A Tactile-visual Conditional Discrimination Task for Testing Spatial Working Memory in Rats

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[Abstract] This protocol describes a novel dual task comparison across two variants of a tactile-visual conditional discrimination (CD) T-maze task, one is dependent upon spatial working memory (SWM; CDWM) and the other one (CD$_{STANDARD}$) is not. The task variants are equivalent in their sensory and motor requirements and overt behavior of the rat. Therefore, differences between the two task variants in the dependent variables such as choice accuracy, neural firing patterns, and the effects of pharmacological or optogenetic inactivation in brain regions of interest can be attributed to SWM, ruling out confounding sensorimotor variables, such as tactile, visual and self-motion cues. The CDWM task protocol is published in Hallock *et al.,* 2013b and Urban *et al.*, 2014.

Keywords: Spatial working memory, Conditional discrimination, T-maze, Encoding, Retrieval

[Background] Our laboratory is interested in exploring the neural mechanisms of working memory. Therefore, we have developed a task that can be used to assess spatial working memory (SWM) ability in rats. Working memory is defined as holding a limited amount of information 'online' so that the information can be used or manipulated to guide goal-directed behavior (Baddley, 1992). Because rodents are naturally inclined to forage for food, they are excellent models to use to probe SWM. Our laboratory has developed and used a conditional discrimination (CD) T-maze task in which floor inserts that vary in texture and color serve as conditional cues for the rewarded goal arm (Griffin *et al.*, 2012; Hallock and Griffin, 2013; Hallock *et al.*, 2013a; Shaw *et al.*, 2013; Hallock *et al.*, 2016). For example, the rats learn to choose the left goal arm if they encounter a mesh insert and the right goal arm if they encounter a wooden insert. To discriminate between the inserts, rats can use visual information (black vs. light brown), tactile information (rough mesh vs. smooth wood), or a combination of both types of information. Because the insert covers the entire floor of the maze and is available when the rat makes a goal-arm choice, this task does not require SWM. More recently, we have developed a working-memory variant of the task (CDWM; Hallock *et al.*, 2013b; Urban *et al.*, 2014). In this variant of the task, the floor insert cues extend only halfway up the central arm of the maze and are not available when the rat makes a goal-arm choice, thus requiring the rats to hold the cue in mind for a brief period of time in order to make a correct choice and receive food reward. In an ongoing experiment, we have found that it is possible to train rats on both variants of the task, giving us a powerful way to identify behavioral correlates of SWM while ruling out confounding sensorimotor variables such as visual, tactile, and self-motion cues.

Materials and Reagents

1. Male Long Evans Hooded (Harlan, Indianapolis) rats, weighing between 250 g and 500 g upon arrival (approximately 90 days of age)
2. Chocolate Sprinkles are used for food reward. Our lab uses the Chef's Quality brand
3. 70% ethanol for cleaning the maze between daily training sessions

Equipment

1. Wooden T-Maze that consists of a central stem (117 x 10 x 5 cm), two goal arms (56.5 x 10 x 5 cm) and two return arms (112 x 10 x 5 cm). The floor of the maze is covered with *Roppe* black vinyl (3 mm thick) (Figure 1)
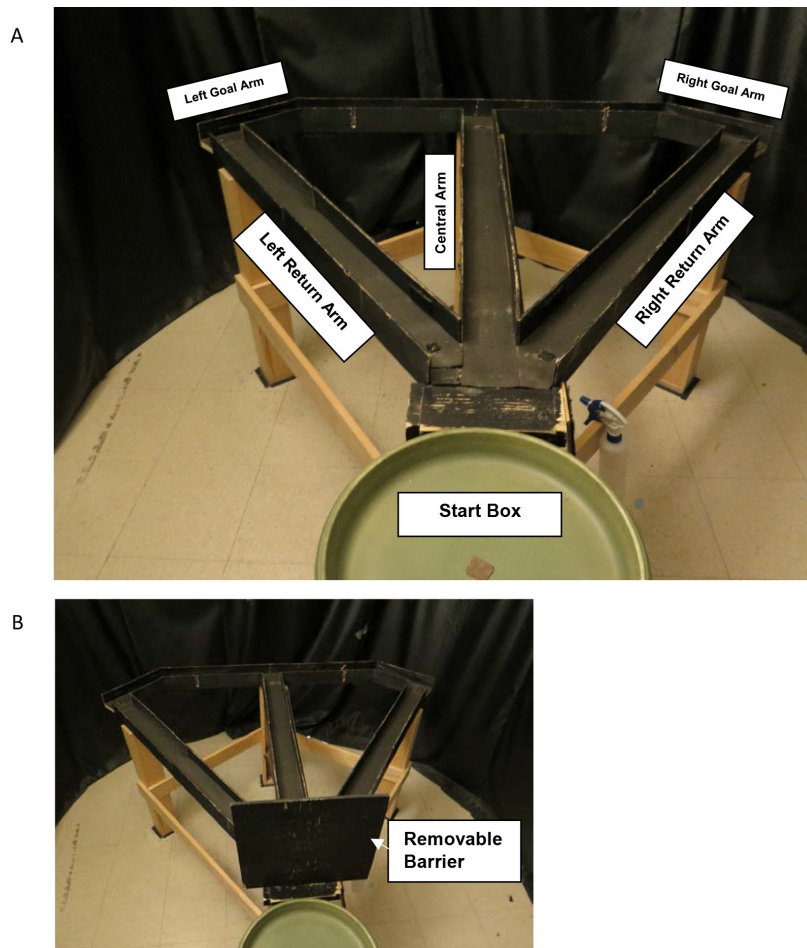   *Note: The T-maze was custom-built by members of our lab.*

Figure 1. T-Maze used for both variants of the CD task shown with (A) and without (B) the removable barrier is used to confine the rat to the start box during the intertrial interval

2. A wooden stool (height: 69 cm) with a plastic saucer (diameter: 38 cm) attached to the seat

serves, as the start box, is positioned at the base of the maze. The start box is separated from the maze by a removable 6-cm tall wooden barrier

3. Three removable wooden floor inserts covered with black plastic mesh on one side and smooth wood on the other serve as conditional cues (Figure 2). The black mesh was glued to one side of the inserts with superglue, and consisted of 4 x 4 mm mesh squares. For the CDWM variant of the task, the central arm insert (74 x 8 cm) extends halfway from the start box to the T-intersection. For the CD$_{STANDARD}$ variant of the task, the central arm insert (117 x 8 cm) covers the entire length of the central arm from the start box to the T-intersection. The three goal arm inserts, one large insert (61 x 8 cm) and two small inserts (26 x 8 cm) are placed at the ends of the goal arms next to the reward cups
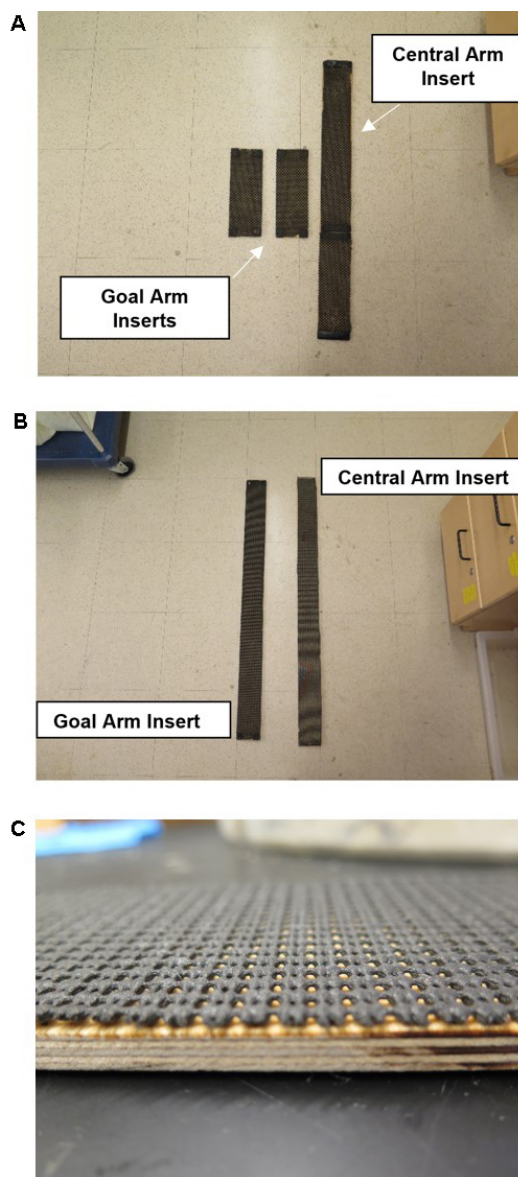

Figure 2. Removable wooden inserts shown mesh side up used for the CDWM (A) and CD$_{STANDARD}$ (B) variants of the task, and close up view of the insert (C)

4. Black curtain, surrounding entire behavior room, 51 cm away from the maze
5. Distal cues taped to black curtain, 142 cm from the floor, located behind the start pedestal, left and right reward cups (Figure 3). The cues are a pink triangle (38.1 x 29.2 cm), a red X (40.6 x 35.6 cm) and a blue cube (43.2 x 41.9 cm) made out of colored tape
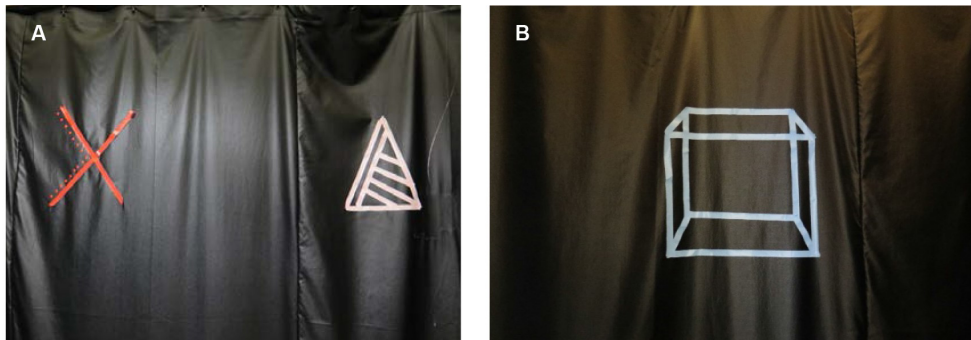

Figure 3. Distal cues taped to the curtains that surround the maze above the reward cups (A) and above the start box (B)

6. Plastic cups (3 cm diameter; 1 cm depth) located at the end of each goal arm where chocolate sprinkles (4-5 pieces) are delivered. The caps were 20 oz. water/soda bottle caps
7. One 60 W incandescent lamp attached to the curtain track of the back middle wall, near the ceiling. The lamp is facing up towards the ceiling, therefore the room is dimly lit with no direct lighting on any portion of the maze

Procedure

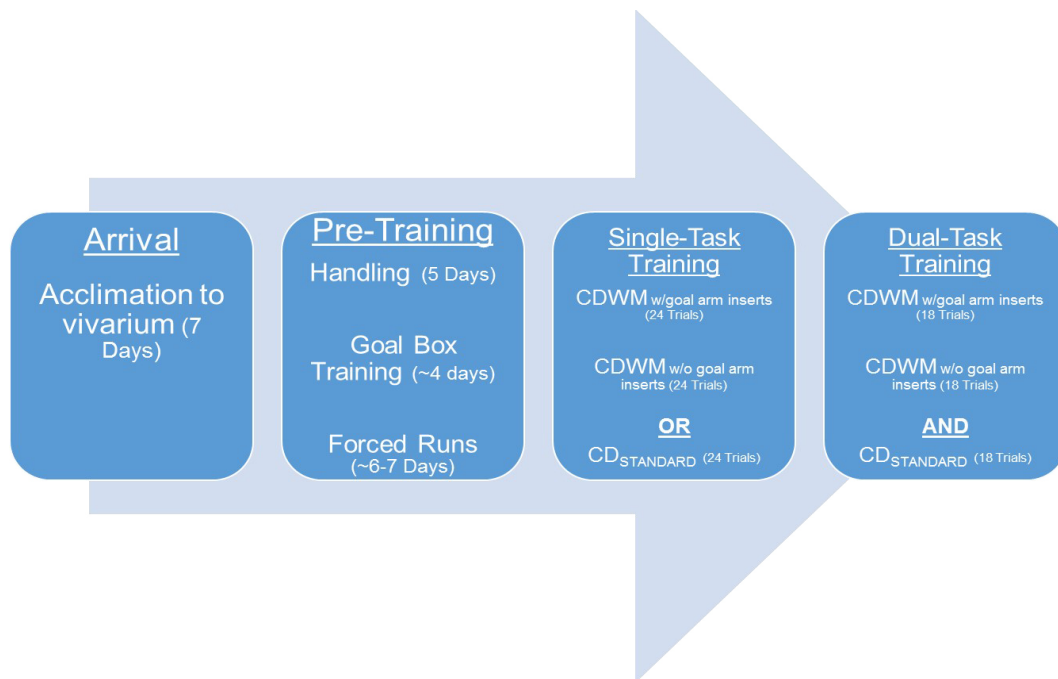See Figure 4 for a flowchart of the experimental procedures.

Figure 4. Flowchart of the experimental procedures, starting with acclimation of the rats to the vivarium through dual-task training

1. Rats are housed 2-3 per cage in a temperature and humidity-controlled animal vivarium and kept on a controlled light cycle (7:00 AM-7:00 PM). Rats are given *ad libitum* access to food (Prolab RMH 3000 rat chow pellets) and water during their acclimation to the colony room.

2. After a 7-day acclimation period in the vivarium, rats are individually housed and brought to the laboratory daily for 5-7 days and handled for 15 min per day by experimenter. The experimenter handles the rat by placing a diaper over their lap and placing the animal on top of the diaper, allowing the animal to move freely. After handling, the experimenter places a plastic cup of chocolate sprinkles in the home cage. The rats are given 20 min to eat the sprinkles, and after 20 min the cup is removed. Rats are then put on food restriction (4-5 food pellets daily) to maintain them between 80 and 90% of their free-feeding body weight and given *ad libitum* access to water. Rats are food restricted starting at pre-training (*i.e.*, the handling period) until the end of testing. Starting weight is recorded on the first day of handling and rats are weighed weekly for the duration of the experiment.

3. Next, rats undergo goal box training. In this phase, rats are brought to the behavior room where the T-maze is located. The animals are confined to the goal arms on the maze and allowed to eat chocolate sprinkles from the reward cups for a total of 6 daily trials (3 per goal arm). A single trial has a duration of 90 sec with an ITI of 10 sec. The trial is terminated early if the animal consumes the food reward in less than 90 sec. Once rats consume the reward in less than 90 sec on every trial for two consecutive sessions, they progress to forced-run training. It typically takes 3 to 5 days for animals to reach goal box training performance criterion.

4. Forced run sessions consist of 12 trials per day, with 6 left and 6 right trials given in a

pseudorandom sequence. Prior to each trial, the experimenter places a removable wooden barrier at the entrance of one of the goal arms. At the start of the trial, the rat is confined to the start box with a second wooden removable barrier. The forced-run trial begins when the start box barrier is removed. The rat is encouraged to run from the start box, down the central arm, turn down the available goal arm, consume the reward, and return to the start box via the return arm. The rat is discouraged from turning around at any point on the maze by blocking his path with wooden stick. This path correction procedure is only necessary on early training sessions after which the experimenter interacts with the rat as minimally as possible. Once rats consume reward on 80% of forced run trials for 2 consecutive sessions, they progress to the single-task phase of training. Forced run training typically lasts for 6 to 7 days.

5. Rats are trained on the CDWM task (Figure 5) or the CD$_{STANDARD}$ task (Figure 6). The starting task variant is counterbalanced between rats, resulting in two groups; the CDWM group and the CD$_{STANDARD}$ group.
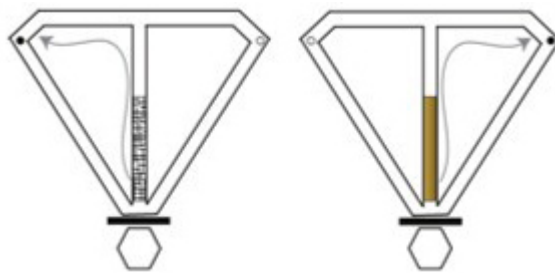

Figure 5. Schematic of CDWM task. Removable wooden insert with plastic black mesh on one side and smooth wood on the other, line the first half of the central arm. See Video 1.

Video 1. Example of three trials (2 mesh trials followed by 1 wood trial) of the CDWM task. The experimenter sham-flips the insert between the two mesh trials and flips the insert between the mesh and wood trial. She then places the food reward in one of the food cups and places the baited cup at the end of the correct goal arm.
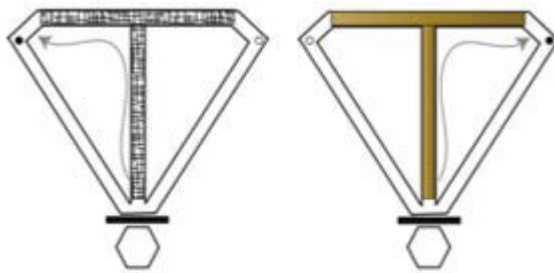
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Figure 6. Schematic of the CD_STANDARD task. Removable wooden inserts covered with plastic black mesh on one side and smooth wood on the other cover the entire central arm and goal arms. See Video 2.

Video 2. Example of three trials (1 wood followed by 2 mesh trials) of the CD_STANDARD task. Similar to Video 1, the experimenter flips the inserts between the wood and mesh trials and sham-flips the inserts between the two mesh trials. She then places the food reward in one of the food cups and places the baited cup at the end of the correct goal arm.

Notes:

a. *Prior to each trial, the experimenter places the floor inserts onto the central stem and both goal arms of the T-maze. The two short left and right goal arm inserts and the half sized central stem insert will be the same color/texture. The goal inserts are used to help the rat learn and maintain the association of the conditional discrimination cue and the location of the food reward (e.g., Wood-Right or Mesh-Left). Our reasoning is that the absence or presence of food will enhance learning of the conditional discrimination if it occurs concomitantly with the presentation of the conditional cue. In the CDWM task, the inserts are half the size of the CD_STANDARD inserts. The central arm insert covers the first half of the central stem of the maze. The goal arm inserts cover the last half of the goal arms, farthest from the T-intersection. One side of the maze insert is covered with black mesh, and the other side is smooth wood (light brown). We have found that it is not necessary to fasten the removable inserts to the maze. The inserts fit snugly in the maze, so they are placed in*

the same location on every trial.

b. *In both tasks, conditional cues are associated with the location of food reward on the T-maze Rats are required to select either the left or right goal based on the texture and color (smooth wood or black mesh) of the floor insert. For example, rats learn to turn right at the T-intersection of the maze when they experience smooth, wood and turn left when they experience black mesh. Trials are presented in a pseudorandom sequence with equal numbers of mesh and wood trials per session (Fellows, 1967). The reward contingency is counterbalanced across rats, with half of the rats in an experiment learning to select the right goal arm on a mesh trial and the left goal arm on a wood trial and the other half of the rats learning the opposite rule (left on mesh, right on wood). The cue inserts are flipped between each trial, even if the same cue is presented on consecutive trials. Flipping cue inserts is done in order to ensure that the rat cannot solve the task by using auditory cues.*

c. *Between each trial, rats wait in the start box for 20 sec while the experimenter prepared the maze for the next trial. A typical training session consisting of 24 trials (12 wood 12 mesh) takes about 30-40 min from beginning to end. Rats are given one session daily of CDWM until they perform the task at a criterion level of at least 80% correct choices on two consecutive sessions.*

6. CDWM task training (during either single or dual-task training) includes an additional phase. After rats reach performance criterion on CDWM with short left and right goal arm inserts and the half sized central arm insert, the short goal arm inserts are removed and rats are trained without them, leaving only the first half of the stem cued via the central arm insert. The same performance criterion is used for this phase of training.

7. Next, rats begin the dual-task phase, learning either the CDWM or CD$_{STANDARD}$ task while also continuing to perform the other task (inter-task interval 15-20 min). In CD$_{STANDARD}$, floor inserts were identical to the CDWM floor inserts except for the size of the inserts. CD$_{STANDARD}$ inserts span the length of the central stem and goal arms of the T-maze. The tasks are identical in every way except for the length of cues available to the animals. Rats are trained on one session of CD$_{STANDARD}$ and one session of CDWM per day. Task type order during dual-task training is counterbalanced. We have found that rats are able to perform daily training sessions of 18 CD$_{STANDARD}$ trials (9 wood trials and 9 mesh trials) and 18 CDWM trials (9 wood trials and 9 mesh trials) for a total of 36 daily trials. A typical dual task session takes ~1.5 h to run per rat. Performance criterion is again set at a performance level of at least 80% correct choices on two consecutive dual-task sessions.

Data analysis

To assess SWM ability, we compare choice accuracy of a single session and across all sessions between the two variants of the task. A selective performance accuracy deficit on CDWM indicates an SWM impairment, see Figure 7 below for performance data from one example dual-task session.

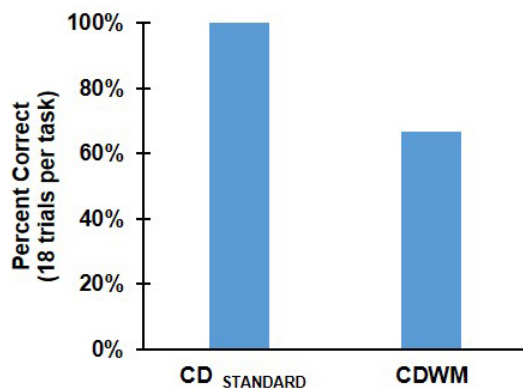We have used both between-subjects and within-subject designs.

Figure 7. Example of choice accuracy for one representative rat in a dual-task session. This rat performed 18/18 correct trials on CD$_{STANDARD}$ and 12/18 correct trials on CDWM.

Notes

1. All procedures are approved by the University of Delaware Institutional Animal Care and Use Committee.

2. For an ongoing experiment, we trained 10 rats on both task variants, with half of the rats first learning CD$_{STANDARD}$, and then adding CDWM and the other half first learning CDWM alone, then adding CD$_{STANDARD}$. It took 18.8 (ranging from 11 to 29) sessions to reach criterion on CD$_{STANDARD}$ and 51.5 (ranging from 35 to 78) sessions to reach criterion on CDWM. Rats that were first trained on CD$_{STANDARD}$ then added CDWM took 34.2 sessions (range: 12 to 69 sessions) on the dual-task phase. Rats that were first trained on CDWM then added CD$_{STANDARD}$ took an average of 13.8 (range: 11 to 18) sessions to reach criterion. In sum, CDWM takes 3-4 times longer to learn than CD$_{STANDARD}$. Additionally, training rats on CDWM in the single-task phase and adding CD$_{STANDARD}$ in the dual-task phase gives us less variability in learning rates than training the rats on the tasks in the opposite order.

Acknowledgments

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