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## Acquisition of a visual discrimination and reversal learning task by Labrador retrievers

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### Abstract

Optimal cognitive ability is likely important for military working dogs (MWD) trained to detect explosives. An assessment of a dog's ability to rapidly learn discriminations might be useful in the MWD selection process. In this study, visual discrimination and reversal tasks were used to assess cognitive performance in Labrador retrievers selected for an explosives detection program using a modified version of the Toronto General Testing Apparatus (TGTA), a system developed for assessing performance in a battery of neuropsychological tests in canines. The results of the current study revealed that, as previously found with beagles tested using the TGTA, Labrador retrievers (n=16) readily acquired both tasks, and learned the discrimination task significantly faster than the reversal task. The present study confirmed that the modified TGTA system is suitable for cognitive evaluations in Labrador retriever MWDs and can be used to further explore effects of sex, phenotype, age, and other factors in relation to canine cognition and learning, and may provide an additional screening tool for MWD selection.

### Keywords

dog; canine; cognition; visual discrimination

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#### Ethical standards

The current experiment complies with the current laws of the country in which they were performed (US). All experimental protocols were reviewed and approved by the NCSU Institutional Animal Care and Use Committee (IACUC) and the DoD US Army Medical Research and Materiel Command (USAMRMC) Animal Care and Use Review Office (ACURO). NCSU research animal facilities are inspected semiannually by the NCSU IACUC, and the CVM is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC, International).

#### Conflict of interest

The authors declare that they have no conflict of interest.

## Introduction

Service and working dogs (*Canis familiaris*) are used by law enforcement, military, and government agencies for a variety of purposes including explosives and drug detection (Gazit and Terkel 2003; Sinn et al. 2010). Military working dogs (MWD) and more specifically Labrador retrievers are currently used by the United States (US) Marine Corps for the detection of improvised explosive devices (IED). Given the international political state and threat of terrorism in recent years, the demand for the use of MWDs continues to grow, and research into working dog selection and training has increased (Rooney et al. 2007; Sinn et al. 2010).

Procuring and training MWDs is a long and costly process and many dogs that enter training programs fail to meet stringent certification requirements, with extensive individual differences contributing to a working dogs' success reported (Maejima et al. 2007; Rooney et al. 2007; Slabbert and Odendaal 1999; Svartberg 2002). Given the time and money invested in training, the ability to predict a working dog's success is highly valuable. Standardized tests measuring personality traits and behavior have been used to quantify behavioral variability and predict future success in a variety of service and working dogs (Goddard and Beilharz 1986; Slabbert and Odendaal 1999; Svartberg et al. 2005; Wilsson and Sundgren 1998). While some tests have successfully predicted adult performance (Slabbert and Odendaal 1999) and linked certain behavioral traits (e.g., boldness) to performance outcomes (Svartberg 2002), others have demonstrated inconsistent results (Goddard and Beilharz 1986; Maejima et al. 2007; Wilsson and Sundgren 1998). Thus, the need to optimize methods in order to further characterize MWDs and predict later success remains (Rooney et al. 2007; Sinn et al. 2010).

To date, most tests aimed at predicting future working dog performance focus primarily on measuring behavioral traits related to temperament that appear to be desirable or undesirable in a working dog (Goddard and Beilharz 1986; Slabbert and Odendaal 1999; Svartberg 2002). Far less research has investigated the possible roles of cognition, learning, and memory in service dog potential. The ability to learn and solve problems, for example, may influence an MWD's training success and operational performance. Cognitive test batteries assessing domains of physical cognition (e.g., spatial or causal relations) and social cognition (e.g., perception of gestures, intentions, and attentional state) have been used to attempt to elucidate differences in cognitive abilities in several species of primates (Herrmann et al. 2007), and have been adapted to dogs in order to compare their performance to chimpanzees (Lambach et al. 2009). Such cognitive testing protocols may provide a useful additional tool to MWD screening and predictive tests. Thus, the aim of the current study was to assess one domain of cognitive function in Labrador retrievers acquired for a MWD training program. We used a modified version of the Toronto General Testing Apparatus (TGTA), a cognitive testing apparatus developed for neuropsychological evaluations in dogs (Milgram et al. 1994). The TGTA presents dogs with objects of different colors and sizes or of varying spatial location and can be used to assess rate of acquisition of simple discriminations, reversals, conditional discriminations, or delayed non-matching paradigms. Previous studies using the TGTA have revealed differences in performance according to age-related disease states, dietary and behavioral enrichment, and treatment

therapies (see Cotman and Head 2008 for review). The present study aimed to apply the TGTA methodology, most commonly used in neuropsychological research and with a smaller dog breed, to a novel canine population.

## Methods and Materials

### Animals and housing

This study used intact male ( $n = 8$ ) and female ( $n=8$ ; 5 intact and 3 spayed) black- ( $n=10$ ) and yellow-coated ( $n=6$ ) Labrador retrievers, ranging in age from 1–3 years old at the start of testing. Dogs were initially acquired by K2 Solutions Inc. canine training facility in Southern Pines, NC from various field-trial breeding kennels throughout the US for an explosives detection training program. Dogs were transported to an environmentally-controlled indoor canine facility at the North Carolina State University College of Veterinary Medicine (NCSU-CVM) Laboratory Animal Resources (LAR) unit where they underwent a period of adjustment and health monitoring for several weeks before experimental testing began. Dogs were housed in individual, indoor, temperature and humidity controlled kennels (1.5 m x 2.4 m) with solid floors and raised resting platforms. Dogs were fed a balanced canine dry ration twice daily (Iams Mini Chunks, P & G Pet Care, Cincinnati, OH, unless otherwise prescribed), and provided water *ad libitum*. Enrichment was provided by allowing access to hard rubber chew toys (Kong Company, Golden, CO) in the runs, predictable and positive daily human social interactions (Lefebvre et al. 2009), and on-leash and/or off-leash exercise opportunities for a minimum of 15 minutes twice a week.

### Apparatus

The Toronto General Testing Apparatus (TGTA) used in this study (CanCog Technologies, Toronto, Canada) was similar to that developed for beagles (Milgram et al. 1994) with appropriate adjustments in chamber size (66 cm x 178 cm x 91 cm), floor height (64 cm), and ramp (114 cm x 58 cm) made to account for the larger size of the Labrador retrievers. Stainless steel bars separated the dog from where the stimuli were presented, and created openings allowing the dog's head to access the stimuli and obtain food rewards from the wells. The experimenter manipulated a black, sliding plastic stimulus presentation tray with three adjacent wells that was used to hold and present the test stimuli to the dog. The chamber and equipment were lightly sprayed and wiped with a dry cleaning towel in between subjects and then thoroughly washed with a disinfectant solution (Virkon® S, E.I. duPont de Nemours Co., Wilmington, DE) after all subjects completed testing for the day. Stimuli were sprayed with the disinfectant solution and wiped with a dry cleaning towel between each subject. Data were collected using DogCog™ software (CanCog Technologies) on a computer running a Windows 7 interface. The software recorded responses (as indicated by a keystroke from the experimenter), randomized stimulus and reward positions, and controlled trial timing.

### Stimuli

Stimuli included solid white blocks (CanCog Technologies) for object-displacement training (see below) or plastic children's play blocks (MEGA® Bloks, Inc., Montreal, Canada) for

the object approach and object discrimination phases. All objects were mounted onto 10-cm wide white plastic coasters using Velcro® (Manchester, NH, US).

### Cognitive Test Protocol

All subjects underwent a standard pre-training cognitive testing protocol using previously published procedures (Milgram et al. 1994, 2005) with modifications to the number of trials, correction procedure, and performance criteria. Pre-training involved procedural learning tasks (reward approach and object approach) designed to train animals to use their nose to displace an object on a tray and obtain approximately 2 cm of a Pup-Peroni® Original bacon flavor treat (Del Monte Foods, San Francisco, CA) (Milgram et al. 2005). Each task consisted of one or more 21-trial sessions, until pre-training performance criterion was met (9 consecutive trials or 16/21 total trials correct).

A preference test was then conducted in which two objects (3 cm yellow cube and a 9 x 3 x 3 cm blue rectangle) were simultaneously presented for 10 trials such that object position was balanced between the right and left positions. Objects were then designated as either the S+ or S- for the subsequent object discrimination phase depending on an individual dog's preference or a coin flip when a choice preference was not observed. Next, 20-trial object discrimination sessions were conducted five days a week. A tone signaled the start of a trial as the hinged door was opened and the stimulus presentation tray was inserted into the inspection chamber for a 3 s inspection interval. The tray was then fully inserted into the chamber and the dog was allowed 30 s to make a response. Displacing the S+ object revealed a food reward in the well underneath. Responses to the S- were not rewarded and the dog was allowed to then respond to the S+ and obtain the reward on each trial. A food reward was affixed underneath the S- coaster to control for food odor cues. If a response was not made within the 30 s time allotment, the tray was withdrawn, a non-response was recorded, and the next trial began. The software recorded each response as indicated by a keystroke from the experimenter, initiating a 30 s inter-trial interval (ITI) before the next trial began. Object position throughout the session was balanced such that each object was presented on the left and right side an equal number of times, with no more than 3 trials in a row containing the S+ on one side. Criterion was met when the subject responded correctly on 16/20 trials or better in one session, followed by a total of at least 28/40 correct responses over two consecutive sessions. After reaching criterion for the initial object discrimination task, the S+ and S- were switched to test for reversal learning. The procedure was the same as the previous phase except that the object previously designated as the S+ became the S- and vice versa.

### Data Analysis

Total number errors and total number of trials to until criterion was met was calculated. Trials in which a non-response occurred were not counted as correct or incorrect and disqualified the session from counting towards meeting criterion. The data were compared by tests for homogeneity of variance (Levene's test), and a multivariate analysis of variance (ANOVA) that examined the effect of coat color and/or sex as group factors on a test parameter. If the Levene's test was significant, the data were analyzed using a Welch's ANOVA. Statistical analyses were performed using SAS statistical software (JMP 9.0, Cary,

NC). A probability value of 0.01 was used for Levene's test, while  $< 0.05$  was used as the critical level of significance for all other statistical tests. Unless otherwise noted, data presented represent mean ( $\pm$  SEM) values.

## Results

### Pre-training

All dogs ( $n=16$ ) completed all pre-training phases. Individual data for each dog showing number of errors made and total trials until criterion for the reward approach and object approach phases of pre-training are presented in Table 1. Black-coated dogs committed significantly more errors during reward approach learning ( $17.8 \pm 3.21$ ) than did yellow-coated dogs ( $7.67 \pm 2.33$ ) ( $F(1,14)= 6.5, P= 0.04$ ) and required significantly more trials to criterion ( $56.7 \pm 5.47$ ) than yellow-coated dogs ( $31.5 \pm 7.17$ ) ( $F(1,14)= 7.88, P = 0.01$ ).

### Object Discrimination Learning and Reversal

Individual data for each dog showing number of errors made and total trials until criterion for the object discrimination learning and reversal are presented in Table 1. There were no overall effects of sex or coat color on initial discrimination learning trials to criterion or errors made. One dog ('Piper') was removed during the reversal learning phase due to excessive non-response trials and apparent low motivation for the food reward. This dog also required more trials to complete the initial object discrimination task and exhibited non-responses on early trials during pre-training phases. Aside from this exception, all dogs completed the reversal learning task ( $n=15$ ). All dogs committed significantly more errors during the reversal tasks ( $90.27 \pm 8.82$ ) than on the original discrimination task ( $27.9 \pm 3.04$ ) ( $F(1,29)= 47.03, P = 0.0001$ ). The mean number of trials to reach criterion was also significantly greater on the reversal task ( $197.33 \pm 14.3$ ) than the original discrimination ( $124.12 \pm 8.82$ ) ( $F(1,29)= 19.49, P = 0.0001$ ).

## Discussion

The results of the current study demonstrate that the TGTA testing system originally developed for beagles is suitable for testing Labrador retrievers. Qualitative differences were seen when comparing performance of Labrador retrievers in the current study to the performance of beagles of comparable ages on a similar task (Milgram et al. 1994); on average, beagles and Labrador retrievers acquired the object discrimination task in 48 and 124 trials, respectively, and committed 16.5 and 27.93 errors until criterion, respectively. Labrador retrievers also made more errors and required more trials than beagles in the reversal learning task. However, direct comparisons are difficult because of differences in number of trials per session, performance criteria, correction procedures, inter-trial intervals, actual stimuli used, and other procedural variations.

Consistent with previous reports in rats (Dufort et al. 1954; Kendler and Lachman 1958), monkeys (Lai et al. 1995; Rap et al. 1990) and beagles (Boutet et al. 2005), Labrador retrievers required more trials to criterion during reversal learning than the original discrimination. This finding likely reflects the increased difficulty of reversal tasks, which require subjects to inhibit responses to previously rewarded stimuli and shift to a new

stimulus-reward contingency within the same perceptual dimension (Tapp et al. 2003). Thus the increase in errors during reversal tasks is largely due to perseverative responding (Boutet et al. 2005). Additionally, the development of positional responding in which an animal disproportionately preferred one side was seen in several dogs during reversal and typically occurred after a period of perseverative responding to the previously rewarded object (Milgram et al 1994).

We also evaluated whether sex or coat color was related to performance. Significant differences were seen between coat colors in the reward approach phase of pre-training, with black-coated Labrador retrievers requiring nearly twice as many trials to reach criterion and committing more errors than yellow-coated retrievers. No differences of sex or coat color were found in the object discrimination or reversal learning phases. Taking into consideration these inconsistent results and our small group size, it is difficult to draw robust conclusions about the relationship between coat color and learning in Labrador retrievers. Future studies could use age-, sex- and coat color-matched dogs to explore the effect of these factors on canine cognition. Because genetics play a role in canine cognition (Hare and Tomasello 2006), and coat color has been linked to behavioral variability (Houpt and Willis 2001; Kim 2010), it is possible that a relationship also exists between coat color phenotype and cognition. An improved understanding of whether cognitive function is related to coat color or other phenotypic variation may help lead to the identification of genetic loci that are associated with these traits. In addition, variation in personality may affect cognitive performance, and vice versa (Carere and Locurto, 2011). Future studies comparing results of established temperament and personality tests to cognitive performance may shed light on the relationship between animal personality and cognition, an area deserving further investigation (Sih et al. 2004).

Most research aimed at measuring and predicting important qualifying traits in MWDs and other service dogs has focused on behavioral and temperament tests. Cognitive processes such as attention, learning, and memory may also influence the success of a MWD, yet investigations into such possibilities are scarce. The TGTA system used in our study may be an effective cognitive screening tool for Labrador retrievers and other MWDs. Although not within the scope of the current experiment, studies linking performance on these tests with later certification or field performance outcomes may be valuable. Additionally, future directions including testing MWDs on a variety of other cognitive tasks and comparing findings to those of commonly used behavioral predictor tasks may reveal relationships between cognition and behavior important to an MWD's success.

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Table 1

Number of trials and errors to criterion for individual dogs

Dog name	Color	Sex	Reward approach			Object approach			Discrimination			Reversal		
			Errors	Trials	Trials	Errors	Trials	Errors	Trials	Errors	Trials	Errors	Trials	
Acc	B	M	24	63	17	63	16	80	74	140				
Annie	Y	F	5	21	13	42	41	140	149	300				
Baxter	B	M	34	84	24	42	34	140	80	176				
Bullet	Y	M	8	42	26	42	12	80	45	140				
Dakota	B	F	6	42	15	42	17	100	83	200				
Honey	Y	F	5	21	21	63	26	147	85	218				
Hunter	B	M	21	63	20	63	23	100	60	160				
Jimmy	B	F	9	42	18	42	33	120	148	280				
Macks	B	M	6	42	12	42	40	140	67	140				
Mercy	B	F	31	84	10	42	26	140	91	200				
Piper	Y	F	4	21	25	63	57	205	-	-				
Reno	Y	M	5	21	16	42	24	120	56	160				
Rip	B	M	17	42	27	63	40	160	130	240				
Ruby	Y	F	19	63	26	42	17	100	60	140				
Valentine	B	F	9	42	12	42	26	100	134	280				
Wizard	B	M	21	63	21	63	15	100	92	180				
Overall			14.0 ± 2.5	47.3 ± 5.3	18.9 ± 1.4	49.9 ± 2.6	27.9 ± 3.0	123.3 ± 8.2	90.3 ± 8.8	196.9 ± 14.3				
Sex														
		M	17.0 ± 3.6	52.5 ± 6.9	20.4 ± 1.8	52.5 ± 4.0	25.5 ± 4.0	115.0 ± 10.5	75.5 ± 9.3	167.0 ± 11.9				
		F	11.0 ± 3.3	42.0 ± 7.9	17.5 ± 2.1	47.3 ± 3.4	30.4 ± 4.7	131.5 ± 12.6	107.1 ± 13.5	231.1 ± 21.8				
Coat Color														
		B	17.8 ± 3.2 <sup>†</sup>	56.7 ± 5.5 <sup>‡</sup>	17.6 ± 1.7	50.4 ± 3.4	27.0 ± 3.0	118.0 ± 8.1	95.9 ± 9.7	199.6 ± 16.4				
		Y	7.7 ± 2.3 <sup>†</sup>	31.5 ± 7.2 <sup>‡</sup>	21.2 ± 2.3	49.0 ± 4.4	29.5 ± 6.8	132.0 ± 17.8	79.0 ± 18.7	191.6 ± 30.6				

<sup>†</sup> or <sup>‡</sup> Indicate significant difference observed between values with the same symbol ( $P < 0.05$ ). Dashes indicate that this dog did not complete the reversal learning phase due to motivational issues. Coat color denoted as black (B) or yellow (Y); sex denoted as male (M) or female (F)