking, concept training does not allocate one feature to control responses, but allows the animal to identify the odor feature that distinguishes the target stimuli from the non-target stimuli. There is a large literature on concept learning in pigeons utilizing both simple and complex stimuli. For example, pigeons have been trained to discriminate the presence and absence of people (Herrnstein & Loveland, 1964), impressionist art versus cubist or abstract art (Watanabe, Sakamoto, & Wakita, 1995), and natural vs. manmade pictures of items across four categories (Bhatt, Wasserman, Reynolds, & Knauss, 1988).

Training Procedures for *Cricetomys Ansoergei*

Mahoney et al.'s (2014) research purpose was twofold: (a) to determine whether *Cricetomys ansorgei* could reliably detect the presence of tobacco in cigarettes versus controls with similar odor properties; (b) to test generalization to different forms of tobacco products not directly trained in order to show useful application in illicit tobacco trade detection. Mahoney et al., (2014) used a soaking sampling method, which consisted of placing filter papers in close proximity to items in containers for several days. Soaked filters were then used to test different types of tobacco products (cigarettes, snuff, and fire-cured leaf) and tobacco products with

additional scents (e.g., snuff plus coffee beans) after training first on one tobacco product (cigarettes) and then a combination of tobacco products with or without additional scents. The results of Mahoney et al.'s study indicated rats were readily able to detect tobacco samples compared to non-tobacco samples after direct training (mean of 95%). However, the rats showed low levels of responding to untrained tobacco products (mean of 10%), and only increased responding to novel targets once the complexity of the discrimination task was decreased. The researchers pinpointed methodological training concerns regarding the number of training sessions conducted for each product and provided recommendations for future studies, including an active method of sample collection. An active sampling method would require samples to be rapidly drawn from shipping containers and provided to the rats for detection at a remote location. It is unclear if this type of sampling, pulling air from a container, would provide enough odor for the rats to detect.

Based on Mahoney et al.'s (2014) procedures, the present study also utilized a concept formation procedure during discrimination training to expose the rats to a large number of target and non-target odors. Outlined in the present literature, concept formation relies on large sets of stimuli to form functional stimulus classes in which multiple stimuli evoke a similar response. Also, in keeping with Mahoney's research rats were trained to emit responses to cigarette-soaked filters but not to filters soaked with different odors in a go/no-go discrimination procedure. To better examine whether rats can detect cigarettes in a remote scent tracing (RST) application an active sampling methodology was developed. To better examine how to improve generalization the number of training stimuli and the presentation of stimuli were altered. Selected targets were trained for extended periods of time and testing sessions occurred each time a brand met stability criteria.

In sum, the purpose of this research was to provide further evidence regarding the ability of giant African pouched rats to detect illicit tobacco, specifically cigarettes, and to investigate strategies to maximize generalization to untrained cigarette brands using an active sampling methodology outlined in the discussion section by Mahoney et al. (2014).

METHODS

Subjects

Seven adult pouched rats (*Cricetomys ansorgei*) five male and two female, served as subjects in this study. The subjects (R. Bravo, R. Camel, R. Habreeze, R. Harrison, R. Iceberg, R. Marlboro, and R. Myron) were four to five years old at the start of the study and had been subjects in a previous study described by Mahoney et al. (2014). Mahoney trained the rats to emit a response in the presence of cartons of cigarettes, snuff, and fire-cured leaf tobacco, but not in the presence of non-targets (e.g., tea, coffee). The subjects were obtained from APOPO's breeding colony and were weaned at 4 weeks of age followed by socialization through extensive handling, and stimulus exposure. The rats' weights were maintained between 1075 – 1275 g for males and between 957 – 1157 g for mature females. During the study food pellets were earned within sessions and if additional food was needed to maintain the health of the animal fresh food was delivered two hours post session. Sessions were conducted five days a week Monday through Friday, and every Friday at 2:00pm the rats were fed a mixture of fresh food (e.g., avocado, peanuts, banana) calculated by weight and nutritional needs. Any unconsumed food was removed on Sunday afternoon.

APOPO's Animal Welfare Assurance was approved by the Office of Laboratory Animal Welfare (A5720-01).

Apparatus and Materials

Sessions were conducted in a semi-automated elevated line cage with Plexiglas walls and a stainless steel floor measuring 55 cm wide, 55 cm tall, and 205 cm long. Ten holes 2 cm in diameter with metal sliding covers were spaced equidistant along the floor near the front wall. An infrared photo beam sensor located (1 cm deep) in each hole detected when a rat "indicated" by placing and holding their nose above a tobacco positive sample. When a rat's nose broke the sensor for a set duration, a pellet dispenser (ENV-203-94, Med Associates, Georgia, VT) located on the small wall of the cage (near the last hole evaluated) and 7 cm from the floor of the line cage dispensed three 45-mg banana flavored rat pellets (Test Diet Omnitreat: Test Diet, 1050 Progress Dr. Richmond, IN 47374). Plastic pots 3 cm in diameter and 5 cm tall containing filters (arranged in a stainless steel bar) were placed beneath the holes for the rats to evaluate. The bars containing filter samples locked into place below the holes. Once a rat evaluated all ten of the holes the bar was manually removed and the next bar was locked into place.

A laptop computer running custom written software (MS Visual Basic[®]) connected to the line cage through a USB port and was used to run sessions and control reinforcer delivery. Planned sessions saved on a flash drive were uploaded to the laptop daily and once opened the program displayed a 6 x 10 grid of cells on the screen which represented the samples prepared for that day's sessions. Indications were displayed in cells as check marks. As a rat evaluated samples from left to right, nose-poke indications meeting individual threshold requirements were

marked on the screen in the grid as either correct indications, resulting in the immediate delivery of food pellets preceded by a “click” or incorrect indications, which earned no programmed consequences. During training sessions, cell locations of known positive samples (rewarded) were displayed in blue; during test sessions, cell locations of generalization samples were not displayed on the screen but entered as blind samples. At the end of each day session data were saved on a flash drive and analyzed in MS Excel[®].

Sample Preparation

Target sample sources (unopened ten-packs of cigarette cartons with polypropylene wrapping) and non-target sample sources (e.g., screws, clothing, spices) were stored in 5 or 8 L individual plastic containers with locking lids. Sources were placed in containers a minimum of ten days prior to the start of the study to allow odor vapor to accumulate. Two 2 cm circular holes were cut into the lid of each container and covered by rubber stoppers allowing for access to the source during sample preparation. Rubber stoppers were only removed during sample preparation and remained in place when the container was not in use. During sampling, three to five samples were pulled from each source per session.

Samples were prepared the day prior to the planned session, and sampling for target and non-target samples consisted of drawing air through filters with a vacuum pump (BIOSENS[®]) placed at the circular opening of source containers for 5 s. Soaked cylindrical filters were then placed into plastic sampling pots with labels and lids for evaluation. Sample preparation required two people: a sample handler, and a sample collector. The sample collector removed rubber stoppers and operated the vacuum pump while the sample handler loaded filter samples into

sampling pots. During sample preparation sessions all non-target samples were prepared first and target samples second. Both the sample handler and sample collector changed latex gloves between sample types to avoid cross-contamination. The vacuum pump nozzle and hose was wiped with methylated alcohol and dried between sample preparation sessions.

Training Sessions

All training sessions consisted of a randomized set of 60 samples comprising six bars with six target samples and 54 non-target samples. Samples were pulled from source containers in sets of three, resulting in the presentation of two cigarette training brands per session. Prevalence of target samples remained constant at 10%. Filter samples were evaluated in an elevated cage in which rats sniffed holes from the left to right, pausing to “indicate” by holding their nose in the hole for 1 to 2 seconds. Indication thresholds (the length of time required for a rat to have its nose in a hole) were based on individual rat performance. Indication thresholds increased by 200 ms if correct indications (hits) averaged $\geq 80\%$ and incorrect indications (false alarms) averaged $\geq 10\%$ over three consecutive sessions.

To better capture indication responses in the experimental chamber each sample was evaluated twice. A Plexiglas wall separated the front of the cage, which housed the sampling holes, from the back of the cage, which served as a walkway. When a rat correctly indicated a target sample it was trained to approach the food hopper, located on the right short wall, and walk behind the Plexiglas divider to circle back and continue sampling from the remaining holes. Researchers covered sampling holes after an indication response or after a failure to indicate on

the second evaluation. Once all ten holes were covered the bar was manually removed and the next bar was locked into place.

Training was considered complete and generalization tests were conducted once responding met group or individual stability criteria (described below).

Test Sessions

Test sessions commenced once training stability criteria were met. All test sessions consisted of a randomized set of 60 samples comprising six bars with six target samples, 45 non-target samples, three novel target samples (generalization samples), and six novel non-target samples. Prevalence of target samples remained constant at 10%. Generalization samples were programmed as unrewarded blind samples. Generalization tests occurred every other day with training sessions on non-test days. During testing, one untrained cigarette brand (three filter samples pulled from one cigarette brand source) was evaluated each session. Thirty novel non-target samples were included to differentiate responding to novel tobacco versus responding to all novel stimuli. Table 1 shows the cigarette brands trained and tested during each phase of the study, as well as the novel non-target sources.

Phase I

Prior to this study, Mahoney et al. (2014) exposed the rats to seven cigarette brands, four types of tobacco (e.g., loose-leaf tobacco), and combinations of tobacco plus controls (e.g., loose-leaf tobacco and coffee).

Table 1

Training target sources and testing target and non-target sources for each phase

| Phase | Rats | Cigarette targets trained | Cigarette targets tested | Novel non-targets |
|-------|--------------------------|---------------------------|--------------------------|-------------------|
| I | Bravo | Portsman | Embassy Light | Socks (2) |
| | Camel | Iceberg | Club Menthol | Shoes (2) |
| | Habreeze | Marlboro | Safari Filter | Curry Powder (2) |
| | Harrison | Embassy | Crescent & Star | Coffee (3) |
| | Iceberg | Benson Hodges | Club Filter | Tea (4) |
| | Marlboro | Embassy King | Safari Menthol | Cumin |
| | Myron | Marlboro Light Camel | Marlboro Light Camel | |
| II | Bravo | Embassy Light | Dunhill Switcher | Book |
| | Camel | Club Menthol | Vogue Frission | Coins |
| | Habreeze | Safari Filter | Dunhill Light | Washers |
| | Harrison | Crescent & Star | Dunhill | Sand |
| | Iceberg | Club Filter | Silk Cut | Sticker rolls |
| | Myron | Safari Menthol | Rothmans King | Water bottle |
| | | Sweet Menthol | Chunghwa | Sisal rope |
| | | Portsman | Kent Blue | Cola |
| | | Iceberg | | Bug spray |
| | | Marlboro | | Comb |
| | | Embassy | | Newspaper |
| | | Benson Hodges | | Screws |
| | | Camel | | Pencils/Pens |
| | | Embassy King | | Tissue paper |
| | | Marlboro Light | | Handkerchief |
| III | Bravo ¹²⁴ | Chunghwa* | Dunhill Switcher | Book |
| | Camel ¹ | Vogue Frission* | Vogue Frission | Coins |
| | Habreeze ¹² | Dunhill Switcher* | Kent Blue | Pencils |
| | Harrison ¹²³⁴ | Kent Blue* | Silk Cut | Cola |
| | Iceberg ¹²³⁴ | Dunhill Light | | Sticker rolls |
| | Myron ¹²⁴ | Rothmans King | | Screws |
| | | Embassy Light | | Water bottle |
| | | Club Menthol | | Pens |
| | | Safari Filter | | |
| | | Crescent & Star | | |
| | | Club Filter | | |
| | | Safari Menthol | | |
| | | Sweet Menthol | | |
| | | Portsman | | |
| | | Iceberg | | |
| | | Marlboro | | |
| | | Embassy | | |
| | Benson Hodges | | | |
| | Camel | | | |
| | Embassy King | | | |
| | Marlboro Light | | | |

Note. Numbers in parentheses indicate the number of different types or brands of that item. Asterisks designate source samples that served dual roles in training and testing during Phase III. Superscripts represent which tests each rat participated in during Phase III (e.g., R. Bravo

completed training on three training targets during phase III and advanced to generalization testing after training on Chunghwa, Vogue Frission, and Kent Blue).

In phase I of this study, training continued with the seven previously used cigarette brands plus one additional cigarette brand and 26 control sources until responding remained unchanged for five days. Generalization tests consisted of seven novel cigarette brands and 14 novel non-target sources and testing occurred over a 3-week time span.

Phase II

The number of cigarette brands trained prior to testing was increased in Phase II as increasing the number of trained stimuli has been shown to increase generalization during testing (Stokes & Baer, 1977). Seven new cigarette brands, the same brands used for generalization tests in Phase I, were trained to stability. A more strict two part stability criteria was in place for Phase II, stating that: 1) the hit rate and false alarm rate needed to be $\geq 85\%$ and $\leq 10\%$, respectively, and 2) the sub-means of the first and last three sessions could not vary more than 10% of each other nor the mean of last six sessions combined. All individual rats needed to meet these criteria simultaneously for the group to advance to testing due to sample preparation restrictions. Generalization tests consisted of eight novel cigarette brands and 16 novel controls tested over a 3-week time span.

Phase III

Cigarette brands were trained one at a time, in succession, in Phase III with the same stability criteria in place from Phase II. Novel cigarette brands were tested after each new brand met criteria. Testing occurred more often with this methodology and exposed the rats to the same

unrewarded novel targets multiple times. Of the eight novel cigarette brands tested in Phase II, four brands were trained one-by-one to stability. Rothman's King and Dunhill light cigarette brands had high generalization test scores (a mean hit rate of 100%) in phase II, and were added to the pool of previously trained cigarette brands for continued training in phase III. Dunhill was erroneously presented during one of the training sessions and removed from the study.

Target brands were trained according to generalization test scores in Phase II, and targets with higher test scores were trained first. Training consisted of the same numbers of targets (six) and non-targets (54) but differed in the number of sources used during sample preparation for the target samples. Previously, three filter samples were taken from two randomized target sources during training sessions. In Phase III one of the target sources remained constant, while the second source continued to be chosen randomly from the numerous cigarette brands previously trained. This allowed rats to continue to come into contact and earn rewards for indications on past target brands, and at the same time, allowed more exposure to the new cigarette brand being trained. Generalization tests occurred once the rats met training criteria for both the new cigarette brand being trained and the cigarette brands previously trained. Generalization tests consisted of four untrained cigarette brands and eight untrained controls.

RESULTS

Phase I

In Phase I, eight cigarette brands were trained concurrently, and during training six positive samples from two target sources were presented each session. Each brand source was presented approximately three times across 13 training sessions, resulting in nine filter samples per target source. After 13 training sessions the mean hit rate and false alarm rate stabilized at 91% (range, 82% – 99%) and 2% (range, .1% – 4%), respectively.

Generalization test sessions consisted of six rewarded target samples, 45 non-target samples, three blind generalization samples, and six novel non-target samples. Figure 1 shows mean test results across seven novel cigarette brands, which are displayed in order of testing and are as follows: Embassy Light 67%, Club Menthol 38%, Safari Filter 2%, Crescent & Star 24%, Club Filter 29%, Safari Menthol 14%, and Sweet Menthol 81%. Figure 2 shows the rats' overall hit rate for generalization samples as 38% with individual rates between 19% and 52%. Two novel non-target sources were included (three filter samples each) each test session, 14 total. Of the 294 presentations of novel non-target samples three were indicated resulting in a rate of 1%.

During testing the mean hit rate for trained targets remained high at 92% (range, 87% – 95%), and the false alarm rate for trained non-targets remained low at 2% (range, 1% – 3%). Similarly, the continued training sessions conducted between generalization test days resulted in an overall hit rate of 92% (range, 83% – 100%) and false alarm rate of 2% (range, 1% – 4%).

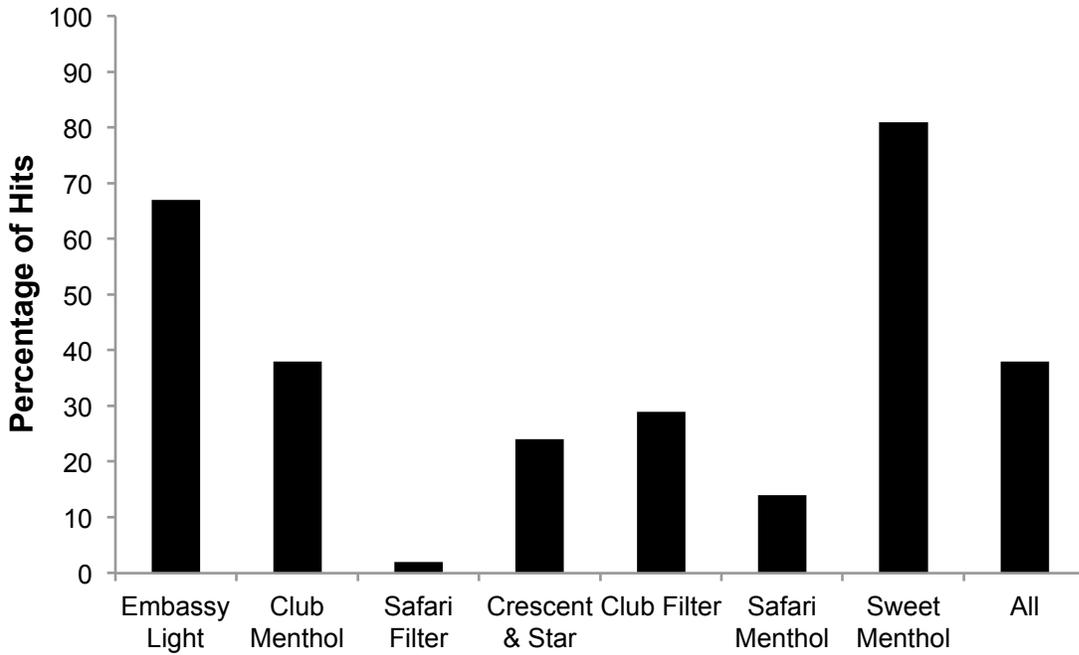


Figure 1. Indications on generalization samples across cigarettes; Phase I. Percentage of generalization samples indicated during phase I testing across cigarette brands.

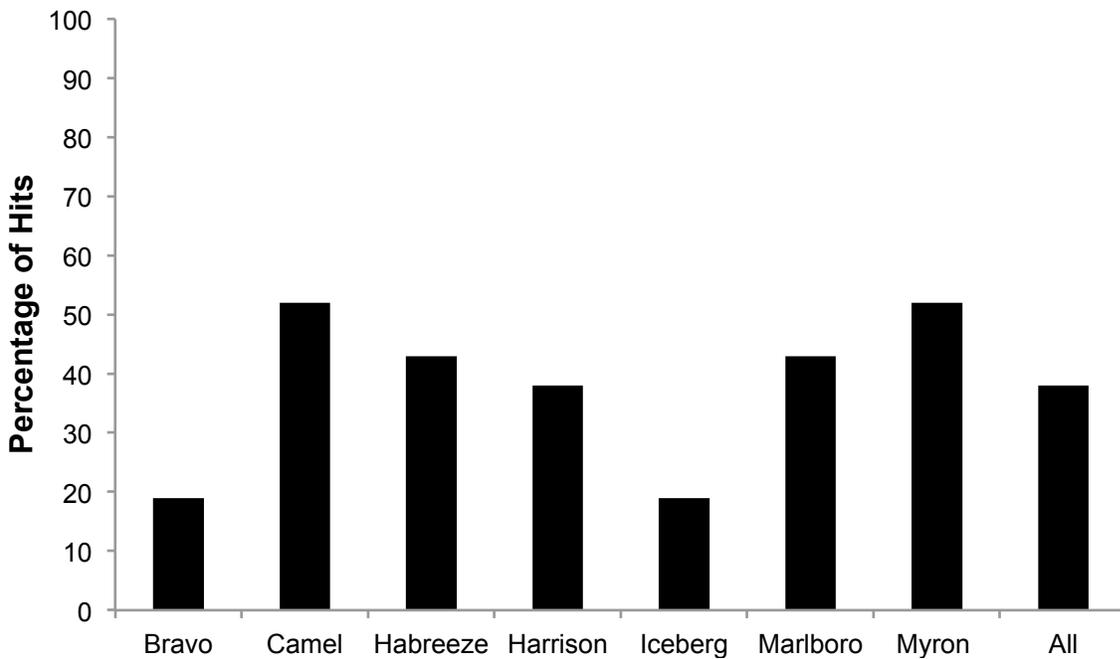


Figure 2. Indications on generalization samples across rats; Phase I. Percentage of generalization samples indicated during Phase I testing across rats.

Phase II

One rat (R. Marlboro) died shortly before the start of Phase II, and data was collected on the remaining six rats. In Phase II, seven new cigarette brands were trained concurrently with eight previously trained cigarette brands. Again six positive samples from two target sources were presented each session. Each brand source was presented approximately six times across 42 training sessions resulting in 18 filter samples per target source. Training on the seven cigarette brands resulted in an overall hit rate of 93% (range, 87% – 96%), and false alarm rate of 4% (range, 4% – 9%) across rats.

Sample distribution for generalization test sessions remained the same as Phase I (described above). Figure 3 shows test results across eight novel cigarette brands, which are displayed in order of testing and are as follows: Dunhill Switcher 6%, Vogue Frission 50%, Dunhill Light 100%, Dunhill 44%, Silk Cut 0%, Rothmans King 100%, Chunghwa 94%, and Kent Blue 0%. Figure 4 shows the rats' overall hit rate for generalization samples as 49% with individual rates between 42% and 58%. Only one novel non-target sample out of 288 (.3%) total samples was indicated during testing, a box of pencils.

The rats' hit rates and false alarm rates remained above accuracy criteria during testing and continued training at 89% (range, 83% – 94%) and 96% (range, 89% – 100%), and 2% (range, 0% – 4%) and 2% (range, 0% – 3%), respectively.

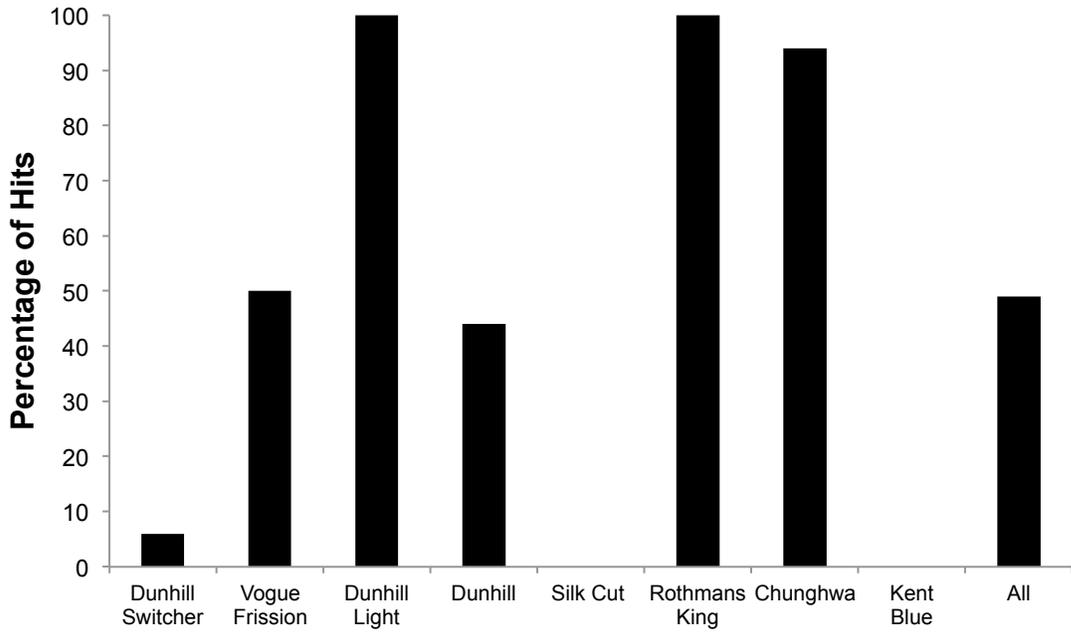


Figure 3. Indications on generalization samples across cigarettes; Phase II. Percentage of generalization samples indicated during Phase II testing across cigarette brands.

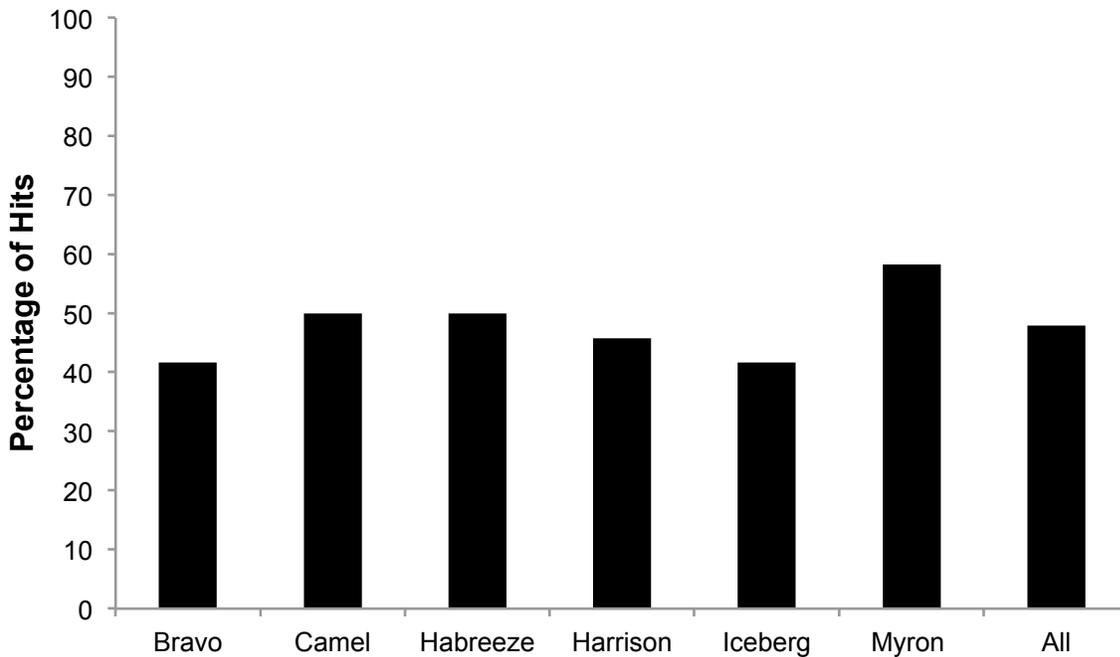


Figure 4. Indications on generalization samples across rats; Phase II. Percentage of generalization samples indicated during Phase II testing across rats.

Phase III

Given the results from the Phase I and II generalization tests, in Phase III we once again increased the number of target exemplars, but altered the training by exposing the rats to new target stimuli in a stepwise fashion. The rats' performance on generalization samples in Phase II determined the sequence of training targets for Phase III, with cigarette brands with high hit rates trained before brands with low or zero hit rates. The rats indicated on 100% of these brands in Phase III testing and were added to the collection of previously trained cigarette brands. Targets were trained in the following order: Chunghwa, Vogue Frission, Dunhill Switcher, and Kent Blue. The Phase II stability criteria were in place requiring a minimum of six sessions and a hit rate $\geq 85\%$.

Generalization tests were conducted after training on each target as described in the methods above. Following training on Chunghwa, four untrained cigarette brands were tested for generalization (Dunhill Switcher, Vogue Frission, Kent Blue, and Silk Cut). Following training on Vogue Frission, three untrained cigarette brands were tested for generalization (Dunhill Switcher, Kent Blue, and Silk Cut). This continued until four new cigarette brands were tested and trained.

Figures 5 – 8 show training sessions for all rats during Phase III. The number of training sessions required to reach stability criteria differed greatly between the first two training targets and the second two training targets. The combined hit rate and false alarm rate for training sessions on Chunghwa are shown in figure 5.

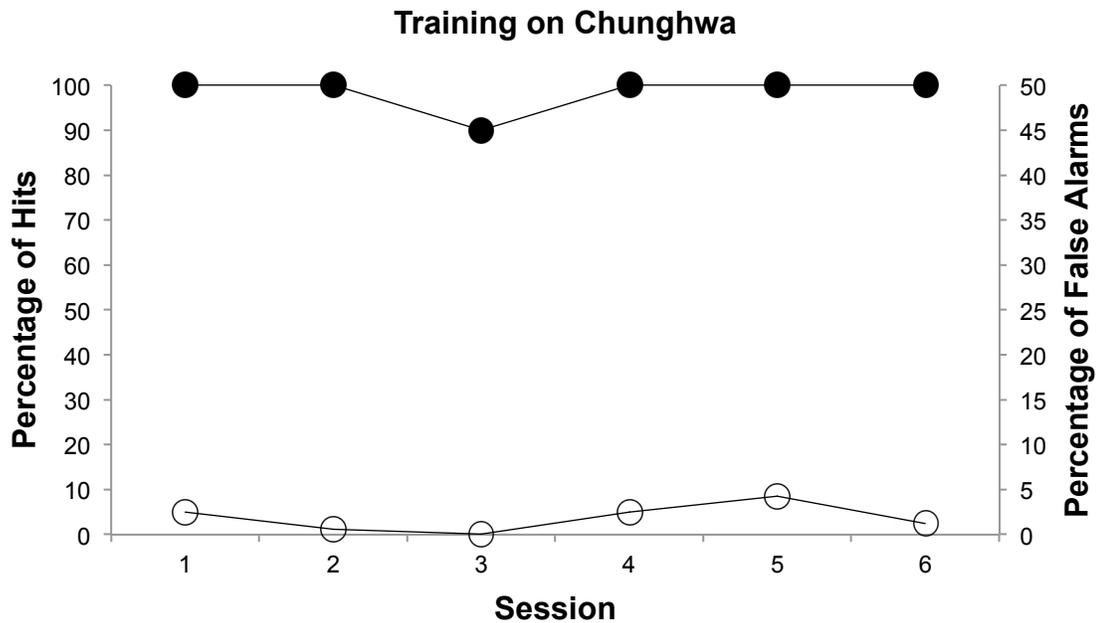


Figure 5. Training sessions for Chunghwa cigarettes during Phase III. Closed circles represent the rats' combined hit rate, while the open circles represent the rats' combined false alarm rate.

Training sessions were comprised of three target samples from Chunghwa and three target samples randomized from one of 18 previously trained sources. Training resulted in six presentations of Chunghwa (18 samples) and a hit rate of 98%. Training sessions for Vogue Frission cigarettes are shown in figure 6. Training sessions were comprised of three target samples from Vogue Frission and three target samples randomized from one of 19 previously trained sources. Training resulted in six presentations of Vogue Frission (18 samples) and a hit rate of 97%. Both Chunghwa and Vogue Frission were trained in the minimum six sessions needed to meet criteria with average hit rates of 98% (range, 92% – 100%) and 97% (range, 92% – 100%), and average false alarm rates of 2% (range, 1% – 3%) and 4% (range, 2% – 5%), respectively.

The combined hit rate and false alarm rate for training sessions on Dunhill Switcher are shown in figure 7.

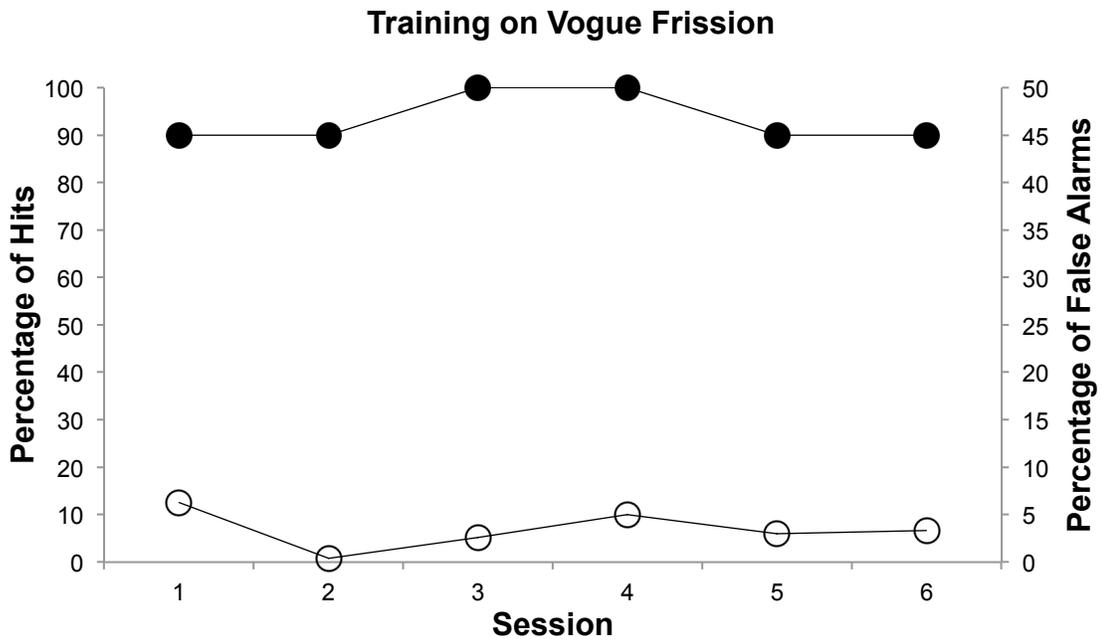


Figure 6. Training sessions for Vogue Frission cigarettes during Phase III. Closed circles represent the rats' combined hit rate, while the open circles represent the rats' combined false alarm rate.

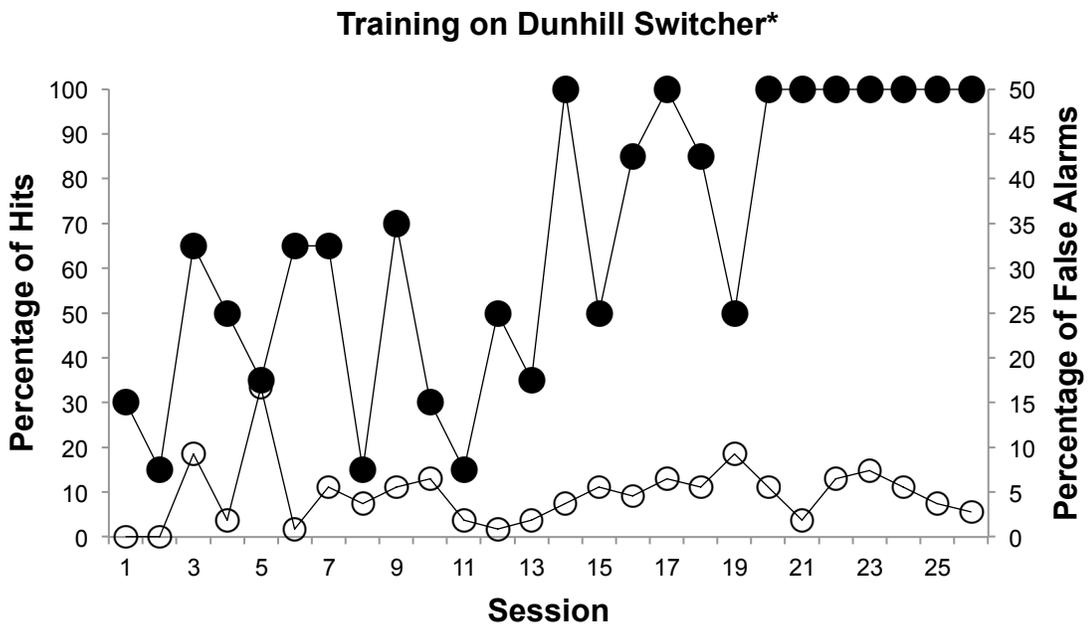


Figure 7. Training sessions for Dunhill Switcher cigarettes during Phase III. Closed circles represent the rats' combined hit rate, while the open circles represent the rats' combined false alarm rate. Note, this graph only includes training data for the two rats that met stability criteria.

Twenty-six training sessions were needed for the rats to meet training criteria on Dunhill Switcher. Training sessions were comprised of three target samples of Dunhill Switcher and three target samples randomized from one of 20 previously trained sources. Training resulted in 26 presentations of Dunhill Switcher (78 samples). After six weeks of training on Dunhill Switcher, two rats (R. Harrison and R. Iceberg) met stability requirements while three rats (R. Bravo, R. Habreeze, and R. Myron) failed to meet requirements due to variability and low average hit rates. One rat, R. Camel, was removed from training on Dunhill Switcher for health reasons. Two rats, R. Harrison and R. Iceberg averaged a 100% hit rate, and averaged false alarm rates of 3% and 5%, respectively.

Training sessions for the final target source, Kent Blue, are shown in figure 8.

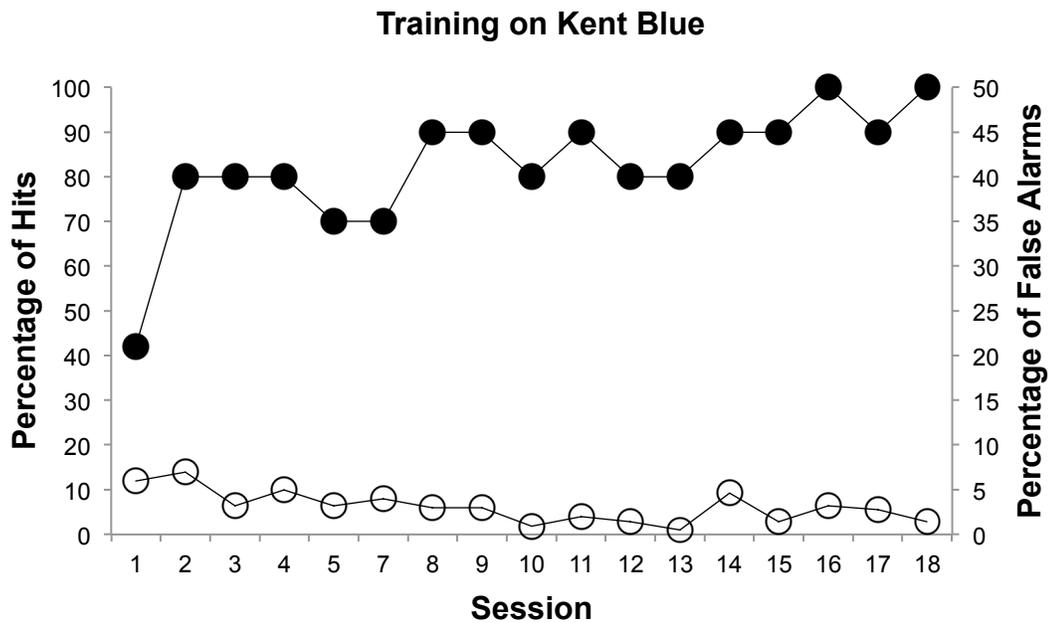


Figure 8. Training sessions for Kent Blue cigarettes during Phase III. Closed circles represent the rats' combined hit rate, while the open circles represent the rats' combined false alarm rate. Table 2.

Eighteen training sessions were needed for the rats to meet training criteria on Kent Blue. Training sessions were comprised of three target samples from Kent Blue and three target samples randomized from one of 21 previously trained sources. Four rats (R. Bravo, R. Harrison, R. Iceberg, and R. Myron) met stability requirements on Kent Blue after 18 training sessions (54 samples) averaging a hit rate of 96% (range, 86% – 100%) and a false alarm rate of 3% (range, 1% – 5%). R. Habreeze suffered an injury and was removed from training on Kent Blue. The rats continued to respond well on trained targets during generalization tests and continued training sessions with a 91% hit rate (range, 79% – 95%), while false alarm rates remained below criterion at 2% (range, 1% - 2%).

Table 2 shows results across generalization tests and cigarette brands.

Table 2

Generalization performance on different brands of cigarettes for all rats during Phase III

| Trained: Tested | Vogue Frission | Dunhill Switcher | Kent Blue | Silk Cut |
|---|----------------|------------------|------------|-----------|
| Results from Phase II | 50 (0-100) | 6 (0-33) | 0 | 0 |
| Chungwah: Vogue Frission, Dunhill Switcher, Kent Blue, Silk Cut | 83 (67-100) | 6 (0-33) | 61 (33-67) | 0 |
| Vogue Frission: Dunhill Switcher, Kent Blue, Silk Cut | | 33 (0-67) | 67 (0-100) | 13 (0-33) |
| Dunhill Switcher: Kent Blue, Silk Cut | | | 100 | 33 (0-67) |
| Kent Blue: Silk Cut | | | | 33 (0-33) |

Note. Numbers in parentheses represent range.

The average hit rate across novel cigarette brands increased from 14% to 35% after training on Chunghwa, from 35% to 38% after training on Vogue Frission, and from 38% to 67% after training on Dunhill Switcher, however the average hit rate dropped from 67% to 33% after training on Kent Blue. Only two novel non-target samples out of 282 (.7%) total samples were indicated during testing, in Phase III.

The results of generalization tests across all three phases of research are shown in Figure 9.

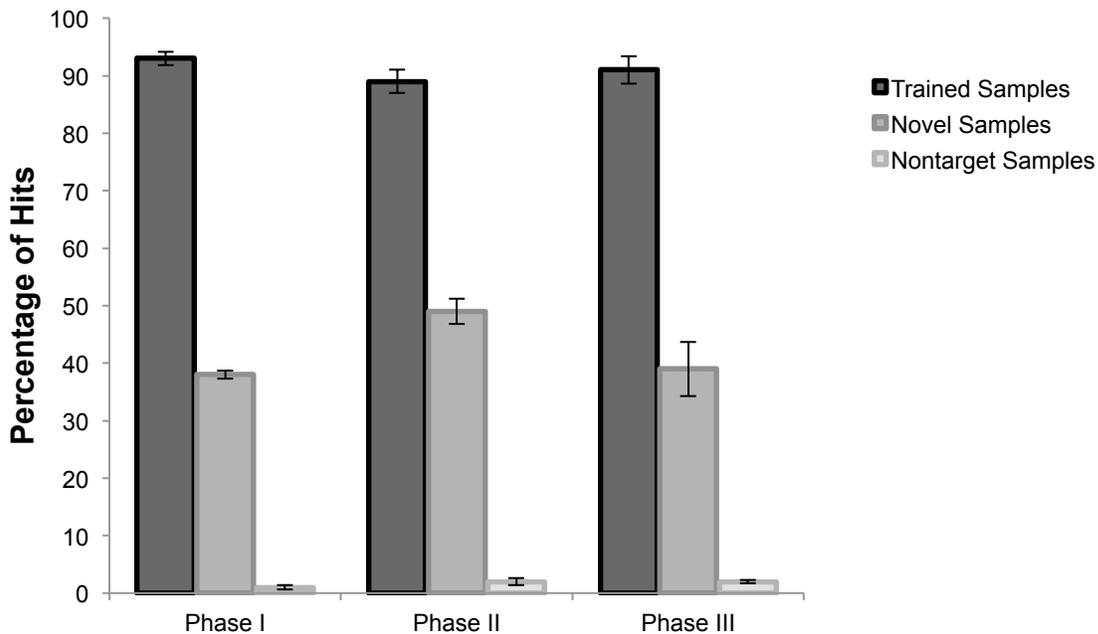


Figure 9. Average performance across target types for all rats and all phases.

During Phase I, the average hit rate of trained samples, novel samples, and non-target samples was 93%, 38%, and 1%. During Phase II, the average hit rate of trained samples, novel samples, and non-target samples was 89%, 49%, and 2%. Finally, during Phase III, the average hit rate of trained samples, novel samples, and non-target samples was 91%, 39%, and 2%.

Two rats R. Harrison and R. Iceberg completed all phases of training. Figures 10 – 12, show the performance of R. Harrison and R. Iceberg on cigarette filter samples during generalization tests across all three phases and all six tests. R. Harrison responded to eight novel cigarette-soaked samples across four cigarette brands (a 38% hit rate), and R. Iceberg responded to five novel cigarette-soaked samples across five novel brands (a 24% hit rate) in Phase I displayed in Figure 10.

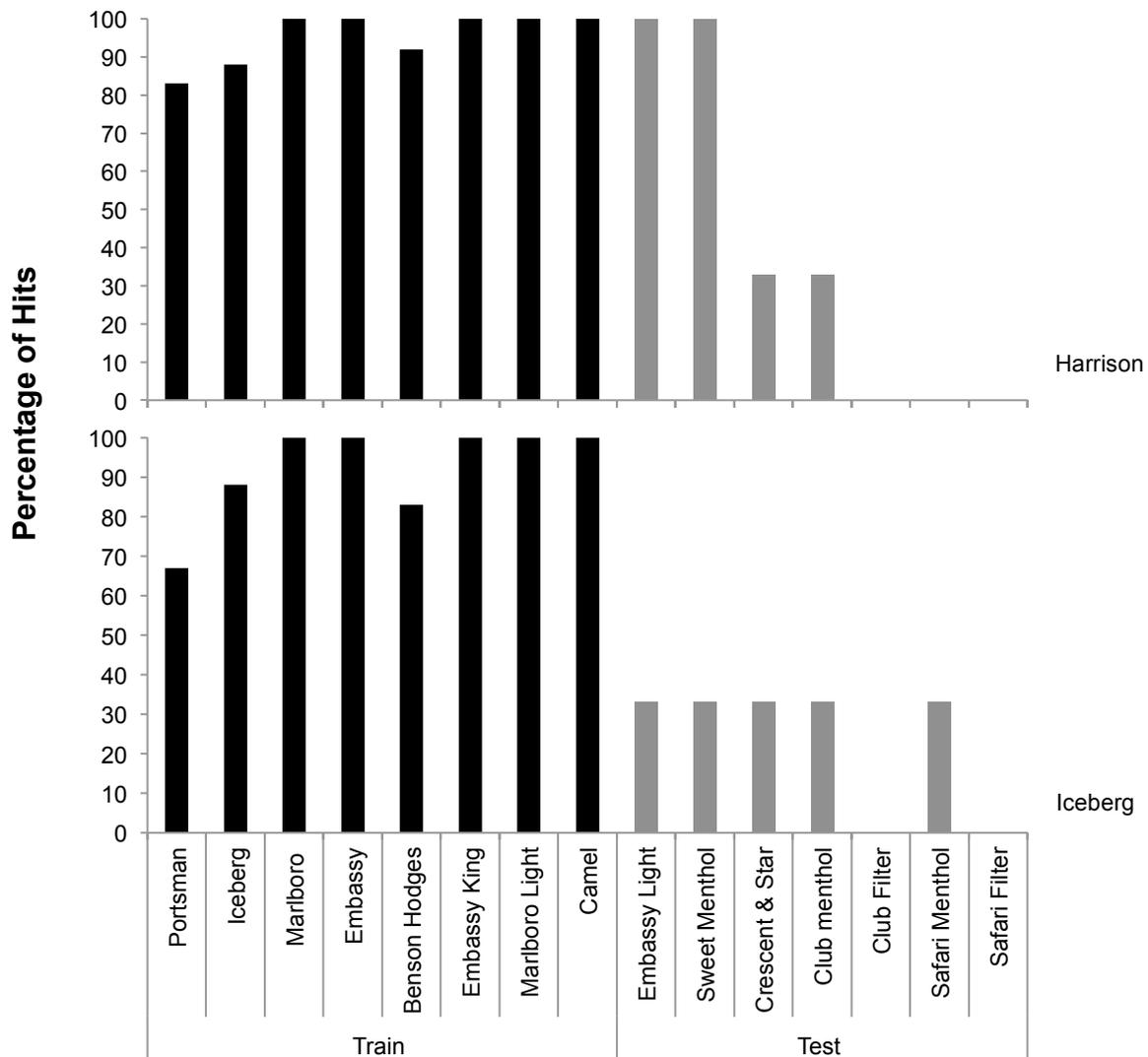


Figure 10. Phase I hit rate results. Percentage of hits on cigarette brands across trained and tested targets for two rats.

R. Harrison responded to 12 novel cigarette-soaked samples across five novel brands (a 50% hit rate), and R. Iceberg responded to 10 novel cigarette-soaked samples across 4 novel brands (a 42% hit rate) in Phase II as displayed in figure 11.

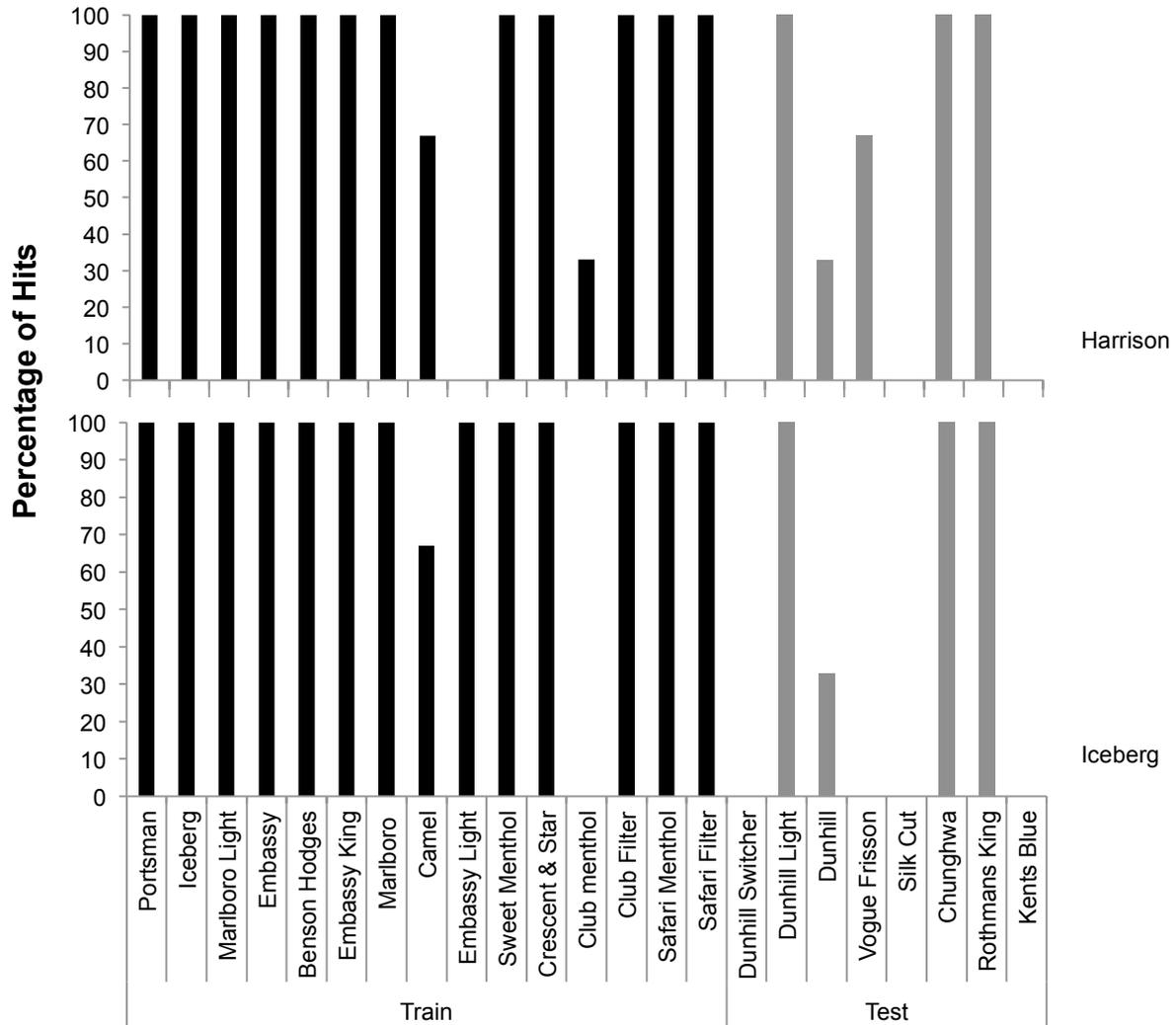


Figure 11. Phase II hit rate results. Percentage of hits on cigarette brands across trained and tested targets for two rats.

Figure 12 shows indications on cigarette-soaked samples for Phase III. Across all four tests R. Harrison responded to 11 novel cigarette-soaked filters across six cigarette brands (a 37% hit

rate), and R. Iceberg responded to 15 novel cigarette-soaked filters across 7 brands (a 50% hit rate). Throughout all tests R. Harrison and R. Iceberg's hit rates for trained cigarette brands remained above 85%.

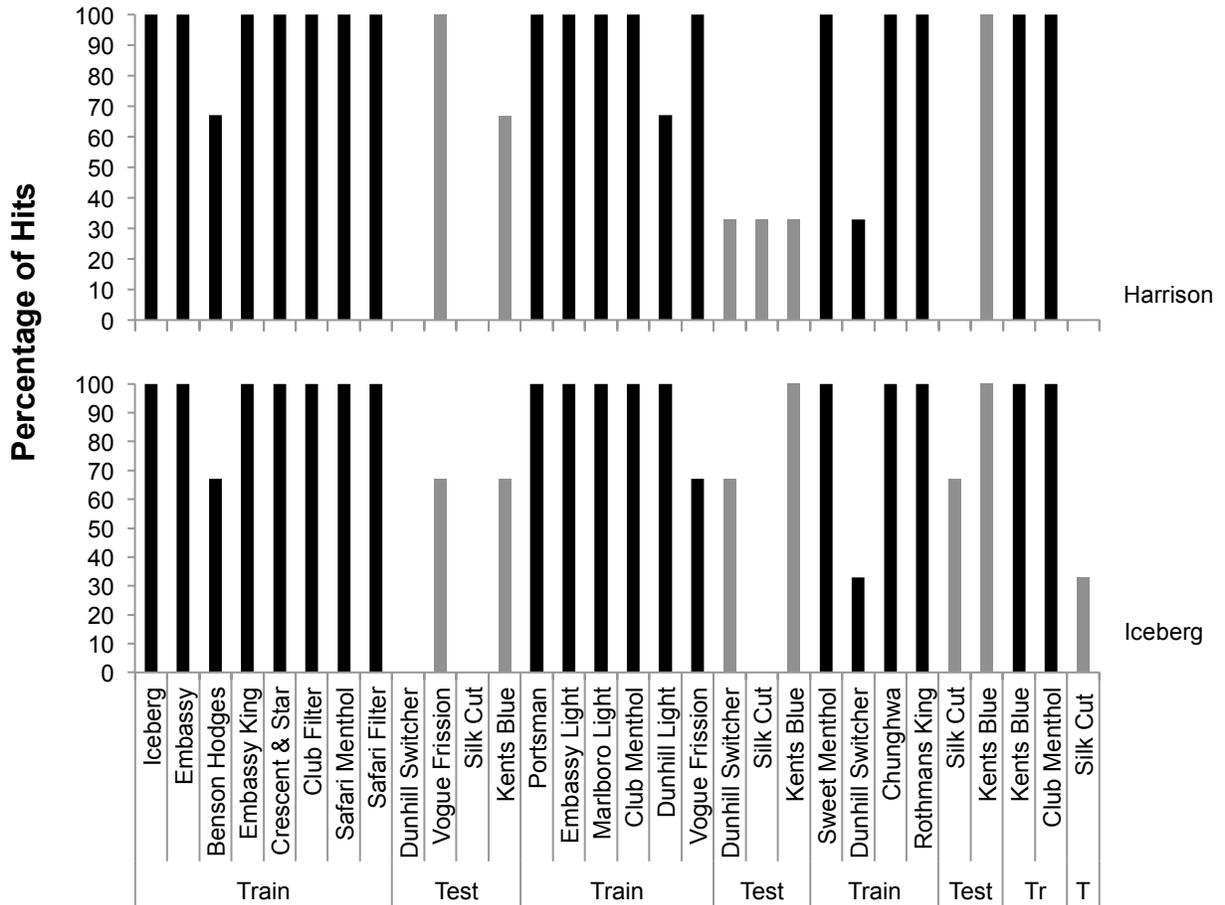


Figure 12. Phase III hit rate results. Percentage of hits on cigarette brands across trained and tested targets for two rats.

DISCUSSION

Cigarettes continue to be smuggled due in part to our inability to detect them, and the adverse health effects of these cigarettes are a growing global concern. This research sought to provide further evidence giant African pouched rats are able to discriminate tobacco filters and to generalize performance to novel cigarette brands.

An active sampling procedure was used in which a vacuum pump pulled air from a source container through a filter for evaluation. This sample preparation method is common in research settings for mine detection, and has been shown to be effective in presenting cigarette odor to rats.

The hit rate for trained cigarette brands remained high following training meeting stability criteria (range, 91% – 100%), in later generalization tests (range, 79% – 95%), and between test sessions (range, 92% – 96%). As the hit rate remained high the false alarm rate remained low following training (range, 2% – 4%), in later generalization tests (2%), and between test sessions (range, 1% – 2%). The hit rates and false alarm rates show clear discrimination between filters with cigarette odor and filters with other non-target odors. During generalization tests 864 novel non-target samples were presented, which resulted in six indications (a hit rate of .7%). The low rate of responding on novel non-target samples shows the rats were responding to similar properties of the novel targets when indicated, not to all novel stimuli.

The number of training sessions required to reach stability differed greatly between phases (range, 6 – 78). The difference in the number of sessions needed to show stability could be a result of the different training criteria in place. In Phase II, the training criteria were more

stringent requiring a stable hit rate and false alarm rate over a minimum of six days. However, the change in stability requirements does not account for the performance variability seen during training. There was an increase in the number of exemplars in Phase II, as well as an increase in the number of sessions. Additional sessions were necessary in order to continue to present two target sources per session while exposing the rats to seven new exemplars. However, the additional training sessions recorded for Phase II surpassed the number of sessions needed to present the new targets. Research with pigeons and humans has similarly shown increasing the number of training exemplars slows acquisition of training stimuli (Wasserman, 2016). There is also evidence to suggest how training stimuli are presented to animals could effect training outcomes. Wasserman, Brooks, and McMurray (2015), arranged concurrent training on categories of pictorial stimuli with pigeons rather than training stimuli in succession, and training and generalization results indicated the task was more demanding for the pigeons. Twice as many presentations of each target source were necessary for stable responding across rats in Phase II when training concurrently.

The number of sessions required to train each cigarette brand, in Phase III, seemed to be related to the disparity between training and test stimuli. New target brands were trained according to their scores in Phase II with higher test scores trained first. Cigarette brands with higher generalization scores required fewer training sessions, while cigarette brands with low generalization scores or scores of zero required twice as many training sessions. Dunhill Switcher was particularly hard to train requiring 78 sample presentations with only two of the five rats meeting criteria. Dunhill Switcher cigarettes contain an added bead of menthol used to alter the flavor of the cigarette while being smoked. This added bead of menthol could have altered the odor bouquet, possibly masking the odorant(s) controlling each rat's behavior.

Research on masking in rats has found a sharp decrease in performance, not a gradual decrease, as soon as the training stimulus is no longer detected within a mixture (e.g., Laing, Panhuber, & Slotnick, 1989). Masking, in part, could also account for generalization performance throughout this study. Performance on novel cigarette brands across phases did not rise above 49%. After training additional exemplars Phase II tests resulted in the highest hit rate (49%), yet rats failed to respond to cigarette-soaked samples from two of the eight novel cigarette brands (Kent Blue and Silk Cut). It is possible many of the cigarette brands tested contained strong novel odor features (e.g., a bead of menthol) blocking the trained odor feature and hence detection.

Testing untrained targets multiple times during Phase III provided multiple generalization scores for each cigarette brand, shown in Table 2. Scores across five generalization tests for Kent Blue show an increase in hit rate as the tests progressed, moving from 0% to 100%. Generally, hit rates increased across all untrained cigarette brands as the number of training stimuli increased. Novel target samples were presented as blind samples during generalization tests and were unrewarded. Rats were exposed to untrained (c.f. novel) cigarette brands multiple times and instead of a decrease in responding, exposures led to an overall increase in responding.

An increase in performance to untrained cigarettes was seen on the second generalization test displayed in figure 5, and across tests in Phase III as seen in Table 2. Generalization test results were not analyzed for statistical significance as it is clear through visual analysis responding was not robust enough to show clinical significance for APOPO's operational purposes. Scent-detection rats employed in illicit cigarette detection operations would be expected to examine thousands of filter samples and to correctly indicate across various cigarette brands with high accuracy. In this study, after training on 21 cigarette brands the rats still failed to respond to Silk Cut (33% hit rate). In the future, researchers could utilize this method of

frequent generalization testing to possibly categorize cigarette brands and arrange for more training for the cigarette brands that show the least generalization.

Herrnstein, Loveland, and Cable (1976) measured independent yet similar responses among a group of pigeons during concept training, and found high levels of concordance among subjects. Concordance for the rats in this research was not calculated, but can be visually analyzed by examining figures 1 – 4. Performance in Phase I across brands varied from 81% (Sweet Menthol) to 2% (Safari Filter) a 79% difference, while performance across rats varied from 52% (R. Myron) to 19% (R. Iceberg) a difference of 33%. Again, performance in Phase II tests varied more across cigarette brands (0% – 100%) than performance across rats (42% – 58%). Performances on cigarette brands across rats compared with individual rat performance across phases seem to indicate similar sources of stimulus control.

It was out of the scope of this research to ascertain the stimulus control that resulted for each rat during concept training. Procedures such as altering the strength of the training stimulus and conducting a component analysis would provide information on which odor feature(s) control a response. If either of the above procedures had been implemented during this research, it is possible we would have a more complete understanding regarding test performance. Without this additional information it is unclear which odor features came to control indication responses and if masking occurred.

Odor is not measured by the physical property of a material, but by the molecules in the air above a material termed vapor. Complex odors consist of multiple volatile chemicals combining to emit vapor bouquet; it is this vapor the rats detect. Cigarettes contain hundreds of chemicals (Bates, Connolly, & Jarvis, 1999) no doubt leading to complex odor bouquets. Discrimination training in this study was conducted without selecting an odor feature to train

rather each rat identified an odor feature that distinguished the target stimuli from the non-target stimuli. Hit rates and false alarm rates clearly show discrimination between cigarettes and non-target odors and modest generalization to untrained cigarette brands. If there were too large of a disparity between training and test stimuli generalization would not be expected. Mahoney et al., (2014) found after training cigarette-soaked filters rats did not generalize to new types of tobacco such as snuff, and posited the disparity between trained and tested stimuli was too great. It is possible the odor of the novel cigarettes in this study were too dissimilar to the trained cigarettes.

Stimulus generalization is valuable as it circumvents the need to present all possible variants of a target odor during training. Finding an odor discrimination method of training that results in robust stimulus generalization to complex odor bouquets is key for humanitarian operations at APOPO. Given the results of Mahoney et al.'s 2014 research on tobacco generalization and the results of this research it is clear tobacco products such as cigarettes comprise complex odor bouquets making it difficult to identify and train all possible exemplars.

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Appendix A
Institutional Animal Care and Use Approval Letter



Western Michigan University
Graduate College
Kalamazoo, MI

12 June 2015

RE: IACUC APPROVAL OF APOPO'S RAT INSTITUTIONAL PROTOCOL

This letter affirms that APOPO's 'Tobacco Discrimination & MDR Training' project has been approved by the organization's Institutional Animal Care and Use Committee. The Institutional Animal Use Protocol Number is 2015-06. 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 7 giant African pouched Rats (*Cricetomys ansorgei*) Will be conducted in accordance with the US National Research Council's 2011 Guide of the Care and Use of Laboratory Animals and applicable Federal regulations.

Prof. Apia W. Massawe
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Appendix B
Session Quality Assurance Checklist

