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The use of a T-maze to measure cognitive–motor function in cats (*Felis catus*)

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Abstract Few tests have been developed to evaluate the cognitive and motor capabilities of domestic cats, despite the suitability of cats for specific studies of neuroanatomy, infectious diseases, development, aging, and behavior. The present study evaluated a T-maze apparatus as a sensitive and reliable measure of cognition and motor function in cats. Eighteen purpose-bred, specific pathogen-free, male, neutered domestic short-haired cats (*Felis catus*), 1-2 years of age, were trained and tested to a T-maze protocol using food rewards. The test protocol consisted of positional discrimination training (left arm or right arm) to reach a predetermined criterion, followed by 2 discrimination reversal tests. The 2 reversal tests documented the ability of the subjects to respond to a new reward location by switching arms of the T-maze. Data were collected on side preference, number of correct responses, and latency of the responses by the subjects. Aided by a customized computer program (CanCog Technologies), data were recorded electronically as each cat progressed from the start box to the reward arm. The protocol facilitated rapid training to a high and consistent level of performance during the discrimination training. This learning was associated with a decrease in the latency to traverse the maze to a mean of 4.80 ± 0.87 seconds, indicating strong motivation and consistent performance. When the rewarded side was reversed in the test phase, the cats required more trials to reach the criterion, as expected, but again showed reliable learning. The latency to the reward in the first session of reversal increased 86% from the first to the last trial, indicating that it may provide a useful index of cognitive processing. Latencies subsequently decreased as the new reversal paradigm was learned. This paradigm provides a relatively rapid and reliable test of cognitive–motor performance that can be used in various settings for the evaluation of feline cognitive and motor function.

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Introduction

There is a paucity of quantitative information available on cognitive and motor function in domestic cats, despite their domestication over millennia and their ubiquity as pets. Although the use of cats in neurobiological studies is well documented, their use in cognitive–motor assessment paradigms is widely viewed as challenging. Sensitive and reliable measures of cognition (Dore et al., 1996) and motor function in cats could provide valid and sensitive end points for studies of feline aging (Levine et al., 1987), diet, and disease states. We have been particularly interested in using cognitive and motor tests to distinguish behavioral effects of feline immunodeficiency virus (FIV), aiding in our understanding of the pathophysiology and pharmacologic management of the disease (Meeker, 2007). For example, FIV serves as an important animal model for human immunodeficiency virus, with neurologic dysfunction observed in both diseases. Despite progress in the development of retroviral treatment agents, cognitive decline remains a persistent and debilitating problem among HIV-infected individuals (Sacktor et al., 2002; Robertson et al., 2007; Moore et al., 2011). However, although of critical importance, early subtle behavioral effects of the disease in cats have not been fully addressed, limiting the ability to investigate early interventional therapies.

Several recent studies have attempted to reveal cognitive and motor abilities of cats, with mixed success. For example, a hole-board test was developed as a spatial memory test for cognitive ability to distinguish FIV-infected from uninfected cats (Steigerwald et al., 1999). A simplified version of the test has also been applied to aging studies but may not be sensitive enough to identify the effects of aging on cognitive function in cats, if these effects exist (McCune et al., 2008). Cats failed to "show causal understanding" in a string-pulling task (Whitt, 2009) or to distinguish 2 from 3 dots in a quantity discrimination test, although alternative explanations were suggested (Pisa and Agrillo, 2009). Feline motor function has been evaluated using a plank-walking test (Steigerwald et al., 1999). This test revealed motor differences between cats infected as kittens with FIV and uninfected controls but did not identify aging effects on motor function in cats (McCune et al., 2008), leading to uncertainty about the sensitivity of the test. More recent tests have used increasingly sensitive measures of cognitive and motor function in FIV-infected cats. Increases in gait width, greater errors in a stepping task, and increased maze completion times in a modified T-maze were found to correlate with inflammatory markers and FIV burden in the central nervous system (Malingat et al., 2009). These studies reveal the potential of behavioral studies to assess neural function in cats but also highlight the need for more sensitive and standardized approaches.

The goal of these studies was to develop a simple, yet sensitive, test that could be used for the assessment of disease-associated cognitive–motor decline as well as the efficacy of novel therapeutic agents. The T-maze has been used as a standard tool for the assessment of cognitive processes (Haley and Raber, 2011), such as spatial memory and associative learning, as well as motor function in many species (from mollusks [Painter et al., 1998] to rats [Carillo-Mora et al., 2009] to primates [Easton et al., 2003]). Levine et al., (1987) used the T-maze to examine the effects of aging in cats. The T-maze has also been used in feline ablation studies to document limitations to sensory discrimination and spatial learning (Norrsell, 1983; Burgess et al., 1986). The objective of the present study was to develop a reliable and sensitive T-maze protocol that could be used to quantify cognitive and motor function in cats.

Materials and methods

Subjects

The subjects were 18 specific pathogen-free, purpose-bred, neutered male domestic short-haired cats (*Felis catus*) aged 1-2 years. The cats were maintained in individual pens (188 cm high, 147 cm deep, 91 cm wide) in a laboratory animal facility on a 12/12-hour light–dark cycle, fed a measured balanced feline dry ration after testing each day, and maintained at body weights consistent with initial body weights and low-to-normal (3/9-4/9) body condition score, as referenced on a standard score chart (Purina Body Condition Score Index, http://www.purina.com/cat/weight-control/bodycondition.aspx). At the time of initial training, all cats were naive to cognitive testing. Housing and test protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Apparatus

Constructed of plywood sealed with polyurethane to conform to laboratory standards, the feline-adapted T-maze was designed by CanCog Technologies (Toronto, ON, Canada) to provide a simple test of cognitive and motor ability (Figures 1 and 2). The outside dimensions of the T-maze were 183 cm × 99 cm, with a height of 77 cm. The maze components included a start box that opened to a runway at the end of which was a decision point, and a left and right reward arm, each leading to a reward area with a reward well where a food reward could be placed. Doors, positioned in each reward arm, were closed to prevent path reversal after the arm choice was made. These doors had magnetic latches that kept the doors open and could be remotely closed by the tester using a switch that released the magnet once the cat had passed. Doors out of and into the start box were guillotine style, operated manually by the tester. Partial wooden panels obscured the view of the reward well until the cat had committed to entering a reward

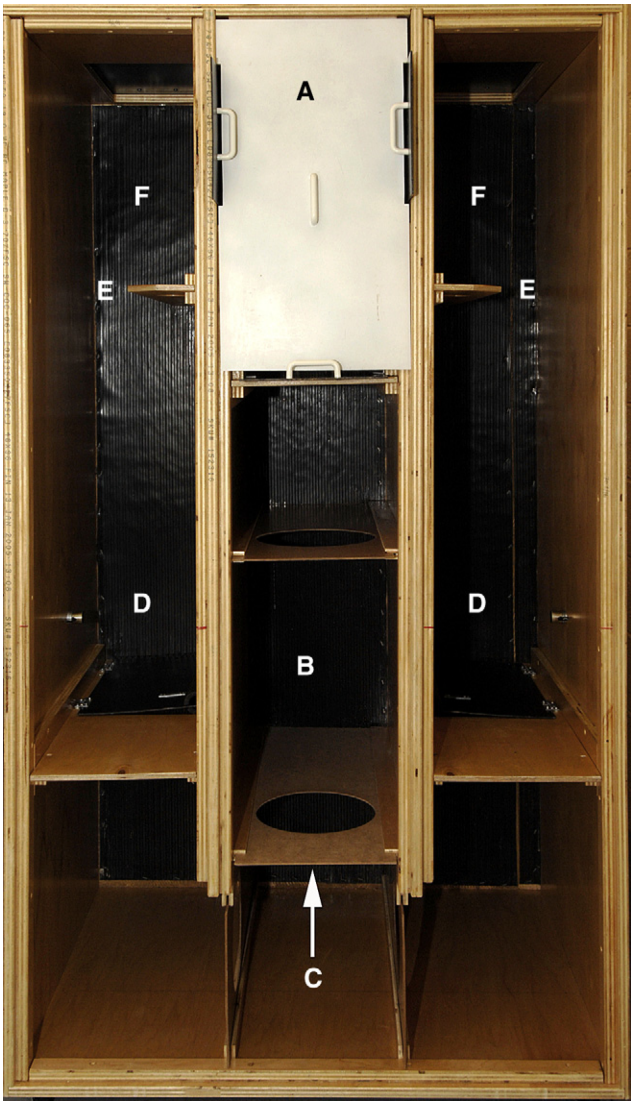

Figure 1 Feline adapted T-maze architecture. Components of the T-maze: A, start box; B, runway; C, decision point; D, left and right reward arms; E, predetermined end point; F, left and right reward area containing reward wells.

Figure 2 Feline subject navigating its way through high hoops in the runway portion of the T-maze.

arm and passed the threshold for door closure. Each reward area was directly connected to the start box. Thus, at the end of each trial, the subjects were able to directly enter the start box from either reward area when a connecting door was raised. Fitted acrylic sheets covered the top of each section of the maze to prevent escape but allowed the animals' behavior to be continuously observed. The tester sat on a stool adjacent to the start box, positioned at the middle point, and could visualize the cat but did not provide cues or interact with the subject. Special vertical tracts permitted the insertion of partial impediments to the path of travel, such as partitions (weaves) or low or high hoops, used to increase motor difficulty after the maze paradigm was learned. A specific computer program (CatCog) was developed by CanCog Technologies to record the number of correct choices and latency to response (in milliseconds). The computer was positioned outside the cat's range of view from within the box. The start and end of the timer as well as the closing of the reward arm doors were manually controlled by the experimenter. The order of testing was randomized daily. Intertester reliability between the 3 trained testers was evaluated regularly during the study using video recordings of tester performance.

Behavioral conditioning

Using food rewards, the cats were conditioned to handling and transport using reward-based training. Transport consisted of voluntary entry into a standard commercial cat carrier, transport to a behavioral test room, and return via carrier after the testing. When the cats were fully conditioned to the carrier and transport, serving as voluntary participants, the T-maze was introduced. In addition, during this process, the cats became familiar with those human individuals who performed the T-maze test protocol. Those individuals did not participate in restraining, anesthesia, surgery, or sample collections, a fact that we consider important in reducing fear responses and optimizing cooperation on the part of the test subjects. Food motivation was high; rations were reduced during testing to induce some hunger but maintain weights within 90% of baseline weights. The cats were trained and tested from 8 AM to 11 AM using highly palatable food rewards (Pounce cat treats, Del Monte Foods, Pittsburgh, PA; Whiskas cat treats, Mars, Inc, McLean, VA; various flavors) and were fed a measured ration of dry chow based on their body weight after testing at 3 PM.

T-maze protocol

The test protocol had previously been developed by CanCog Technologies (unpublished data) and consisted of 6 stages: adaptation, reward approach, preference testing, discrimination training, reversal 1, and reversal 2 (described later in the text). The cats were tested 6 d/wk by technicians who were both familiar to and with the cats during the behavioral conditioning period. The maze was cleaned with a neutral-odor disinfectant (Trifectant, Virkon Corporation, Saugerties, NY) between the sessions and left to air-dry.

Adaptation and reward approach

Adaptation allowed the cats to become familiar with the configuration of the T-maze and to be rewarded for exploratory motor behaviors. The duration of each adaptation session was variable, ranging from 10 to 15 minutes once daily, depending on each cat's responses. Small food rewards were strewn throughout the maze, all doors in the maze were fixed open, and each cat was placed in the maze at the start box. After the initial sessions, as the cat moved through the maze purposefully, the maze doors, including those to the start box, were opened and closed manually by the experimenter to acclimate the cats to the sound and associated air movement. Enough time was allowed for each cat to fully explore the maze each day and receive food rewards from left and right reward arms. The criterion for each cat's completion of the adaptation stage was when the subject would reliably move throughout the maze, from the start box to both reward arms, and ingest treats 10 times in 1 session. The number of days required for this stage was variable owing to the behavioral qualities of each cat: more timid or reactive cats required more time to adapt to the apparatus and the behavioral protocol.

Reward approach involved reducing the number of treats placed in the maze, with treats always present in both reward wells at the far end of the reward arms. As the cat progressed with the process of moving through the maze, the technician began to require the cat to wait in the start box before being released into the rest of the maze to pursue a route to 1 reward well. Eventually, treats were restricted to the reward wells of both arms of the T-maze, and high hoops were positioned in the maze for all subsequent trials. Two high hoops were placed in the runway and 1 high hoop was placed in each arm to increase the motor difficulty of the task. High hoops were 75-cm solid barriers with a 21-cm-diameter round opening, with the bottom of this opening set at a height of 40 cm. These were used in the discrimination training and the reversal tests presented here because we had determined in pilot studies that high hoops increased the motor challenge and significantly affected latency times. Despite the rewards being present in both the reward wells during the reward approach phase, the cats naturally began to show a directional preference, choosing one side more frequently than the other (typically, the first rewarded side). The side preference was not uniform among the cats, with some showing preference for the left side and some for the right side. Completion of the reward approach phase was when the cat would traverse the maze successfully from the start box to either reward arm 10 times in 1 session, with the high hoops in place and food rewards located only in the reward wells.

Preference testing

Following successful completion of the reward approach stage, each cat had 1 day of preference testing to determine its preferred side. This was established empirically as the side that a cat went to ≥ 6 times during 1 session of 10 trials when both arms contained rewards. A contingency was planned for the cats that did not show a preferred side (5/5 split) such that their preferred side would be determined by a 2/3 coin toss; however, this was not needed. Each cat's preferred side was used by default as the first rewarded side in the discrimination training to facilitate and standardize the initial reward training. Using each cat's preferred side allowed us to establish a strong response pattern before the introduction of reversal 1 and reversal 2 tests. Establishing consistent performance was an important consideration because individual variation in performance is often a limiting variable in attempts to establish reliable test paradigms.

Discrimination training and reversal 1 and reversal 2 tests

The general protocol for discrimination training and reversal 1 and reversal 2 tests was as follows: the test cat was positioned in the start box, and the tester started the software timer the instant the cat was released (when the door out of the start box was opened) and then stopped it the instant all 4 feet of the cat crossed a predetermined point in either reward arm (Figure 1E). When stopped, the software began a 30-second intertrial interval, which allowed the cat time to ingest the reward and return to the start box (Figure 1A), and the tester to reset the rewards in the reward arms. To control for auditory cues, the tester lifted the doors on both the reward arms, placed a reward into the empty reward well, and then closed both the doors. Each cat completed 10 trials (1 session) per day. On each day of the testing, the cats were rewarded on only 1 side of the maze for the entire session of 10 trials. After the first error in side choice, the cats were allowed to traverse the maze to the other (rewarded) side; however, subsequent errors were not followed by an opportunity to correct direction, and the reward arm doors were closed. Latency was recorded by the proprietary software (CatCog) as the time from opening the door to the start box until the backlegs passed the threshold of the reward arm door. The cats had 60 seconds to complete the maze, or the results were recorded as a non-response for that trial. These trials were not included in the latency calculations. For analysis, a ceiling of 20 seconds was placed on the latency measure to minimize skewing of the data. During the discrimination training, the reward was located on the cat's preferred side, and this was then alternated for reversal 1 and reversal 2 stages.

To assure consistency in performance while leaving some flexibility for daily variation, the cats were tested for a minimum of 4 days, and a criterion of 21/30 correct responses on 3 consecutive days was used to advance to the next phase. This was because pilot studies indicated that an individual cat's performance may be variable from session to session. For example, 10/10 correct responses on 1 day may be followed by 8/10 on a subsequent day. Thus, the criterion for the completion of each stage was 9/10 or 10/10 correct responses on 1 day or 8/10 correct responses on 2 consecutive days, followed by 21/30 correct responses on the following 3 consecutive days. After the discrimination training was completed, reversal 1 stage was initiated by placing the reward in the opposite T-maze arm. Reversal 1 was followed by the reversal 2 phase, with the reward returned to the original preferred arm of the T-maze. For each cat, the following measures were collected: preferred side (left or right), number of correct responses, number of trials to reach the criterion, and latency to the reward arm (in milliseconds).

Statistical analysis

Summary statistics (mean ± standard error of the mean [SEM]) were calculated for all cats for the percentage of correct responses per session, the number of trials to reach the criterion, and the latency for each phase of testing (discrimination training and reversal 1 and reversal 2 phases). Descriptive statistics (mean ± SEM) were calculated using Excel worksheets (Microsoft, Redmond, Washington) and GraphPad Prism (GraphPad Software, Inc., San Diego, California) statistical and graphics software. The data were evaluated for normality using both the Kolmogorov–Smirnov and D'Agostino and Pearson omnibus normality tests. Nonparametric statistics were used for data failing both tests. Changes in performance were assessed using a within-subjects repeated-measures design. Changes in performance during each of the 2 reversal tests were compared with those during discrimination training based on the mean number of trials to reach the criterion and a 1-way analysis of variance (ANOVA) across discrimination training and reversal 1 and reversal 2 sessions. Session latencies were analyzed using a 2-way repeated-measures ANOVA to assess changes in running speed within sessions and across conditions (discrimination training and reversal 1 and reversal 2 phases). A *t* test was used to compare the mean latency during discrimination training with that during the preceding preference testing as well as for comparing the latencies at the beginning and end of the first reversal sessions. In addition, the latency changes during the first sessions of the reversal phases were evaluated using a regression analysis of latency versus time to determine whether the slopes were non-negative.

Results

Adaptation and preference testing

Eighteen cats were trained to the T-maze. All successfully completed the training and efficiently traversed the T-maze with high hoops. Adaptation time varied between cats, with 4-14 days required to begin the formal testing (preference test); mean adaptation time (±SEM) was 7.8 (±0.8) days. During preference testing, as a group, the cats failed to show a consistent side preference, with left and right preferences equally split (9/18). However, for individual cats, the side preference was relatively strong, with a mean of 8.78 ± 0.33 responses to the preferred side (*t* test, $t = 11.45$, $n = 18$, $P < 0.001$ relative to chance). However, when we compared the side preference with the choices made during the adaptation phase, there was only a weak relationship, with 11 of the 18 cats showing the same side preference and an r^2 of 0.0897 ($P = 0.227$) for the regression of adaptation side preference onto the results of the preference test. This suggested that most cats did not have a strong intrinsic preference to a particular side. The preferred side for 13 of the 18 cats was on the same side as that in the first rewarded trial, suggesting that they may simply continue with the first rewarded response. The mean latency to pass the reward gate was 7.67 ± 0.57 seconds across all trials within the preference test session.

Discrimination training and reversal 1 and reversal 2 phases

Figure 3 illustrates the average number of correct choices of 10 trials for all cats in each session of the discrimination training (Figure 3A) and reversal 1 (Figure 3B) and reversal 2 (Figure 3C) phases. The cats rapidly transferred from the preference session to the discrimination training, showing a mean of 9.18 ± 0.24 correct responses of 10 within the first session. Performance accuracy remained consistently high in the subsequent testing. The discrimination criterion was reached in a mean of 4.22 ± 0.13 sessions (10 trials per session), with a range of 4-6 sessions (minimum, 4 sessions).

After reversal of the reward to the opposite arm (reversal 1), response accuracy dropped to a mean of 1.82 ± 0.43 correct responses in the first session. However, the cats

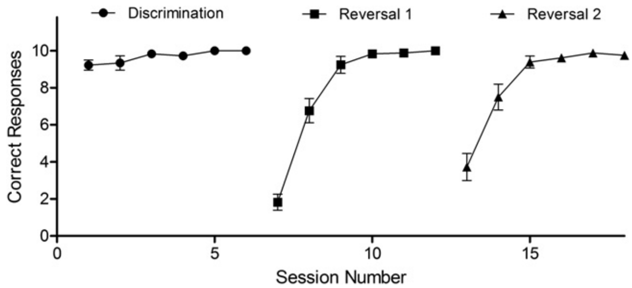

Figure 3 T-maze acquisition curves for discrimination training and reversal 1 and reversal 2 sessions for 18 cats. The X-axis represents the session number, and the Y-axis represents the mean (±standard error of the mean) number of correct responses per session of 10 trials for all cats.

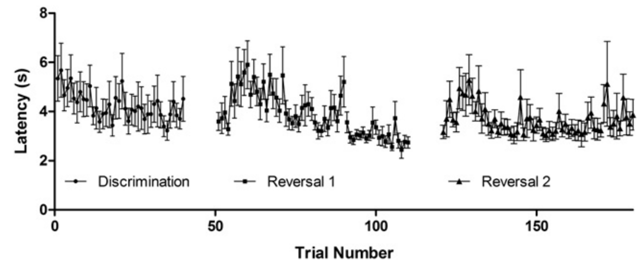

Figure 4 Latency to T-maze arm choice for each trial during discrimination training and reversal 1 and reversal 2 sessions for 18 cats. The X-axis represents the trial number (10 trials per session), and the Y-axis represents the mean (±standard error of the mean) latency (in seconds) to correct choices for each trial.

quickly adapted to the switch in the reward side, showing an average of >90% correct responses by the third session. During the reversal 1 session, the cats took an average of 5.94 ± 0.23 sessions to reach the criterion. A similar pattern was observed during the reversal 2 phase, with the cats taking an average of 5.61 ± 0.20 sessions to reach the criterion. These data did not pass the tests of normality and were compared using a Friedman nonparametric ANOVA. When compared with the discrimination training, the reversal paradigm resulted in a significant increase in the number of trials required to reach the criterion ($P < 0.0001$, n = 18, 3 groups, with Dunn multiple comparison test of reversal versus discrimination, $P < 0.05$). In both cases, the reversal paradigm provided excellent reproducible learning curves.

Response latency

T-maze latency provided an independent measure of cognitive processing, with excellent sensitivity. In almost all cases, the latency data passed the normality test, and parametric statistics were used to evaluate the changes unless otherwise indicated. Latency decreased from an average of 6.86 ± 1.01 seconds during the preference testing to an average latency of 4.80 ± 0.47 seconds for the discrimination training as the cats became more experienced with the maze, a significant decrease of 2.1 seconds (paired t test, $t = 2.60$, n = 18; $P = 0.0188$). Figure 4 illustrates the average latency for each trial across the discrimination training and reversal 1 and reversal 2 conditions. Two-way ANOVA across the groups and the 4 matched sessions was applied. A significant effect of the session latencies over time (repeated measures) was found ($F = 6.421$, degrees of freedom [df] = 3, $P = 0.004$), indicative of a small but continuous decrease in latencies from session to session. No significant effect was observed across the conditions ($F = 1.302$, $df = 2$, $P = 0.281$) or interactions ($F = 0.705$, $df = 6$, $P = 0.646$), indicating that the average latencies and patterns were relatively stable within each condition. However, during the reversal 1 phase, the average latency increased 86% over the first session of 10 trials from an initial fast response time of 3.59 ± 0.38 seconds to 6.69 + 1.22 seconds by the end of the session, suggesting a delay in response time as the cats began to respond to the reversal. By session 5 of the 10 of the reversal phase, average latency was again consistent across the session and reduced to 3.14 ± 0.27 seconds, with a 98.8% arm choice accuracy. During the reversal 2 phase, a similar pattern was observed, with latency increasing from a mean of 3.17 ± 0.28 seconds in the first trial of the session to a mean of 4.64 ± 0.72 seconds in the last trial. Again, by session 5, the cats responded quickly (mean, 3.34 ± 0.44 seconds) and accurately (98.8% correct). To evaluate the potential significance of this trend, we performed a linear regression of latency versus time for each initial reversal session. In each case, the slope of the regression line was significantly nonzero (reversal 1, $F = 8.02$, $P = 0.0052$; reversal 2, $F = 5.426$, $P = 0.0210$). The increase was confirmed by comparing the latency of the first trial with the latency of the last. The latencies for the last individual trials did not pass the normality criteria, and the change was evaluated by the Wilcoxon signed rank test, which was significant for the reversal 1 phase ($P = 0.0069$) but not reversal ($P = 0.0894$).

Although response latencies were generally short, consistent response patterns were occasionally interrupted by a trial with an unusually long latency, often characterized by the subject becoming stationary and exhibiting grooming behavior. Although infrequent, the magnitude of the long latency times contributed disproportionately to the latency variation observed across all trials. The influence of the long latency times can be observed in the trial-by-trial variation in Figure 3. These long latencies were relatively rare: latencies of 4.5% of all trials were >10 seconds and 1.4% of all trials reached the 20-second limit. Across all trials, the average latency was 4.07 seconds (standard deviation, 2.97 seconds; upper 95% confidence limit, 9.90 seconds). By this criterion, a latency of >10 seconds for any individual cat deviated significantly from the normal latency to run the maze. These deviations were distributed across all sessions (discrimination training and reversal 1 and reversal 2 stages) but decreased with increased exposure to the maze, not with difficulty of the task. The long latencies (>10 seconds) were distributed as follows: 16 per session for discrimination training, 9 per session for reversal 1, and 4 per session for reversal 2.

Discussion and conclusion

The findings presented here confirm that cats can be trained successfully on an adapted T-maze that combines both motor and cognitive components. A unique feature of the T-maze design was that the start box was physically connected to the goal box, which allowed repeated testing without having to remove the animal from the maze until testing was complete. After conditioning, individual cats were trained to move from a start box to a decision point, and when the correct arm was chosen, to obtain a food reward. After following a specific training program, all cats (n = 18) successfully reached the criterion for completion in discrimination training and reversal 1 and reversal 2 sessions. Although there was variation in the pattern and rate of learning between cats, the initial discrimination training was rapid, and the group standard errors were low (3%-4% of the mean), allowing for the sensitive assessment of changes in the rate of acquisition in subsequent tests.

Use of the cat's preferred side as the initial rewarded side during discrimination training facilitated consistent and rapid acquisition of the task and provided an equivalent starting point for all cats. The decrease in latency and the strong performance during discrimination training indicated that learning had taken place. The cats' initial side preference did not persist during reversal 1 and 2 phases. During reversal 1 and 2 sessions, the cats learned new sides easily and efficiently, suggesting that the side preferred during preference testing was not an intrinsic bias.

The cats' speed of moving through the T-maze became rapid and relatively consistent by the time the discrimination training was initiated. In each condition, the latencies increased in successive sessions, but the pattern and average latencies were similar between each condition, indicating that performance had stabilized by the beginning of the critical assessments in the reversal sessions. The significant increase in the latencies over the first sessions of the reversal 1 2 stages, although not a primary variable, suggested that the initial response to reversal may be an important parameter sensitive to cognitive processing for further evaluation in subsequent studies. However, one difficulty was the appearance of occasional trials in which the cats appeared to be distracted from the T-maze task. For example, a cat with consistent latencies of 3-6 seconds during 9 of 10 trials in 1 session would display a single trial with a latency that was 3-4 times greater than the mean of the other trials. We were unable to identify any environmental or behavioral phenomena to explain this inconsistency. Although these "distracted trials" constituted <5% of the total trials, they contributed disproportionately to the individual trial variability shown in Figure 4. Further analysis of these distracted trials is warranted to determine whether these trials might reflect attention deficits or responses to specific stimuli.

A limitation of the T-maze is the difficulty in controlling for olfactory cues, either food odors or feline scent trails, that may be present as the cat runs through the maze. Although the odor of the food reward in the reward area could theoretically influence the cat's decision, the fact that the cats showed a directional preference when both arms were baited (Adaptation) and did not immediately choose the side with the reward during reversal learning suggests that this is not the case. It is possible that the cats' own trail through the maze could provide odor cues for themselves. However, during reversal learning, the cats changed direction during a session as they learned the new direction. The accuracy of the responses and an excellent learning curve (Figure 3) make olfactory signaling less likely as a confounding explanation. Despite the unlikelihood of olfactory cuing during the testing, in future studies, an additional safeguard would be to place a small amount of a food reward hidden underneath the reward well on the nonrewarded side. It is unlikely that cats followed the trails of other cats, as the rewarded side varied between cats, and the maze was cleaned between cats and between days.

Decreasing fear responses and behavioral inhibitions were critical to improving motivation, leading to a more reliable performance in the T-maze. Although predators, cats exhibit many behavioral adaptations consistent with a prey species, including increased motor behaviors in novel environments, flight reactions to noise and disturbance, and avoidance responses to unfamiliar individuals (Bradshaw, 2002). The extent of such responses varies from cat to cat. The protracted conditioning phase of our protocol, including establishing positive experiences with individual technicians, was critical to successful testing. In addition, individually customizing the adaptation and reward approach phases of the T-maze was designed to decrease escape responses that could interfere with testing performance.

In all studies of feline cognitive and motor function, maintaining the attention and reward motivation of the feline subjects is an important consideration in data interpretation. The cats in our study were highly motivated by the food reward and showed no signs of satiation over the course of a 10-trial session. Latencies continued to decrease throughout the testing, with times of 3-4 seconds typical of the trials at the end of the reversal 2 session. By testing in the morning and then adjusting dry rations for subsequent feeding, each cat could be tested while maintaining a relatively stable body weight.

In conclusion, this study presents a novel sensitive method of evaluating cognitive and motor function in cats. The adapted T-maze, as presented here, may be applied to studies of feline aging, disease states, and therapeutics to assist the development of new treatment strategies.

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References

Bradshaw, J.W.S., 2002. The Behaviour of the Domestic Cat. CAB International, Wallingford, UK.

Burgess, J.W., Villablanca, J.R., Levine, M.S., 1986. Recovery of functions after neonatal or adult hemispherectomy in cats. III. Complex functions: open field exploration, social interactions, maze and holeboard performances. Behav. Brain Res. 20, 217-230.

Carillo-Mora, P., Giordano, M., Abel, S., 2009. Spatial memory: theoretical basis and comparative review on experimental methods in rodents. Behav. Brain Res. 203, 151-164.

Dore, F., Fiset, S., Goulet, S., 1996. Search behavior in cats and dogs: interspecific differences in working memory and spatial cognition. Anim. Learn. Behav. 24, 142-149.

Easton, A., Parker, K., Derrington, A.M., Parker, A., 2003. Behaviour of marmoset monkeys in a T-maze: comparison with rats and macaque monkeys on a spatial delayed non-match to sample task. Exp. Brain Res. 150, 114-116.

Haley, G.E., Raber, J., 2011. Spatial learning and memory in animal models and humans. In: Animal Models of Behavioral Analysis. Neuromethods series Vol. 50, 91-109.

Levine, M.S., Lloyd, R.L., Fisher, R.S., Hull, C.D., Buchwald, N.A., 1987. Sensory, motor, and cognitive alterations in aged cats. Neurobiol. Aging 8, 253-263.

Malingat, F., Vivithanaporn, P., Zhu, Y., Taylor, A., Baker, G., Pearson, K., Power, C., 2009. Neuorobehavioral performance in feline immunodeficiency virus infection: integrated analysis of viral burden, neuroinflammation, and neuronal injury in cortex. J. Neurosci. 29, 8429-8437.

McCune, S., Stevenson, J., Fretwell, L., Thompson, A., Mills, D.S., 2008. Ageing does not significantly affect performance in a spatial learning task in the domestic cat (*Felis silvestris catus*). Appl. Anim. Behav. Sci. 112, 345-356.

Meeker, R.B., 2007. Feline immunodeficiency virus neuropathogenesis: from cats to calcium. J. Neuroimmune. Pharmacol. 2, 154-170.

Moore, D.J., Letendre, S.L., Morris, S., Umlauf, A., Deutsch, R., Smith, D.M., Little, S., Rooney, A., Franklin, D.R., Gouaux, B., Leblanc, S., Rosario, D., Fennema-Notestine, C., Heaton, R.K., Ellis, R.J., Atkinson, J.H., Grant, I., 2011. Neurocognitive functioning in acute or early HIV infection. J. Neurovirol. 17, 50-57.

Norrsell, U., 1983. Unilateral behavioural thermosensitivity after transection of one lateral funiculus in the cervical spinal cord of the cat. Exp. Brain Res. 53, 71-80.

Painter, S.D., Clough, B., Garden, R.W., Sweedler, J.V., Nagle, G.T., 1998. Characterization of *Aplysia* attractin, the first water-borne peptide pheromone in invertebrates. Biol. Bull. 194, 120-131.

Pisa, P.E., Agrillo, C., 2009. Quantity discrimination in felines: a preliminary investigation of the domestic cat (*Felis silvestris catus*). J. Ethol. 27, 289-293.

Robertson, K.R., Smurzynski, M., Parsons, T.D., Wu, K., Bosch, R.J., Wu, J., McArthur, J.C., Collier, A.C., Evans, S.R., Ellis, R.J., 2007. The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS 21, 1915-1921.

Sacktor, N.C., Degaonkar, M., Herskovits, E., et al., 2002. Magnetic resonance spectroscopic imaging in individuals with HIV-associated cognitive impairment initiating highly active antiretroviral therapy: correlation of metabolite concentrations with clinic, virologic, and immune activation marker. Neurology 58, A405-A406.

Steigerwald, E.S., Sarter, M., March, P., Podell, M., 1999. Effects of feline immunodeficiency virus on cognition and behavioral function in cats. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 20, 411-419.

Whitt, E., 2009. Domestic cats (*Felis catus*) do not show causal understanding in a string-pulling task. Anim. Cogn. 12, 739-743.

Purina Body Condition Score Index. Available at: http://www.purina.com/cat/weight-control/bodycondition.aspx. Accessed February 1, 2011.