Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE63152R1
Full Title:	A Behavioral Task Modeling 'Everyday Memory' in an Event Arena to Foster Allocentric Representations for Rodents
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TITLE:

A Behavioral Task Modeling 'Everyday Memory' in an Event Arena to Foster Allocentric Representations for Rodents

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KEYWORDS:

behavior, event arena, everyday memory, episodic-like memory, allocentric, spatial learning

SUMMARY:

The goal of this optimized 'everyday memory' protocol in an event arena was to employ a stable home-base that encourages the use of allocentric spatial representations. This animal model provides an effective test-bed for future research into the formation and retention of event memories using behavioral and physiological techniques.

ABSTRACT:

The event arena provides an optimal platform to investigate learning and memory. The appetitive everyday memory task described in this paper provides a robust protocol for the investigation of episodic and spatial memory in rodents, which specifically fosters allocentric memory representation. Rats are trained to find and dig for food during the encoding phase and, after a time delay, rats are given a choice to find the reward food pellet in the correct location. There are two key elements that promote the use of an allocentric strategy in this protocol: 1) rats start from different start locations within and between sessions, 2) a stable home-base is deployed where rats have to carry their food to eat. By means of these modifications, we effectively encourage the rodents to use allocentric spatial representations to perform the task. In addition, the task provides a good paradigm for within-subject experimental design and allows experimenters to manipulate different conditions to reduce variability. Used in conjunction with behavioral and physiological techniques, the resulting rodent model provides an effective test-bed for future research into memory formation and retention.

INTRODUCTION:

To investigate the neurobiology of learning and memory, invasive techniques are required that are not generally feasible in humans. Thus, for over a century, behavioral protocols have been designed for laboratory animals to model various forms of human memory. The design and choice of both task and apparatus are central to the success of effective models of human memory. Numerous paradigms have been developed with diverse complexity, ranging from simple classical and instrumental conditioning protocols[1,2,3] to mazes such as the T-maze[4], radial arm maze[5], Barnes maze[6], watermaze[7], and the cheese-board maze[8]. Yet, while these tasks capture facets of associative learning and spatial navigation, they cannot be used unambiguously to study the memory representation of momentary events (i.e., episodic-like memory). And, although novel object recognition[9] and permutations of this spontaneous memory task, such as object-place memory[10], have provided valuable insights into recognition memory, they do not test explicit recall of events. To address this demand, the event arena was specifically developed, and its use has enabled research into long-term, paired-associate memory encoding and recall[11,12,13] as well as the encoding and recall of discrete events happening in a familiar space[14,15,16,17,18]. The latter theme is the focus of this manuscript.

The event arena is a large, square, open-field area where events occur for rodents. The size of the arena can be scaled to accommodate either rats or mice, and rodents are encouraged to enter and explore. A typical example of an event that takes place within the arena is the finding and retrieval of food from a sandwell at a specific location. The event arena is designed for such appetitive tasks, in which rats or mice are trained to search for, find, and dig up food. It capitalizes on their natural tendency to carry food rewards back to a dark box, which in this case is located adjacent to the arena, where they then eat it. After minimal training to dig for food, rodents take to this task naturally in the memory encoding trials and after a delay, perform in the recall choice trial. In a choice trial, several sandwells (i.e., locations for digging) are available, but only one is rewarded.

Different tasks can be arranged within the event arena (e.g., spatial memory, episodic-like memory, and paired-associate learning). Given the interest in developing effective models of episodic-like memory, a protocol is developed in which there is a daily change in the location where food can be dug up. Thus, rodents are required to remember where the event of digging up food happened most recently. The protocol outlined below entails an encoding trial in which rats search for a sandwell in a new place each day followed, after a delay, by a choice trial, where the recently encoded sandwell location is rewarded, while the other, alternative sandwells in different locations do not contain accessible food. Remembering where the food was on a previous day is not helpful: the correct location has to be encoded and remembered, at least for a while, each day. Accordingly, we have introduced the term 'everyday memory' to capture the form of memory modeled in this task, which we, as humans, use on a daily basis. A human example of everyday memory is remembering where one has parked one's car at the shopping mall (**Figure 1A**) or has put one's glasses down around the house. In this protocol, intra- and extra-arena cues are all stable, just as they are in the settings of our everyday lives (i.e., homes, offices, car parks, etc.). Thus, rodents must remember where something happened most recently within a familiar environment (**Figure 1B**). The task is analogous to, but an improvement on, the delayed-matching-to-place (DMP) task in the watermaze[19]. Being an appetitive task, it exploits rodents' natural behavior to

forage for food[20], instead of their desire to escape from the water. However, as in the watermaze[7], there are no local cues differentiating correct from incorrect locations; animals must use recall rather than recognition to locate the correct sandwell location after varying memory delay durations.

The event arena has already been successfully utilized in investigations of 'everyday memory'. These are memories that are automatically encoded each day, retained in long-term memory, but often forgotten after relatively short time periods. Bast et al.[14] showed monotonic delay-dependent event memory from excellent memory at short intervals through to chance levels at 24 h. The retention of memory can, however, be successfully enhanced by post-encoding novelty or, with multiple encoding trials, with extended trial spacing[15,17].

The event arena is versatile and relatively non-stressful; no aversive stimuli are used. The size of the arena, and the tasks it accommodates, can be adapted for both rats[14,15] and mice[16]. Also, as a land-based task, it is amenable to physiological recording and calcium imaging studies, unlike the watermaze[21]. Moreover, in accordance with the principles of the 3Rs (reduction, refinement, replacement), studies employing the event arena require fewer animals to obtain statistical power, as within-subject experimental designs are feasible (in which each animal serves as its own control for pharmacological interventions, optogenetic stimulations, etc.) and no aversive stimulation is required for motivation. Although initial training demands more time and occurs over more sessions than in, for example, novelty recognition tasks, once animals achieve a stable, asymptotic level of task performance, manipulations such as drug, vehicle-control, or optogenetic stimulation may be interspersed with a relatively small number of additional training sessions[17]. In addition, distinct facets of representation come under direct experimental control in the event arena, such as the nature of the spatial representation employed when solving the task.

The issue of representation concerns the mental framework employed by rats when remembering where recent events happen[18]. Do they remember where the food is located? Or do they only remember how to get to the food? Rats can use allocentric (map-like) or egocentric (body-centered) spatial representations to solve an appetitive task within the arena[18]. However, to control and identify the spatial strategy employed by each experimental subject when performing the task, there are distinct training protocols that are able to selectively promote the use of only one spatial representation. Usually, an egocentric-based representation is employed when rats take their food reward back to the same location from which they started the day's trial, which allows several opportunities to remember the reward location during runs back and forth. This spatial strategy can be employed regardless of whether the start location is changed from day to day or kept constant. In contrast, an allocentric representation is favored when rats are required to carry food reward to a fixed home-base location at the side of the arena, which is different from the changing starting locations. There are numerous advantages of allocentric representations with respect to the brain's storage capacity.

In this paper, we have outlined the home-base protocol, which encourages the employment of only an allocentric representation, and have provided representative results for this task, which clearly illustrates the advantages of using this rodent model of 'everyday memory' in the investigation of learning and memory and highlights how allocentric representations of

episodic-like spatial memory can be promoted.

PROTOCOL:

The methods described in this paper have been approved by the University of Edinburgh Ethical Review Committee; they are compliant with the UK Animals (Scientific Procedures) Act 1986 and the European Communities Council Directive of 24 November 1986 (86/609/EEC) legislation governing the maintenance of laboratory animals and their use in scientific experiments.

NOTE: The experimental subject of the protocol outlined below is Lister-hooded rats, but it can be adapted for other rodent strains.

1. Animal handling, housing, and food control

1.1. Allow 1 week for Lister-hooded male rats to settle after arrival. During this time, handle them daily by gently stroking and tickling them in their cages. Once they are settled, start picking them up for approximately 5 min each day.

1.2. Record each rat's weight upon arrival and every 2–3 days per week. Tailor their food intake, so that each rat's weight is gradually reduced to approximately 85%–90% of their free-feeding body weight; this can be estimated using an established growth curve for free-feeding rats. Maintain the rats within this food-restricted weight range throughout the experiment.

1.3. House the rats in a 12 h (light on)/12 h (light off) light cycle and conduct all experiments during the light phase (7 am–7 pm).

2. Setting up the apparatus

2.1. Experimental rooms, control rooms, and event arena

2.1.1. Experimental and control rooms are either one room separated into two parts or two adjacent rooms separated by a curtain or door and are required for this experiment.

NOTE: This separation will prevent the experimenters from influencing or disrupting the animals while they perform and learn this complex behavioral task.

2.1.2. Dedicate one room to the event arena, the static environmental cues, and the experimental procedures (i.e., the experimental room), and use the other room to record the rats' performance by the experimenters (i.e., the control room).

NOTE: An event arena is a square-shaped, open-field area where event-place associations can be studied (**Figure 2A**). The name 'event arena' derives from the fact that this apparatus is an arena (i.e., an open but constrained space) in which 'events' happen (e.g., digging up buried food pellets in sandwells; **Figure 2B**)[17,18]. To prevent overwork and injury (i.e., back strain) to the experimenter, the arena is elevated above the floor (~1 m).

2.1.3. Using transparent Plexiglass, build a square (160 cm x 160 cm) arena. The floor of the arena comprises a 7 x 7 grid of 49 movable white Plexiglass tiles (20 cm x 20 cm; **Figure 2A,B**). Modify five additional tiles with a central hole (6 cm diameter): these will hold the sandwells within the arena. The five locations of these modified tiles are arranged in the configuration outlined in each session's sandwell map.

NOTE: The configuration of the five sandwell locations changes with each new session (see step 4.2).

2.1.4. Place the event arena in the designated experimental room. Set up the intra-arena cues: position two distinctive landmarks with distinct tactile surfaces (e.g., a glued stack of golf balls (30 cm (h) x 11 cm (w) x 11 cm (l)) and a black water bottle (22 cm (h) x 9 cm (d))) at two locations within the arena: row 4, column 2 and row 4, column 6 (**Figure 2C**).

2.1.5. Keep the objects used for the intra-arena cues and their position constant throughout the experiment but clean them daily with 70% ethanol.

2.1.6. Set up the 3D extra-arena cues: position distinctive landmarks (e.g., patterned spherical lantern (40 cm (d)); red star lantern (60 cm (w)); blue lantern (70 cm (h) x 35 cm (w))) and patterns around the perimeter of the event arena—which is positioned in the center of the experimental room (**Figure 2D**).

2.1.7. Keep the objects used for the extra-arena cues and their position constant throughout the experiment.

2.2. Black boxes

2.2.1. To enable the animals' access to the arena, build four identical black boxes from black Plexiglass (length: 30 cm, width: 25 cm, height: 35 cm per box; **Figure 2E**). Each black box should have a remote-controlled sliding door on one-length surface. This will give the experimenter control over the rats' entry to the arena.

NOTE: The black Plexiglass creates a dark interior, which is preferred by the rats over the bright environment of the open-field event arena.

2.2.2. Place these black boxes midway along each of the four walls of the arena. These black boxes will be identified by their location relative to the top of the live video feed—captured by the camera and received by the computers in the control room—using the cardinal points North (top), East (right), South (bottom), and West (left) of the event arena.

2.2.3. Allow rats to enter the arena from one of the three black boxes, which is referred to as startbox (e.g., East, South, and West; **Figure 2A**, orange rectangles). Use the remaining black box (e.g., North; **Figure 2A**, blue rectangle) as a home-base, which the rats will enter to eat the food reward (i.e., pellets) they retrieve from the arena.

NOTE: Any black box location (i.e., North, East, South, West) can be designated as the home base, but it must be kept constant throughout the entire experiment: the stability of its

location is critical for the successful encouragement of allocentric spatial representations.

2.2.4. In the startboxes and home-base, put two small, transparent, flat-based wells, one for water and one for food pellets (in the case of the home-base, this is used for reward in the habituation stage only) and place sawdust in each startbox and the home-base.

2.3. Sandwells

2.3.1. Use transparent acrylic plastic, with an inner diameter (d) of 6 cm and a total depth (h) of 6 cm to make sandwells used to conceal the food reward that the rats retrieve (i.e., locate, dig up and take to the home-base to eat). Insert a spherical, perforated plastic bowl 4 cm from the top. Insert the sandwells into the adapted tiles within the arena.

NOTE: The plastic bowl creates an accessible part (6 cm (d) x 4 cm (h)) for rewarded pellets, which the rats have access to, and an inaccessible section (6 cm (d) x 2 cm (h)), to which the rats do not have access (**Figure 3A,B**).

2.3.2. In the rewarded sandwells, for both encoding and recall choice trials, place four 0.5 g pellets in the accessible section and eight food pellets in the inaccessible section (**Figure 3C**). In the non-rewarded sandwells, place twelve pellets in the inaccessible section (**Figure 3C**).

NOTE: Both the rewarded and non-rewarded sandwells contain a total of 12 pellets and are filled with specially prepared sand, which conceals the pellets in the sandwells.

2.3.3. Fill sandwells with a mixture of sand and masala powder (2.5 g masala/2.5 kg sand) to mask any odor emanating from the food pellets. Freshly prepare the sand/masala mixture at the start of every session (**Figure 3D**).

NOTE: Steps 2.3.2 and 2.3.3 are designed to mask any olfactory artifacts emanating from the sandwells during encoding and choice trials. This will ensure that the rats' search for the correct sandwell location, and their resulting task performance, is guided solely by their memory of the location where food was dug up, and not by any odor cue emanating from the rewarded sandwell, which could reveal the presence of food-reward.

2.3.4. During a probe trial, which tests the rats' memory for the location of the single sandwell previously rewarded (i.e., contain food pellets), make all five sandwells present within the arena as non-rewarded (i.e., no food pellets are available in the accessible section); including the correct sandwell location.

NOTE: All sandwells present within the arena contain the same number of pellets (n = 12) in their inaccessible section.

2.4. Overall set-up and software

2.4.1. Maintain illumination of the experimental room at a moderate level of brightness using wall-mounted halogen lamps (115–125 lux), and maintain a room temperature between 19 to 23 °C.

2.4.2. Install a charge-coupled device camera above the event arena in the experimental room to record and monitor the rat movements and behavior (**Figure 4A**). The camera provides a live feed to the adjacent control room for both custom video capture and the custom computer software (developed by P. A. Spooner, University of Edinburgh).

2.4.3. Monitor the rats' movements using the custom computer software used to time the rats (**Figure 4B**). This program controls the door of each black box, allowing the experimenters to remotely manage the rats' access to and from the arena from their adjacent control room. Record each animal's latency to locate the correct sandwell and time spent digging at each sandwell during a choice and probe trial.

3. Habituation protocol

NOTE: During habituation, the rats are trained to search for sandwells, dig for a food reward, and explore the event arena.

3.1. Learning to dig for a food reward

3.1.1. Place a small container filled with sand in an individual cage. For the first session (i.e., H1), add three 0.5 g food pellets just under the sand's surface and place one food pellet on top. Thereafter, place all four pellets under the surface (**Figure 5**).

3.1.2. Place each rat in the individual cage with one sandwell, refreshing the sandwell for each rat. Leave the rat in the individual cage until it digs and successfully retrieves all four pellets from the sandwell.

3.2. Habituation Session 1

3.2.1. Place a rat in one of the startboxes (e.g., South) for 30 s and put a pellet (0.5 g) in the home-base's (e.g., North) small, flat-based well designated for food. The placed food pellet acts as a reward and encourages rats to go to the home-base to eat.

3.2.2. Open the startbox door (e.g., South). Close the door when the rat leaves the startbox, enters the arena, and begins exploring this new environment.

3.2.3. If the rat does not leave the startbox within 5 min, intervene in one of the two ways. Either encourage the rat to enter the arena by holding a paintbrush just outside of the startbox door. Once the rat is interested, move the brush further into the arena letting the rat follow it. Once the rat is in the arena and a safe distance from the startbox door, close the startbox door.

3.2.4. Alternatively, take the rat out of the startbox, close the startbox door, and place the rat in the arena, directly outside the startbox door from which it would have entered. If a rat is unmotivated and does not perform the task well (e.g., leave the startbox unassisted, dig effectively, etc.), check the rat's weight and calculate its free-feeding weight (%).

NOTE: If the free-feeding weight is well above 85%, the rat may not be hungry; in this case, its total daily food allowance (g) may require further restriction.

3.2.5. After the rat has explored the arena for 10 min, open the North black-box door (i.e., home-base). If the rat does not enter within 5 min of the door being opened, take the rat out of the arena, close the door of the North black-box, and place the rat in the home-base.

3.2.6. After the rat has finished eating the pellet placed in the home-base at the start of the session, return the rat to its home cage.

3.3. Habituation Session 2

3.3.1. Place a sandwell, with all four food pellets (0.5 g/pellet) buried under the surface of the sand, in the event arena. Change the location of this rewarded sandwell each session hereafter.

NOTE: Having large food pellets (0.5 g), the rats will prefer to carry them to an environment they consider safe (i.e., dark environment) to eat them[22].

3.3.2. Place one cue pellet in the flat-based well, designated for food, in the chosen startbox (i.e., East), followed by a rat.

3.3.3. When the rat finishes eating the cue pellet—after approximately 45 s—open the startbox door (e.g., East).

3.3.4. Close the startbox door once the rat enters the arena and is a safe distance from the door. If the rat does not come out of the startbox, refer to steps 3.2.3–3.2.4.

3.3.5. Let the rat search for the first pellet in the sandwell. To successfully retrieve the food reward, it must dig in the single sandwell now present within the arena.

3.3.6. Once the rat retrieves the first pellet, open the home-base door (i.e., North). The rat should then locate and enter the home-base to eat its reward. If the rat starts to eat the pellet within the arena, gently guide it back to the home-base to eat the pellet.

NOTE: This is critical, as each rat must be encouraged to eat in the home-base; without proper training, they have a tendency to return to the trial's startbox, from which they entered the arena, to eat.

3.3.7. After the rat finishes the first pellet at home-base, allow it to leave the home-base and re-enter the arena to retrieve the second pellet.

3.3.8. Upon retrieving the second pellet, let the rat locate the home-base again to eat the food reward. Once the rat enters the home-base, close the door of the North black box.

3.3.9. After the rat finishes eating the second pellet in the home-base, gently remove it from the home-base and return the rat to its home cage.

3.4. Sessions 3–7

3.4.1. Repeat habituation session 2 (steps 3.3.1–3.3.9) five times, burying the pellets in the sandwell deeper with each session (**Figure 5**). By the end of habituation, encourage all the rats to run quickly to the rewarded sandwell present within the arena, successively collecting the available food pellets and carrying them back to the home-base to eat.

4. Main training protocol

NOTE: Each main training session consists of two memory encoding trials (E1, E2) followed, after a short time delay (~30 min), by one recall choice trial (C1). During all trials, rats are required to successively retrieve two pellets from the rewarded sandwell. After locating each pellet, the rats should locate and enter the home-base to eat this food reward. The location of the correct (i.e., rewarded) sandwell is counterbalanced across sessions for all rats (**Figure 5**).

4.1. Counterbalancing measures

4.1.1. Carefully counterbalance the sequence of the sandwell locations and startbox order used across sessions (**Figure 6**). Before each session, prepare the location map (**Figure 6A**); decide the correct sandwell location for each rat, which must notably change across sessions (**Figure 6B**); and create the counterbalance sheet (**Figure 6B,C**) and recording sheet (**supplementary figure 1**).

4.1.2. Produce three sandwell sets, with five sandwells per set (**Figure 6C**). Each set requires five sandwells because five sandwell locations (one correct and four incorrect) are used in the arena during each recall choice trial, and three sandwell sets are required so that the sandwells used for each trial can be alternated within every session.

4.1.3. During each session, use one sandwell set for a rat's encoding trials (**Figure 6B,C**; Encoding 1: Set 1, Well A; Encoding 2: Set 1, Well B) and another, different sandwell set (**Figure 6B,C**; Recall Choice: Set 2, Well C) for their recall choice trial.

4.1.4. Within each session, use a different combination of sandwell sets for each rat (**Figure 6B,C**), and across sessions, alternate the sandwell set combinations used for each rat.

4.2. Encoding trials

4.2.1. Put a rewarded sandwell in the correct location according to the location map and the counterbalance sheet (**Figure 7**). Never use the intra-arena cue locations, the center tile, or the three tiles directly in front of the four startboxes as a sandwell location.

4.2.2. Place one pellet, followed by a rat, in the startbox (e.g., East) designated for encoding trial 1 (E1); the pellet will act as a cue for the task. Allow enough time (~30 s) for the rat to eat this cue pellet before starting the trial.

4.2.3. Press the on-screen **Start** button to record the trial on the in-house video capture system.

NOTE: It is important to maintain a record of the rats' encoding trials for (1) research transparency (i.e., raw evidence of each animal's task performance), (2) re-scoring, and (3) future reference (i.e., to explore and collect the data for other performance measures).

4.2.4. Open the door of the startbox remotely using the custom computer software (**Figure 4B**).

4.2.5. Start the timer in the custom computer software when the rat enters the arena and close the startbox door.

4.2.6. Give the rat 200 s to look for the correct sandwell, dig, and retrieve its first pellet. If the rat has still not located the correct sandwell or its first pellet after 200 s, retrieve one of the pellets from below the sand and place it on top. If the rat fails to visit the correct sandwell and retrieve this pellet after another 200 s, use a brush to guide it gently to the correct sandwell.

4.2.7. Once the food reward has been found, the rat should carry it to the home-base (e.g., North black box) and eat it once inside. If the rat does not locate and enter the home-base, and chooses instead to eat its first pellet within the arena, quickly take the rat from the arena and place it in the home-base.

4.2.8. After eating its first pellet in the home-base, let the rat enter the arena from the home-base and locate its second pellet from the correct sandwell.

4.2.9. After retrieving its second pellet, let the rat locate and enter the home-base to eat it.

4.2.10. Close the home-base door once the rat is safely inside and give it sufficient time to eat the second pellet.

4.2.11. Stop the custom video capture recording and timer on the custom computer software. Press the on-screen **Stop** button on the custom video capture software. Then, click on the **Stop** button on the on-screen timer on the custom computer software.

4.2.12. While the rat is eating, wipe the arena floor with a cloth soaked in 70% ethanol solution. Do this between every trial.

4.2.13. Prepare the correct sandwell for encoding trial 2 (E2) and place it in the correct location within the event arena.

4.2.14. Take the rat from the home-base and put it in the startbox (e.g., West) designated for E2.

NOTE: The use of an alternate startbox is critical for the effective encouragement of the rats to use only an allocentric spatial solution to perform the task, as the rats cannot rely on a

static viewpoint of the arena or follow their previous path to successfully locate the correct sandwell. Instead, they need to attend to the intra- and extra-arena cues, which promotes allocentric encoding.

4.2.15. Repeat steps 4.3.2 to 4.3.12, and then return the rat to its home cage.

4.3. Recall choice trial

NOTE: Each rat's recall choice trial is run 30–40 min after the second encoding trial (E2) and presents the rats with an arena containing five sandwells.

4.3.1. Place the rewarded sandwell in the correct location assigned for the session, while the four unrewarded sandwells are placed in the four incorrect locations allocated for the session and rat in question (**Figure 8A**). The sandwell location map for the five sandwells in the recall choice trial is altered for each session and counterbalanced across sessions.

4.3.2. Put the sandwell containing four accessible pellets in the correct location. Put four additional sandwells—each unrewarded and containing no pellets in the accessible section—in the incorrect locations set by the session's sandwell location map.

4.3.3. Put the cue pellet and rat in the startbox for the recall choice trial (e.g., South). Ensure that this starting location (e.g., C1: West) is different from those used in the two encoding trials (e.g., E1: East, E2: South).

4.3.4. Start recording the trial using the in-house video capturing system.

NOTE: It is important to maintain a record of the rats' recall choice trials for (1) research transparency (i.e., raw evidence of each animal's task performance, which could be submitted as part of a paper's supplementary material), (2) re-scoring, and (3) future reference (i.e., to explore and collect the data for other performance measures).

4.3.5. On the custom computer software, select the timers matching the sandwells (sandwell timers) to be used on this particular session (**Figure 4B**).

4.3.6. Once the rat has eaten the cue pellet (~30 s), open the door using the custom computer software. When the rat leaves the startbox, close the startbox door and start the timer on the custom computer software.

4.3.7. When the rat digs in a sandwell, click the on-screen **Sandwell** icons to record the time spent digging in each sandwell. Continue to record the rat's dig time in each sandwell visited until the end of the recall choice trial.

4.3.8. Allow the rat to then locate and enter the home-base to eat this food reward.

4.3.9. Use the same procedure as that given for the encoding trials (see step 4.2) for the retrieval of the second pellet from the correct sandwell in the recall choice trial.

4.3.10. Do not click and record the rat's dig time in each sandwell visited during its search for the second pellet. Only record the order of the sandwells (using the number 1–5 allocated to each sandwell location) visited before the rat successfully locates the rewarded sandwell and retrieves its second pellet. This requires concentration.

5. Recall probe test

5.1**.** Use the same set-up as that for a recall choice trial, except that there are no accessible pellets in any of the five sandwells, including the session's previously rewarded, correct sandwell location (**Figure 8A,B**).

NOTE: As in the recall choice trial, all five sandwells are available during the probe test and the rats are free to dig in any sandwell(s) they choose; however, none of the sandwells contain an accessible food reward—instead, all 12 pellets are present in the inaccessible section of each sandwell (**Figure 3C**).

5.2. Put the five sandwells containing no accessible pellets in the arena at the locations provided in the session's sandwell map (**Figure 8**).

5.3. Put the rat in the startbox with a cue pellet. Use the starting position not employed in either of the same session's two encoding trials.

5.4. Set the sandwell timers on the custom computer software to correspond with the session's sandwell map (**Figure 4B**). Ensure the sandwell timers set on the custom computer software correctly correspond to the session's sandwell map.

5.5. Start recording the probe trial in the in-house video capturing system.

NOTE: It is important to maintain a record of the rats' recall probe trials for (1) research transparency (i.e., raw evidence of each animal's task performance, which could be submitted as part of a paper's supplementary material), (2) re-scoring, and (3) future reference (i.e., to explore and collect the data for other performance measures).

5.6. Once the rat has finished the pellet, open the startbox door remotely using the custom computer software (**Figure 4B**).

5.7. Once the rat has entered the arena and is a safe distance from the door, close the startbox door and start the timer in the custom computer software.

5.8. Record the rat's dig time and latency to each of the sandwells visited during the 120 s probe trial, by clicking on each sandwell visited and holding for as long as the rat continues to dig. This 120 s countdown commences when the rat digs in the first sandwell.

5.9. Record the dig times and latencies at 60 s and 120 s by taking a screenshot of the custom computer software at the 60 s and 120 s time mark.

5.10. After the 120 s probe trial has elapsed, put three pellets in the correct sandwell (i.e.,

the location of the rewarded sandwell in the encoding trial) to prevent memory decline. The rat is required to retrieve two of these three pellets. Once a pellet is retrieved, the rat is required to locate and enter the home-base to eat it.

5.11. Press the on-screen **Stop** button on the custom computer software after the 120 s probe test. Clear the file name and note only the rat's latency to retrieve the first and second pellet placed in the now-rewarded, correct sandwell location.

NOTE: Preferential digging at the correct location is used as an index of memory: good memory for the everyday event, experienced in the encoding trials (i.e., encountering the session's correct sandwell location), is indicated by a greater time spent digging in the correct location than the average time spent digging in the incorrect locations.

5.12. Schedule a recall probe test at the beginning of the training to check whether the performance is at chance level. Thereafter, schedule probe tests at specific intervals (e.g., every sixth session), or schedule only when the rats reach a stable task performance: to warrant a probe test, their average performance index (%) needs to be 60% or above for three consecutive sessions. The average performance index is defined in step 7.3.1.

6. Non-encoding control test

NOTE: A non-encoding trial is a control measure used to determine whether the rats are using olfactory artifacts, rather than their memory of the correct sandwell location, to perform the task. As the name suggests, the 'non-encoding control test' means that there are no encoding trials performed prior to the recall choice trial; only the recall choice trial is conducted. The expectation is that without being permitted to encode the location of the everyday memory event, the rats' performance in the choice trial will be at chance level. If this is not the case, and the rats perform well in the non-encoding control test, a re-design of the sandwells, and their accessible and inaccessible compartments may be required.

6.1. Perform a recall choice trial as described in section 4.3 (steps 4.3.1 to 4.3.10).

7. Performance measurement

NOTE: Several parameters are measured and **Supplementary Figure 1** shows an example datasheet.

7.1. Choice of sandwells

NOTE: Choice is defined as the number of sandwells that rats dig in, up to and including the correct sandwell, during the recall choice and recall probe trials. The maximum possible value of the choice is 5, as there are five sandwells in total.

7.1.1. During each trial of the experiment (recall choice trial and recall probe test), determine the number of choices made by a rat: whether it places its front paw(s) on or into a sandwell. If a rat ran past, or merely sniffed quickly in the vicinity of a sandwell, this is not considered a choice.

7.1.2. In rare cases, when it is difficult to tell from the video monitors whether the rats make a choice (as defined above), check at the end of the trial, whether there are any traces of digging: that is if the sand is displaced around the sandwell(s). If there is evidence of digging, however slight, consider this a choice. Pausing at a sandwell and not digging is considered only a visit and should not be counted as a choice.

7.2. Errors

NOTE: Error is defined as the number of incorrect sandwells (unrewarded) that rats visit before locating the correct sandwell. Choice is defined as the number of sandwells that rats dig in, up to and including the correct sandwell, during the recall choice and recall probe trials. The maximum number of errors is four as there are five sandwells in total.

7.2.1. Calculate error using the following formula:

$$Error = (Choice - 1)$$

7.2.2. When a rat re-visits the incorrect sandwell, do not count this as another error as the maximum number of errors is four due to there being five sandwells in total.

7.3. Performance index (PI)

NOTE: Performance index is defined as the number of errors made before the rats locate the correct sandwell in a recall choice trial. With five sandwells, up to four errors can occur. The chance level among five sandwells is therefore two errors (i.e., 50%).

7.3.1. Calculate performance index using the following formula:

$$Performance\ Index\ (\%) = \frac{(Maximum\ number\ of\ errors - Actual\ number\ of\ errors)}{Maximum\ number\ of\ errors} \times 100$$

7.3.2. When a rat re-visits the incorrect sandwell, do not count this as another error as the maximum number of errors is four due to there being five sandwells in total.

7.4. Latency

NOTE: Latency is defined as the time that elapses before digging commences at the correct sandwell(s).

7.4.1. Measure latency from when the rat leaves the startbox until it reaches the correct sandwell. Monitor and record latency using the custom computer software.

7.5. Dig time

7.5.1. Measure the rats' dig time in each sandwell (both correct and incorrect sandwells) in

the recall probe trial.

NOTE: Good memory for the everyday event is defined by the rats' digging in the correct sandwell (n = 1) for a greater proportion of the 120 s probe trial than the average time they spend digging in the incorrect sandwells (n = 4).

7.5.2. Calculate the correct and incorrect using the following formulae:

$$Correct\ (\%) = \frac{Dig\ time\ in\ correct\ sandwell\ location\ (s)}{Total\ dig\ time\ (s)} \times 100$$

$$Incorrect\ (\%) = \frac{Dig\ time\ in\ incorrect\ sandwell\ location\ (s)}{Total\ dig\ time\ (s)} \times 100$$

8. Avoidance of unintended bias

NOTE: The following control measures are implemented throughout the protocol to ensure the reproducibility and reliability of this everyday memory task.

8.1. Counterbalance the sandwell locations across sessions. This avoids garnering any reward bias to a specific side of the event arena.

8.2. Counterbalance the sandwell sets, as well as the sandwells within these sets used in the correct position, across sessions and rats within each session. This discourages the rats from attempting to follow any residual odor trial lingering from the trials of the preceding rat(s).

8.3. Wipe the floor of the event arena between every trial with a cloth soaked in 70% ethanol solution; this will prevent the path of a previous rat(s) from influencing subsequent task performance.

REPRESENTATIVE RESULTS:

This stable home-base protocol has been used to successfully train rats to learn this everyday memory task using allocentric representations. There are two important elements in this protocol. First, animals start from different black boxes (e.g., East, South, and West) within and between sessions (**Figure 7A**). There are two encoding trials and one recall choice trial per session (or probe trial instead of the choice trial in some cases), all starting from an alternate startbox. This encourages the animals to attend to both the intra- and extra-arena cues upon entering the arena. It is not possible to solely rely on idiothetic path integration to perform the task, as different paths are required from each startbox to reach the correct sandwell location. Second, the animals readily learn to eat the food reward pellets in the North-located, stable home-base. During both the encoding trials and recall choice trials, the rats dig and collect their food reward, and then, very naturally, run back to the North home-base to eat the first pellet (**Figure 7A**). After they finish the first pellet, they come out from the North box and look for the second pellet. This is a different path to that of the first pellet and the rats have to re-orient themselves to successfully relocate the correct sandwell. Again, this encourages the animals to consider the intra- and extra-arena cues and promotes the use

of allocentric representations.

Memory formation: We first examined whether rats could achieve a stable performance using this home-base protocol. We found that the rats acquired a good, above-chance performance in this everyday memory task within 16 sessions (**Figure 7B**). The performance index for recall choice trials peaked at around 80%: a level of performance comparable to previous everyday memory protocols, which did not use a stable home-base[14,15,17]. While 80% may not seem as good as the 90% and above reached in two-alternative forced-choice tasks[11], bear in mind that this is a five-alternative choice task. The stability of performance across sessions was also impressive (typically <7%). We also took steps to ensure that this level of performance was based on the rats' memory of the everyday event encoded during the two encoding trials through the use of two non-encoding control tests. If the animals were artifactually relying on cryptic olfactory cues, their performance would be above chance level (50%); however, if the animals were instead relying on their memory of the everyday event, encountered during the two encoding trials, their performance would be poor and fall to chance level (50%). When we conducted these non-encoding control tests, the rats' task performance fell to chance level (**Figure 7B**). From this result, we conclude that the rats were relying on their memory of the everyday event to successfully perform the task.

[Place **Figure 7** here]

Limited retention over time: After establishing that the rats could successfully remember spatially located events in this allocentric protocol, we tested whether they displayed overnight forgetting, characteristic of episodic-like everyday memory (**Figure 8A**). We tested the rats with two retention delays of 24 min and 24 h. We used a within-subject paradigm in which every animal experiences every condition. The rats spent significantly more time digging at the correct location at 24 min than at 24 h ($t(7) = 2.85$, $p < 0.05$) (**Figure 8B**). This delay-dependent forgetting is an essential feature of everyday memory, so this observation ratifies the effectiveness of our task: everyday memory can be effectively modeled by this protocol, as memory decays over the course of 24 h.

Allocentric encoding: Next, we examined whether the rats rely on the intra- and extra-arena cues to successfully perform this memory recall task. After the two encoding trials and before the recall probe trial, a curtain was placed around the arena to remove sight of all intra- and extra-arena cues, followed by the rotation of the arena by 45°. In the probe trial, where all cues (intra- and extra-arena) were hidden or removed, the rats' task performance significantly declined to chance level ($t(7) = 3.37$, $p < 0.05$) (**Figure 8C,D**)—a result that strongly suggests that use of a home-base effectively encourages the rats to employ an allocentric spatial strategy. Furthermore, in the original study, we also performed inter-experimenter correlations to confirm that all the experimenters involved with running this behavioral task recorded the rats' performance similarly[18].

[Place **Figure 8** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Everyday memory. (**A**) Human everyday memory. Schematic showing a green car parked in a car park. After a delay, the driver attempts to remember exactly where she parked

her car. (**B**) Animal everyday memory. Schematic showing a rat digging and retrieving a pellet from a sandwell at a location within the event arena. After a delay, the rat is given a choice trial with multiple incorrect sandwells (gray) and one correct sandwell (green).

Figure 2: The event arena and cues. (**A**) Schematic showing the event arena (Abbreviations: N= North, E= East, S= South, W= West). (**B**) The event arena with intra- and extra-arena cues. (**C**) The two 3D intra-arena cues (left to right): golf ball stack and cylindrical black bottle. (**D**) Several 3D extra-arena cues (from left to right): patterned spherical lantern; red star lantern; blue lantern. (**E**) One of four black boxes is positioned midway along each event arena wall. Three of these black boxes serve as start-boxes, which provide a starting position for the rats at the start of each trial. The fourth black box is a home-base where rats consume the food reward that they retrieve from the arena.

Figure 3: Sandwells. (**A**) Schematic showing an empty sandwell with the accessible and inaccessible sections labeled. (**B**) An empty sandwell with an accessible section and inaccessible section. (**C**) Schematic illustrating the pellet arrangement in a rewarded (left) and non-rewarded (right) sandwell. Both the rewarded and non-rewarded sandwells contain a total of 12 pellets and are filled with specially prepared sand, which conceals the pellets in the sandwells'. (**D**) Series of photographs showing the preparation of a rewarded sandwell, including the correct placement of the pellets in the accessible section (step 1-4).

Figure 4: The experimental setup of the event arena. (**A**) Schematic showing the experimental setup of the experimental and control rooms. (**B**) Screenshot showing a live feed of the experimental room viewed through the custom computer software. The custom computer software allows the experimenters to control the startbox doors remotely and provides other measurements.

Figure 5: The design of habituation sessions. From left column to right column: the habituation session (H1–H7); the startbox used for each session (e.g., H1: South startbox (SB)); the location where rats are required to eat their food reward (i.e., North home-base); the position of the accessible pellets in the rewarded sandwell (in both written and illustrated form; p = pellet), which will be placed in each session's designated sandwell location; the position of the pellets in the flat-based sandwell in the single cage (in both written and illustrated form), which aims to promote digging behavior and strengthen the rats' association between digging in a sandwell and receiving a food reward. The last two columns refer to sandwells in the single cages (outside the arena). Abbreviation: N/A= not applicable

Figure 6: Representative counterbalancing. (**A**) Schematic illustrating how the sandwell location map and correct sandwell location encountered by the rats (e.g., Rat 1) changes across the sessions. (**B**) Example of a counterbalance table for one session (e.g., Session 1). A different startbox is used for each trial within a single session (i.e., encoding trial 1 (E1) started from the South startbox (SB)), but their order of use was the same for each animal (e.g., Rat 1–3). The sandwells used for the correct location (e.g., location 2, 4, 3) and their associated sets, used in full during the recall choice trial, were counterbalanced across each session's trials (e.g., encoding 1, encoding 2, recall choice) and the animals performing the task (e.g., Rat 1–3). (**C**) Table outlining the sandwell sets counterbalanced within and across sessions. There are 15 sandwells in total and three sets (set 1–3) of sandwells, each containing five

wells (A–E). Each rat uses different wells in each encoding and recall choice trial. For example, as mentioned in **Figure 6B**, Rat 1 will use Sandwell 1A in encoding trial 1, Sandwell 1B in encoding trial 2, and Sandwell 2C in the recall choice trial.

Figure 7: Main training protocol and performance index. (**A**) Schematic outlining the experimental protocol for the everyday memory task's main training. During each main training session, two encoding trials (encoding trial 1 and encoding trial 2) were first performed. During each encoding trial, the rats were trained to retrieve two pellets successively (one pellet by one pellet) from the single, correct (i.e., rewarded, green) sandwell located within the event arena. Each encoding trial began from a different startbox (orange). To retrieve the food reward, the rats left the startbox (e.g., South) and located the correct sandwell (green). Once the rats retrieved the food reward from the correct sandwell, they located and entered the home-base (North, blue) to eat the food pellet. After the rats retrieved the second pellet in encoding trial 2, they experienced a short 30 min delay, followed by a recall choice trial. Starting at a different startbox during encoding trials, the rats encountered an arena where multiple incorrect sandwells (gray) and one correct sandwell (green) were now present. (**B**) Graph showing the rats' (n = 17) acquisition data for this stable home-base task. The rats achieved a consistently good task performance by session 16 (ANOVA), which was maintained until session 70 (above chance, *t*-test, $p < 0.05$ or better). Two non-encoding control trials were performed at the start (session 18) and end (session 68) of the main training program (pink arrows). In the absence of the encoding trials, the rats performed poorly: their average performance index (%) fell to chance level (50%, *t*-test, $p > 0.05$). Data are mean ± SEM. This figure has been modified from Broadbent et al[18].

Figure 8: Recall probe test protocols and results. (**A**) Schematic illustrating the experimental protocol for a recall probe test session. Rats were trained in two encoding trials, and after a delay of either 24 min or 24 h, were presented with five sandwells. (**B**) Graph showing the characteristic delay-dependent decay of everyday memory. After a delay of 24 min, the rats' memory for the encoded event was significantly above chance level (t (7) = 2.92, $p < 0.05$) and significantly different from 24 h (t (7) = 2.85, $p < 0.05$); *$p < 0.05$. Data are mean ± SEM. Individual data points are also shown. (**C**) Schematic showing the protocol for the probe test used to assess spatial strategy, where all intra- and extra-arena cues were hidden behind curtains or removed, and the arena was rotated by 45°. The encoding trials were run identically to those performed in a normal probe test (**Figure 8A**, i.e., all environmental cues are present). The delay between encoding trial 2 and the recall probe trial was 24 min, and all environment cues were removed for the recall probe trial. (**D**) Graph showing the results of probe test with and without the event arena's environmental cues. When the intra- and extra-arena cues were removed, the rats performed poorly, digging in the correct sandwell for a significantly lower proportion of the 120 s probe trial than observed when all environment cues were present (t (7) = 3.70, $p < 0.05$). *$p < 0.05$. Data are mean ± SEM. Individual data points are also shown. This figure has been modified from Broadbent et al[18].

Supplementary Figure 1: Example of a recording sheet used to record a single rat's performance during the encoding trial 1, encoding trial 2, and the recall choice/probe trial of a single session.

DISCUSSION:

Humans automatically encode single events in everyday life. We readily recall some events and forget others. The episodic-like everyday memory protocol described above provides a robust method for researchers wishing to investigate this type of memory (episodic memory) in rodents. Because the task involves the daily act of finding and retrieving food pellets from a defined location, the natural instinct of rodents to forage for food is exploited. The task rests on the reasonable assumption that the act of finding and digging up food at a particular but changing place each day is an event for the rat.

Episodic memory is considered to be an integrated memory for an event in time and place. Following the introduction of spontaneous novel object recognition as a method for studying recognition memory[9], an important sophistication was added in the work of Dix and Aggleton[23], which added in location and context as additional associative attributes. There have thence been further developments, including the Langston and Wood[24] studies of object-place-context as a triple association. These are important approaches, but they all rely on recognition memory. The event arena represents a conceptually distinct development as it is a recall task rather than one reliant only on recognition memory. In remembering where an event (digging up food) recently occurred in a specific context, different from where it happened the day before, the animal must approach today's location from its starting position at the edge of the arena without there being any local cues which mark out that sandwell from any other—they all look alike. Consolidated long-term memory is of no value, only a rapidly shifting recency effect. We judge this protocol to be much more analogous to episodic recall such as remembering where one has recently put down one's glasses than would be to choose between a set of objects or images depicting where the glasses might have been placed. We recognize, however, that there are limitations, for example, the lack of any test of context-specificity in the manner of the Dix and Aggleton[23] innovation. However, in still unpublished work, we have shown that rats can perform the event arena task in two separate arenas with distinct extra-maze cues, and will successfully search for the sandwell in the correct position in each context.

Our allocentric-centred episodic-like protocol for everyday memory is reliable and reproducible[18]. As evinced by the representative results above, this protocol effectively precludes any ambiguity regarding the spatial strategy employed by the rats to perform the task by effectively encouraging the use of only an allocentric spatial representation (**Figure 8C,D**). This is achieved by the use of a stationary home-base which rats learn is a safe and accessible place to eat their reward pellets retrieved from the arena. They are, therefore, motivated to learn its position within the event arena using an allocentric representation of this space so that, regardless of the animal's location within the arena, the home-base can be easily found. By having a designated home-base, there is a goal location in the arena that is allocentrically constant across sessions. The rats learn the location of this home-base, and, carrying their food reward, they navigate the open-field environment of the arena using the allocentric cues (intra-arena and extra-arena) and locate the home-base: a dark, safe environment, where they will eat the food reward they retrieved from the correct sandwell. Previously, the implementation of a stable home-base has been shown to successfully encourage the use of an allocentric spatial strategy[18]. In contrast, the place where the daily event happens—the finding and digging up of food pellets from the correct sandwell—varies daily. The rats are encouraged to encode a memory of the sample trial that exploits an allocentric representation of the stable arena, and the recall trial is where the effectiveness

of this memory encoding is tested.

The rats naturally carry the food reward they retrieve from the correct sandwell to this learned location. Another feature of this everyday memory protocol that encourages the use of an allocentric strategy is the employment of a different startbox location for each trial (e.g., East, South, West) so they cannot memorize a specific path and use it on further trials to successfully locate the correct sandwell. Instead, the animals use an allocentric spatial reference frame to navigate the arena, locate the correct sandwell (from a startbox or the home-base) and remember where the everyday event of finding food is happening that day.

Poor performance at an early stage of training is indicative of a mixed or purely egocentric spatial strategy. However, animals that employ this strategy adapt to the protocol's requirements and soon begin employing an allocentric frame of reference. It is the use of the fixed home-base that refines this rodent model of everyday memory and makes it more reliable than its predecessors.

To ensure that the integrity of this home-base measure and the effective encouragement of an allocentric spatial strategy was achieved and maintained, several mandatory control measures and critical steps were incorporated into this protocol. First, to limit the rats' use of olfactory cues to successfully perform the task and accurately identify the correct sandwell in the choice trial, the sand used to fill the sandwells containing the accessible and inaccessible pellets within the arena was re-weighed at the start of each session and a set amount of garam masala powder was routinely added. Additionally, to prevent the correct sandwell and incorrect sandwells from being distinguishable by smell, all sandwells in the arena, including the correct sandwell, held the same total number of pellets, whether accessible or not. And between each trial, the arena floor was cleaned with 70% ethanol solution to prevent any visual or odor trails affecting future task performance. Second, to ensure that everyday memory was effectively modeled by this protocol, the sandwell map, which details the location of the five sandwells, was modified each session and the correct sandwell location for each rat was altered across sessions (**Figure 6**). This careful counterbalancing is essential for the success of this everyday memory model.

Although the long-term training procedure is a major strength of this behavioral protocol, the time it takes can cause procedural limitations. Generally, rats require around 16 sessions of main training before achieving a good, stable level of performance. However, in order to achieve and maintain this level, several other factors, which all have the potential to influence and disrupt a consistent task performance, must be controlled. These include: (1) maintaining a suitable level of food deprivation (85%–90% of normal body weight); (2) ensuring that the rodents' living conditions are consistent for the entire duration of the experiment; and (3) sustaining a structured training schedule, whereby training occurs every day, with minimal breaks or disturbances to the schedule. As mentioned in the protocol, experiment replicability is extremely important. Throughout this paper, we have emphasized the careful design of this everyday memory protocol, including the various counterbalancing measures that determine the sequence of trials and the sandwell maps used, the prevention of olfactory artifacts throughout, and the addition of several distinct control experiments. It is important to note that different experimenters are able to perform the experiment with the same rats and achieve a similar level of performance. In a previous study employing this everyday memory

model, inter-experimenter comparison data showed a high correlation in the scoring of the rats' dig times at each sandwell during the same probe trials[18]. A further limitation is that we have not yet explored having two or more different events during a day (e.g., finding food vs. finding water). Event discrimination in recall would add sophistication to the protocol, but is the subject of future work.

Another highlight of this protocol is the fact that different rat strains can be used. While in the study we report in the representative results that we used Lister-hooded rats to perform the task, another experiment (data not shown) used other rat strains (e.g., tyrosine hydroxylase transgenic rats) and still achieved a good, stable performance index (%). This protocol has also been deliberately developed to accommodate a within-subjects experimental design, making an animal model that is compatible with the values of the 3Rs (reduction, refinement, and replacement). This provides a framework for the important ethical considerations surrounding the use of animals in experiments and is responsive to different experimental interventions. For example, we have used this protocol in conjunction with drug manipulations, optogenetics, and calcium imaging to successfully investigate memory encoding and recall (data not shown).

This protocol has been conducted in our laboratory during the animals' light phase. In a previous study[12] using a similar task in the event arena, we conducted an experiment in the rats' night/dark phase, partly because the study likely involved overnight consolidation. However, since this time, we have conducted multiple event arena tasks during the light phase, and no behavioral differences were found between light and dark phases[13,15,16,17]. The light phase is more practical, in many respects, and training during the animals' night phase nonetheless involves having lights on in the training room so that the animals can see cues relevant for allocentric coding. This creates an ambiguity that is avoided by simply training in the light phase.

Furthermore, weak and strong encoding trials can be applied to this everyday memory protocol to investigate episodic and spatial memories of different strengths. In the current protocol, we have used two encoding trials but the number of encoding trials can be adjusted by researchers to suit different memory manipulations.

To conclude, our stable home-base protocol provides a powerful rodent model for episodic-like everyday memory, which promotes the use of only an allocentric representation and avoids the spatial strategy ambiguity present in its predecessors. We are confident that this protocol provides a reliable and reproducible test-bed for future investigations into the neurobiology of everyday memory.

ACKNOWLEDGMENTS:
This work was supported by Medical Research Council Programme Grants, the European Research Council (ERC-2010-AdG-268800-NEUROSCHEMA), Wellcome Trust Advanced Investigator Grant (207481/Z/17/Z).

DISCLOSURES:
The authors have no conflict of interest to disclose.

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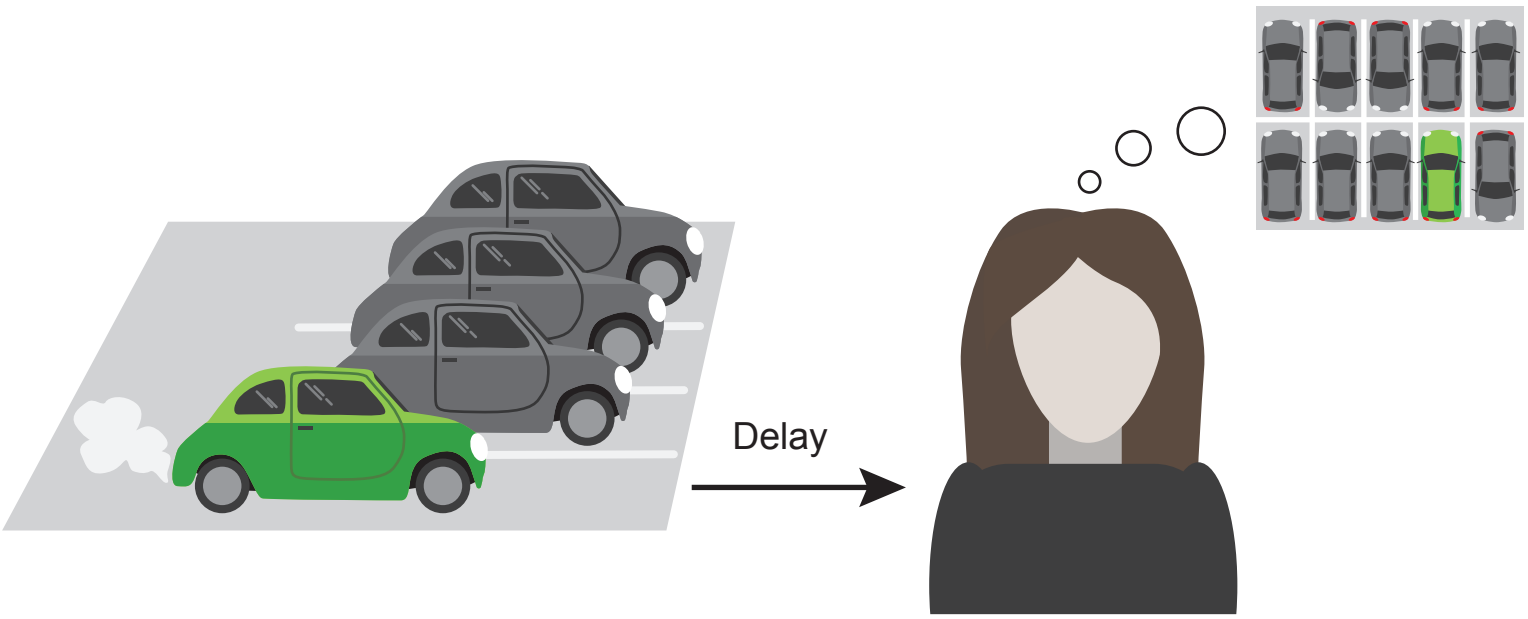
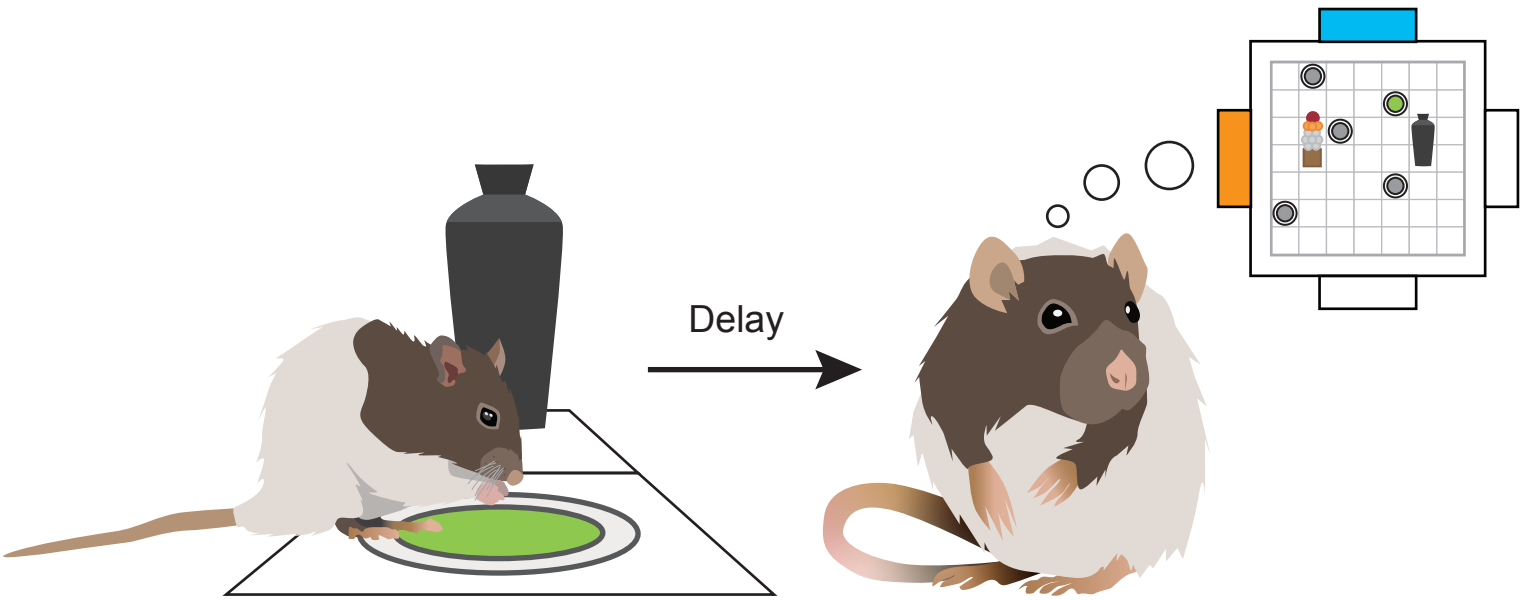
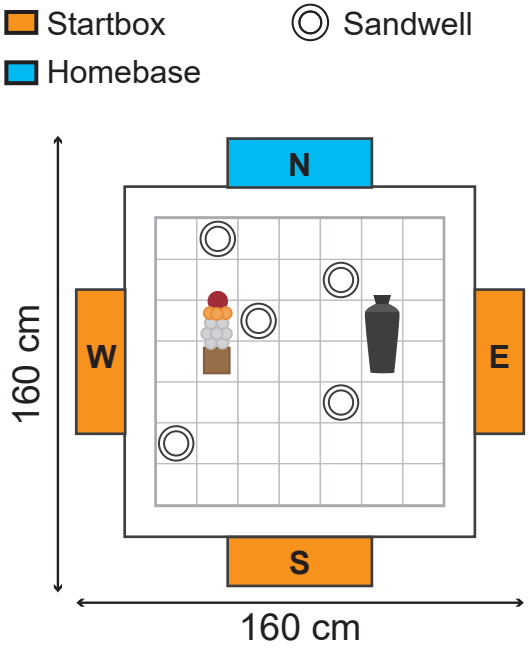
A) Human Everyday Memory: Where did I park my car?



B) Animal Everyday Memory: Where is the food?

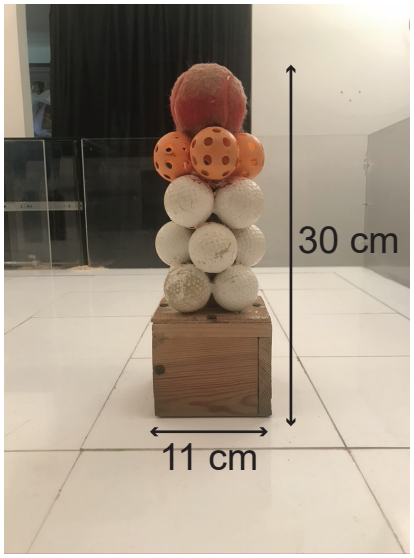


A Event Arena Schematic



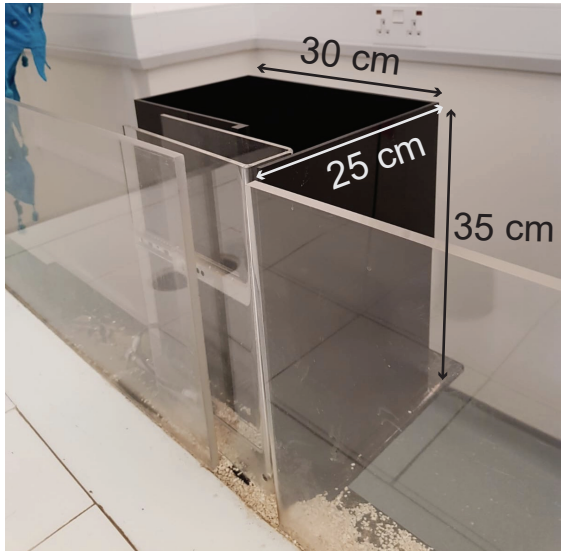
B Event Arena

C 3D Intra-arena Cues

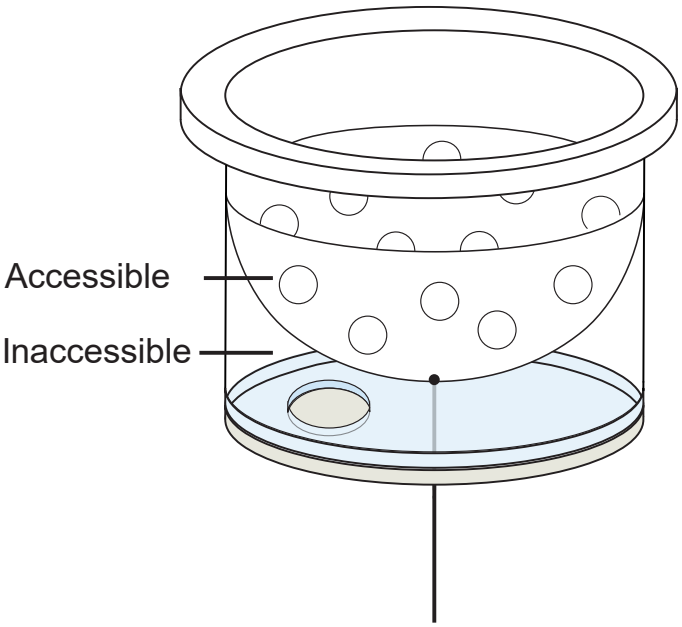


D 3D Extra-arena Cues

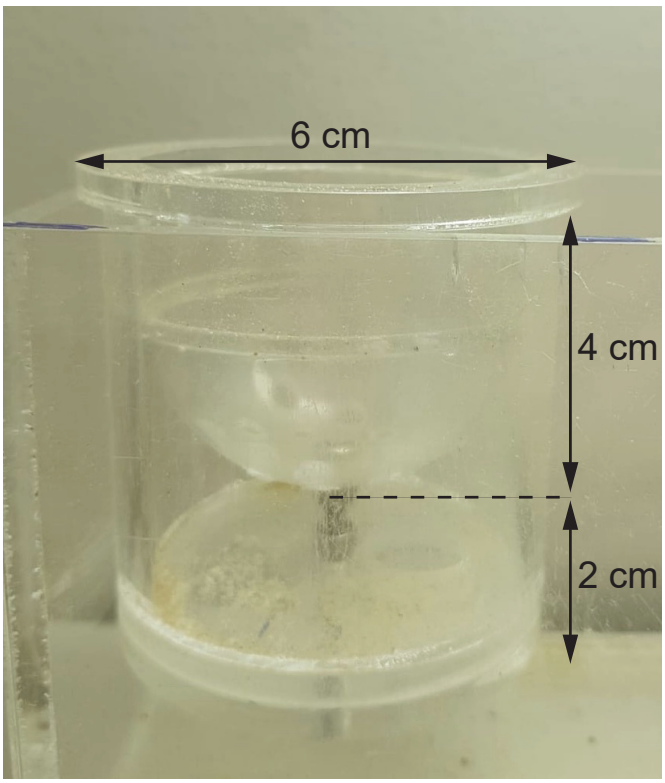
E Black box



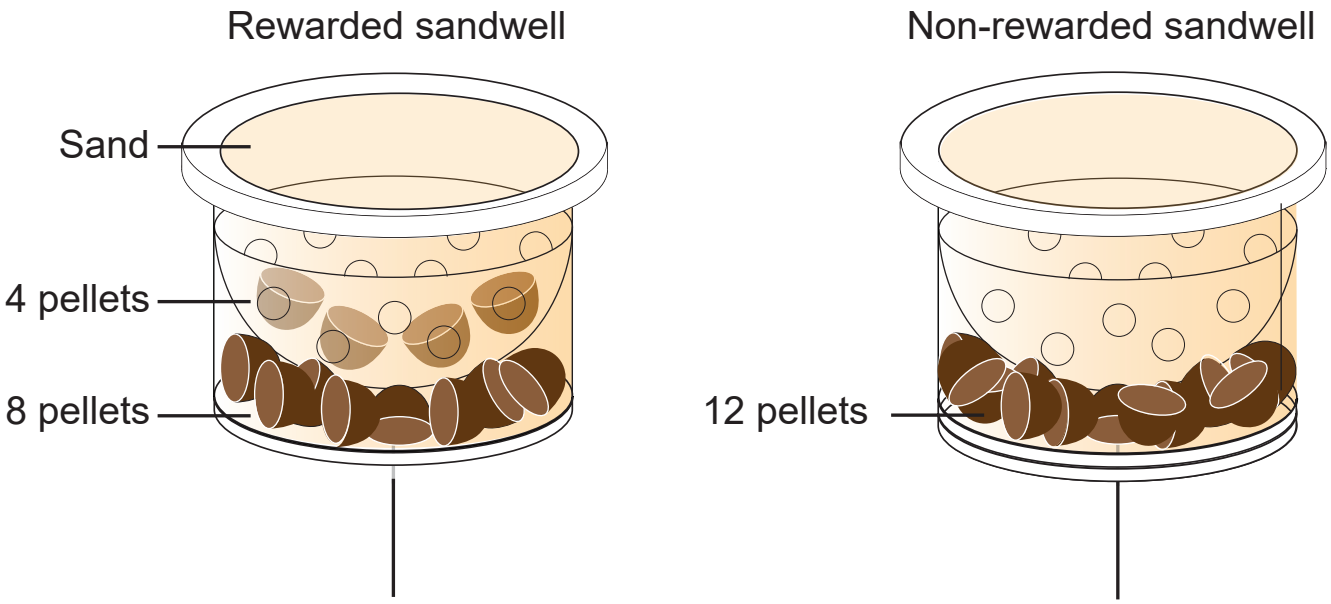
A Schematic of an empty sandwell



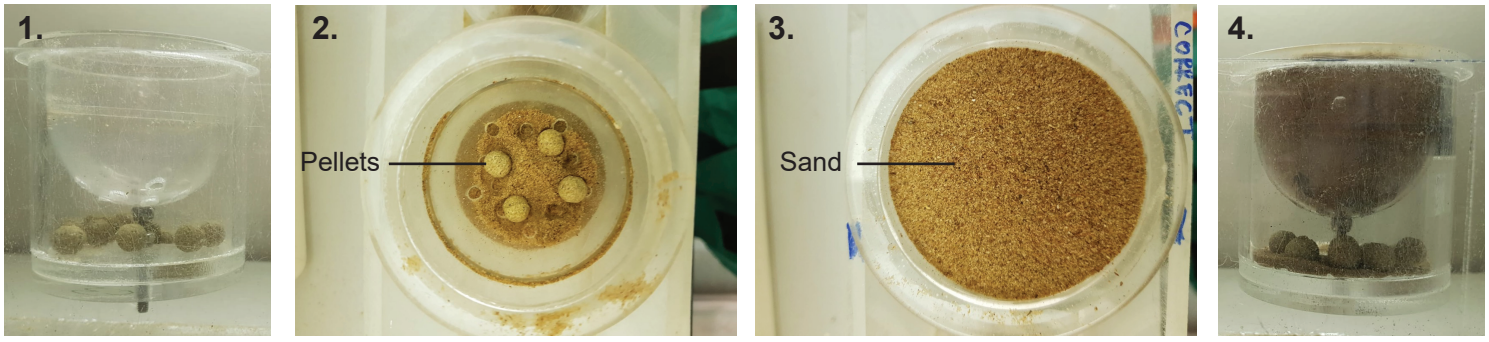
B Empty sandwell



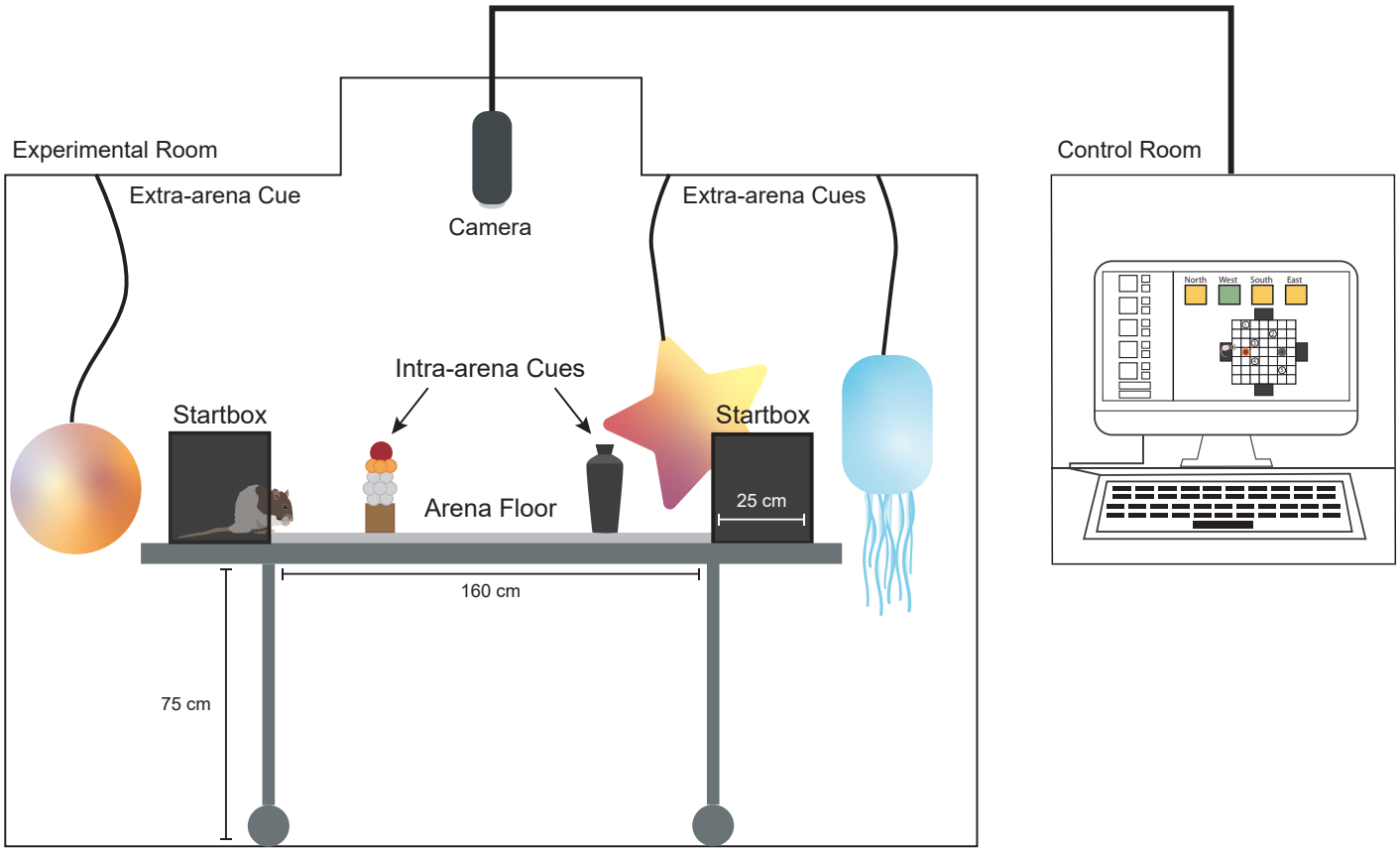
C Arrangement of pellets in rewarded and non-rewarded sandwells



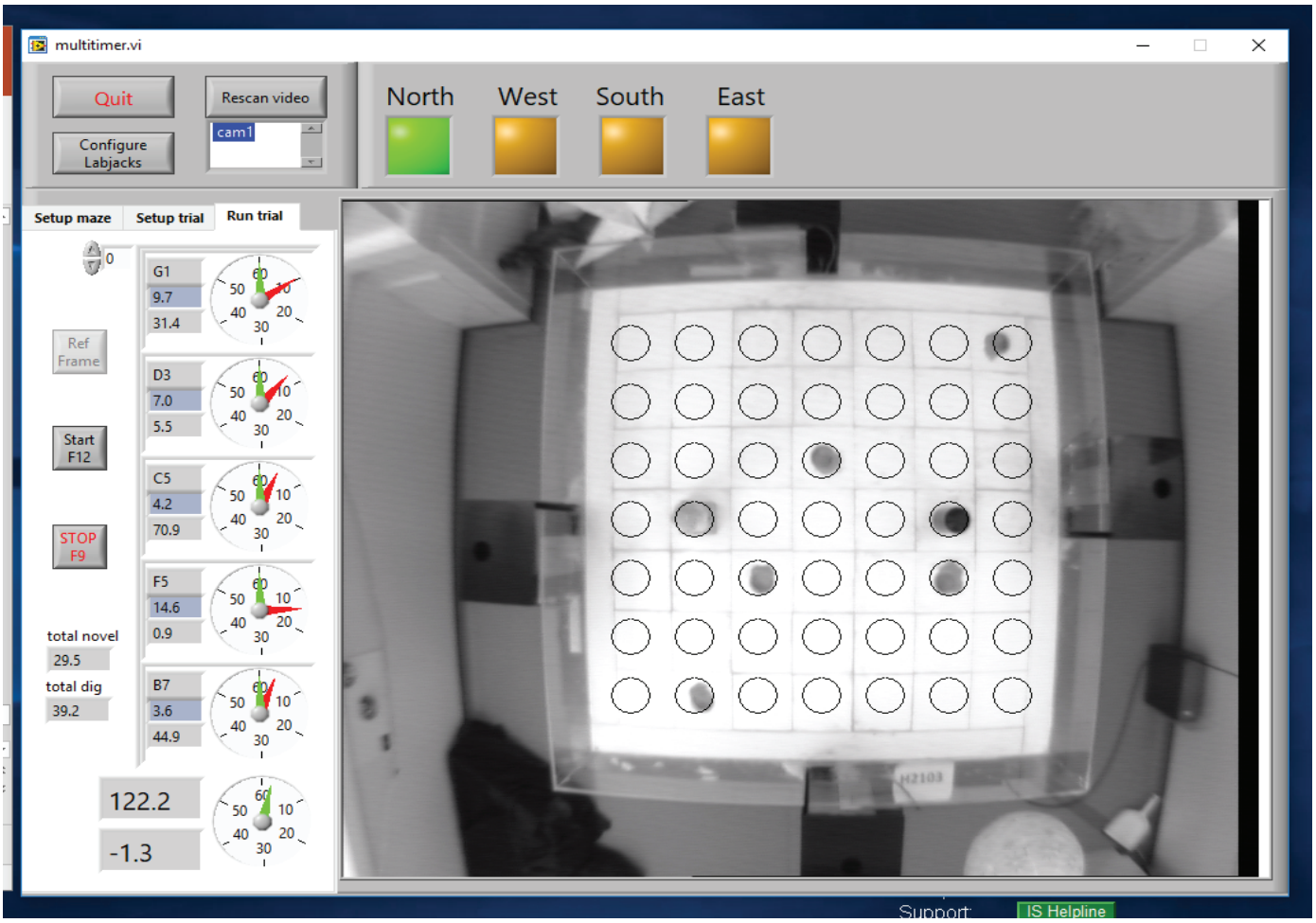
D Preparation of a rewarded sandwell



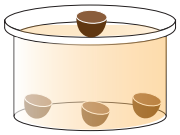
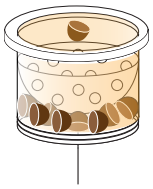
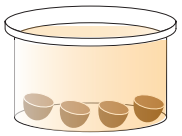
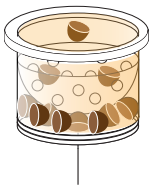
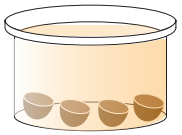
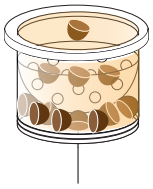
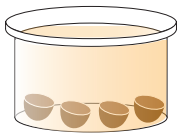
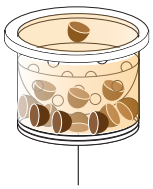
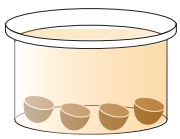
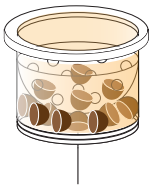
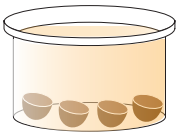
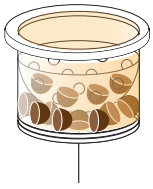
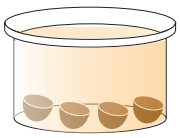
A Event Arena Set-Up (Side View)



B Custom LabView Software *(Multitimer)*



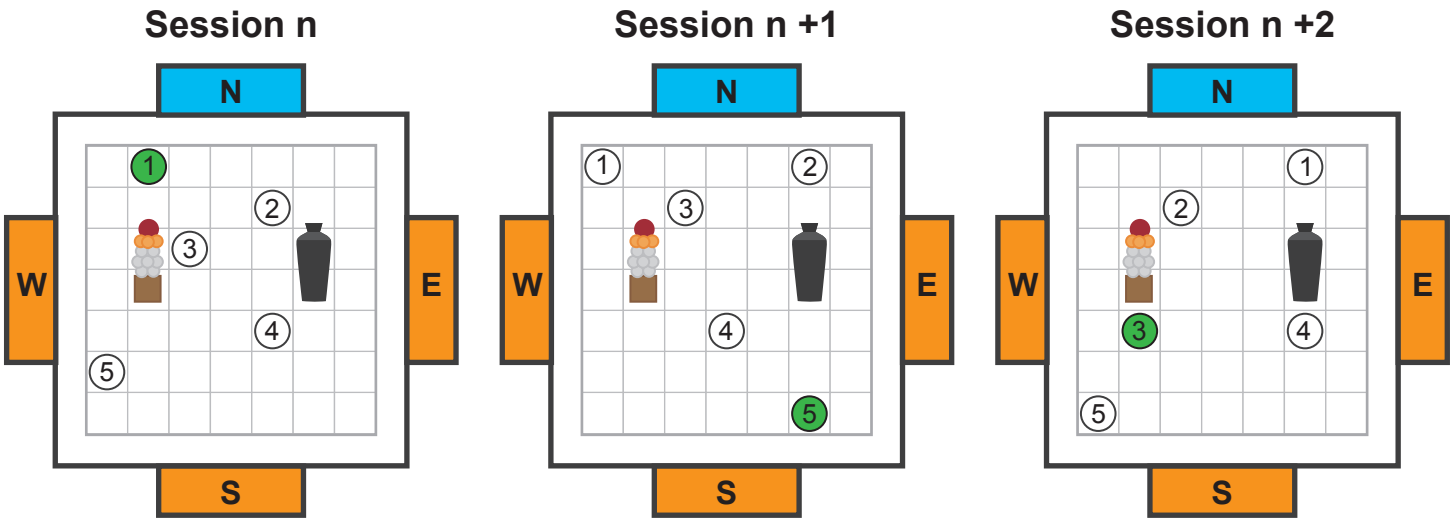
Habituation Sessions Summary

	In Arena				In Single Cage	
Sessions	Start from SB	Eats at	Sandwell		Sandwell	
H1	South	N/A	N/A	N/A	1p top, 3p bottom	
H2	East	SB North	1p top		4p bottom	
H3	West	SB North	1p top, 2p middle		4p bottom	
H4	East	SB North	1p top, 3p middle		4p bottom	
H5	South	SB North	1p top, 3p bottom		4p bottom	
H6	West	SB North	1p middle, 3p bottom		4p bottom	
H7	East	SB North	4p bottom		4p bottom	

A) Rewarded location counterbalanced across sessions

Example: Rat 1

Startbox
Homebase
Sandwell Map Locations (1-5)
Correct Sandwell Location



B) Example of a session's counterbalance table

Session n	Startbox	Rat 1	Rat 2	Rat 3
Encoding 1 (E1)	South	Sandwell Set 1,Well A 1A	2A	3A
Encoding 2 (E2)	East	1B	2B	3B
Recall Choice (C1)	West	2C	3C	1C
Rewarded Location		2	4	3
Near/Far from C1 SB		Far	Near	Near

C) Sandwell sets

	Wells				
	A	B	C	D	E
Set 1	Set 1, Well A 1A	1B	1C	1D	1E
Set 2	Set 2, Well A 2A	2B	2C	2D	2E
Set 3	Set 3, Well A 3A	3B	3C	3D	3E

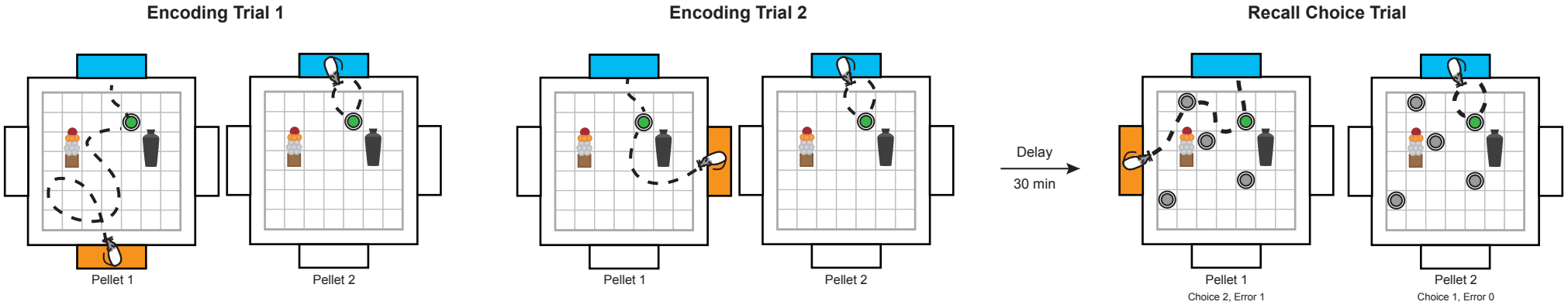
A Experimental Protocol: Main Training

Encoding trials - 1 SW

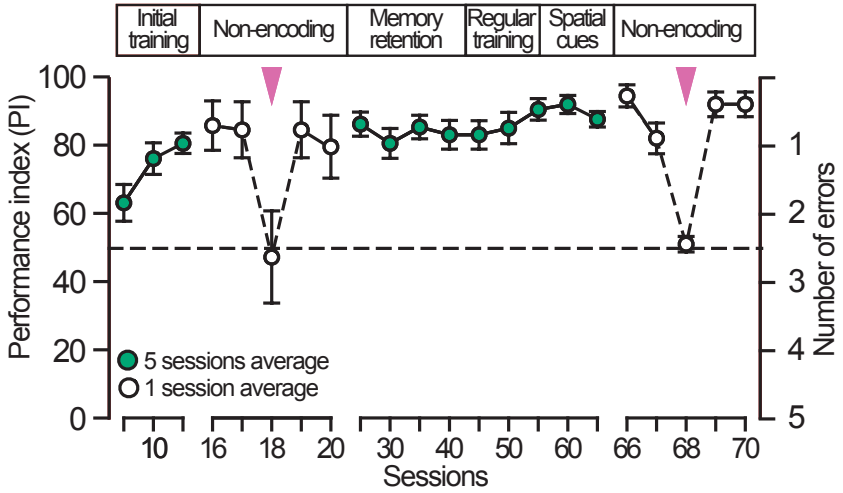
2 trials from different SB and return to Homebase

Rewarded
Non-rewarded
Startbox
Homebase
Animal's Path

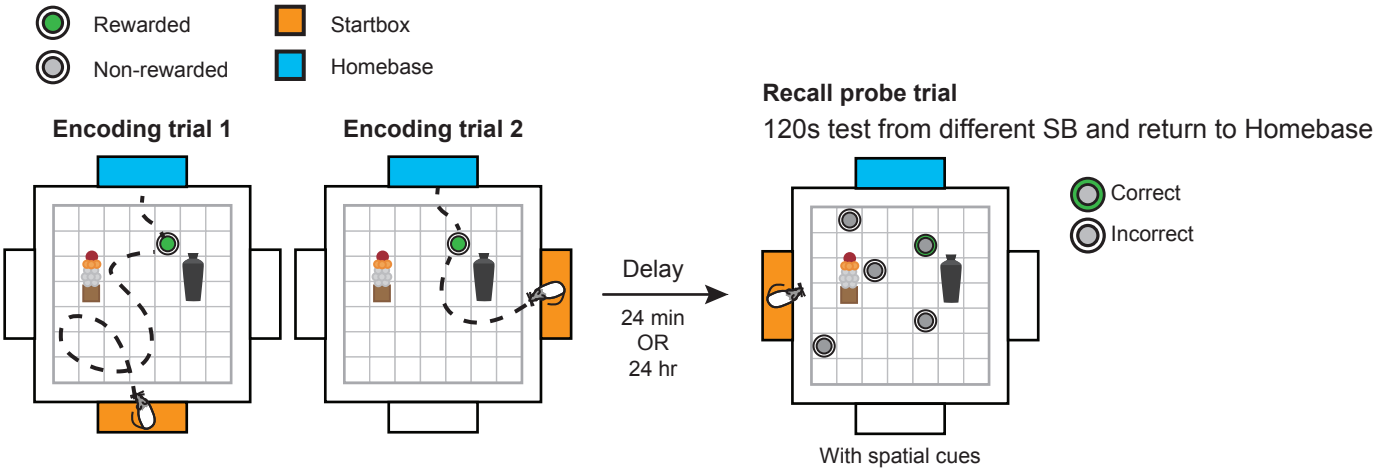
Retrieve 2 pellets per trial (one pellet by one pellet)



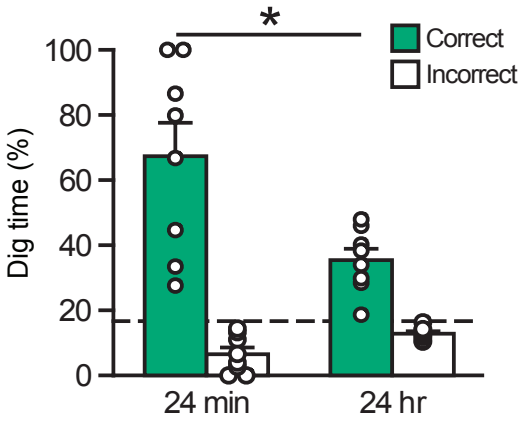
B Main Training Performance



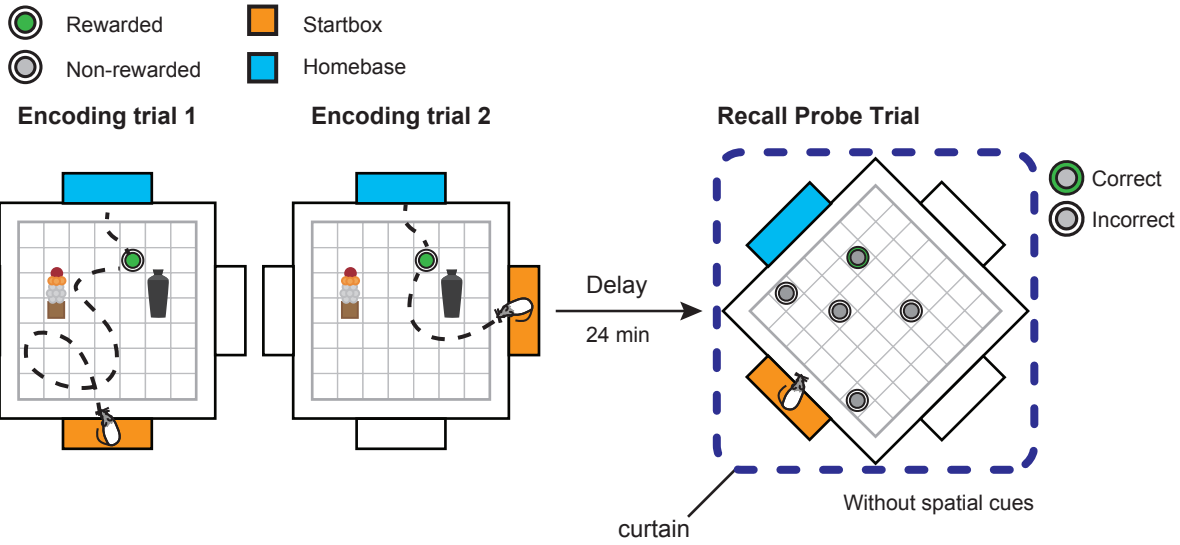
A Experimental Protocol: Recall Probe Test



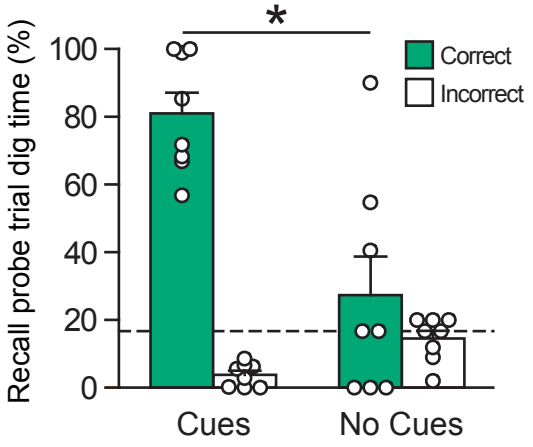
B Recall Probe Test Results: 24 min vs 24 hr delay



C Recall Probe Test Without Intra- and Extra-Arena Cues



D Recall Probe Test: With vs Without arena cues



Experiment _____ – Training Session Date / / /

Experimenters: _____ LUX:_____

Lat. 1: latency to dig in correct well; Lat. 2: latency to retrieve food; Ent. Arena: latency to enter the arena from startbox (1st pellet) or homebase (2nd pellet).

- -

Rat-No.: _____

Encoding Trials

	Location	SB	Ent. Arena	Lat1	Lat 2	Eats@	Time
E1-1st			0				
E1-2nd							
E2-1st			0				
E2-2nd							

Recall Choice/ Probe Trial Time:

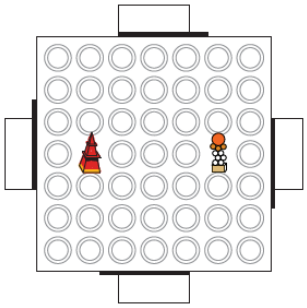
	Location	SB	Ent. Arena	Lat1	Lat 2	Eats@	Order	Choice
C1-1			0					
C1-2								

Additional Time:

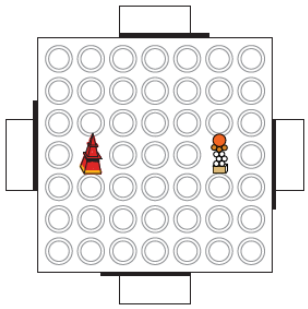
	Location	SB		Lat1	Lat 2	Eats@	Order	Choice
			0					

Encoding 1

Pellet 1

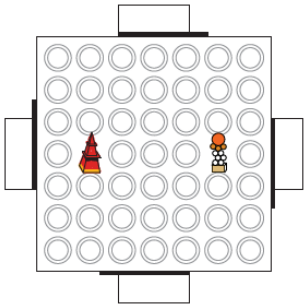


Pellet 2

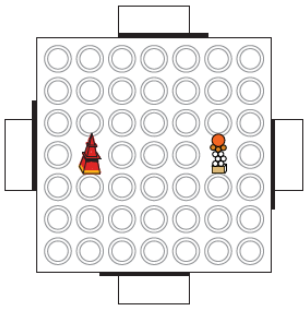


Encoding 2

Pellet 1

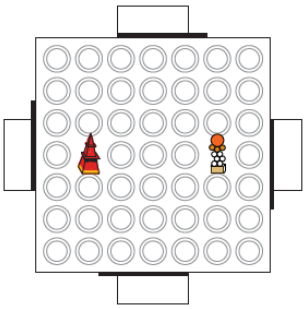


Pellet 2

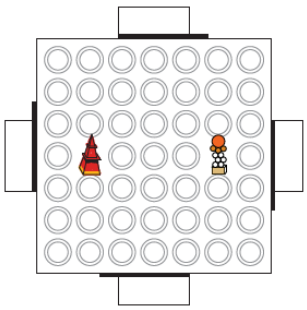


Recall Choice

Pellet 1



Pellet 2



For Probe Trial Use Only

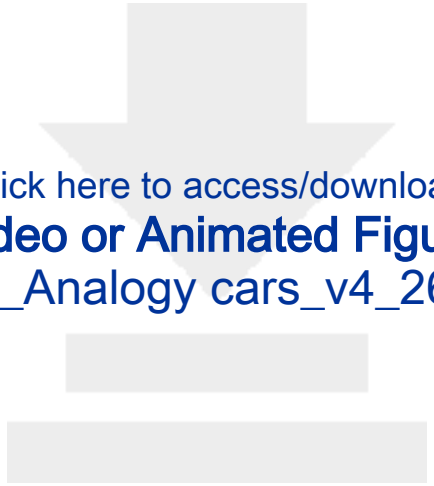
Trial Duration: 60s

Location	Dig Time	Latency
L1		
L2		
L3		
L4		
L5		

Trial Duration: 120s

Location	Dig Time	Latency
L1		
L2		
L3		
L4		
L5		

Figure 1



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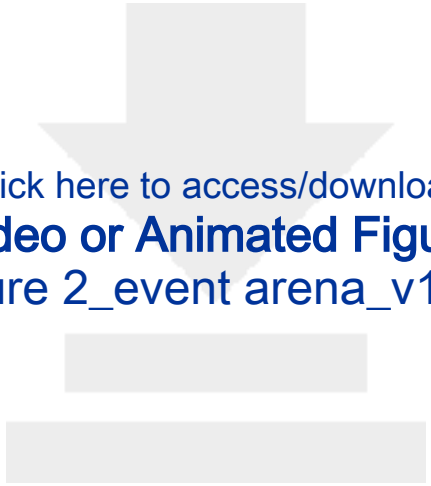
Video or Animated Figure

Figure 1_Analogy cars_v4_260721.ai

Figure 2



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Video or Animated Figure

Figure 2_event arena_v11.ai

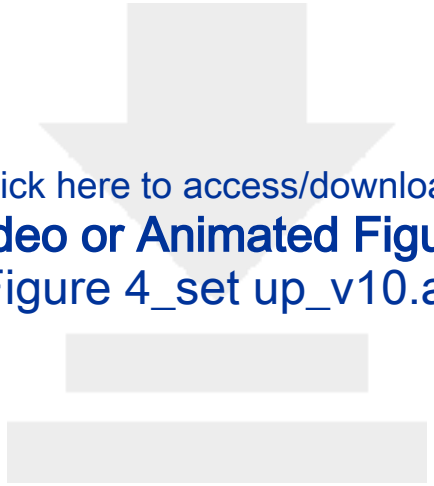
Figure 3

Click here to access/download

Video or Animated Figure

Figure 3_Sandwells_v8.ai

Figure 4



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Video or Animated Figure

Figure 4_set up_v10.ai

Figure 5

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Video or Animated Figure

Figure 5_Habituation Summary_v9(1).ai

Figure 6

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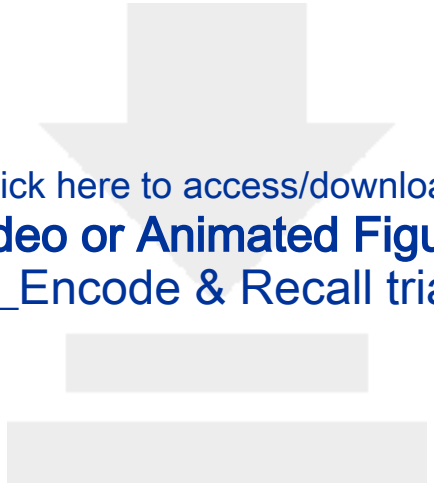
Video or Animated Figure

Figure 6_Counterbalance_v7ai.ai

Figure 7



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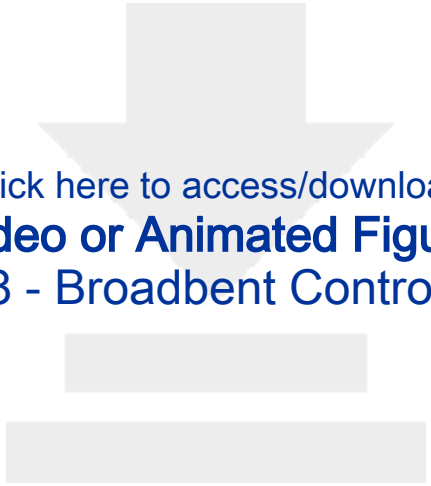
Video or Animated Figure

Figure 7_Encode & Recall trial_v16.ai

Figure 8



Click here to access/download

Video or Animated Figure

Figure 8 - Broadbent Control_v12.ai

Table of materials

Click here to access/download

Table of Materials

JoVE_Table_of_Materials_R2.xlsx

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Email: Anne.Aitken@ed.ac.uk; R.G.M.Morris@ed.ac.uk

JOVE
18th October 2021

Attention of Dr Mittal and the Reviewers

JOVE 63512
Manuscript title: A behavioural task modelling 'everyday memory' in an event arena designed to foster allocentric representations for rodents.

Dear Dr Mittal,

Thank you for your email on 20th September 2021 indicating your interest in publishing our manuscript. We greatly appreciate the time and effort you and the three reviewers have spent reviewing our manuscript; your helpful comments and insightful suggestions have improved our work.

We are grateful to the reviewers for their kind comments to the effect that the manuscript contains a "fine and thorough protocol" (Rev 1), "extremely well presented" (Rev 2), "invaluable and suitable to be published" (Rev 3). We have responded to all of the reviewer and editorial comments, and our responses are described in a point-to-point fashion (see below). Corrected or added portions in the revised manuscript text are in red. We have kept the yellow highlighted text for filming purposes.

The quality and clarity of this revised manuscript is now improved, thanks to the editorial and reviewers' comments and suggestions for this revision. We hope this new version of the manuscript will be suitable for publication.

Sincerely,

Dorothy Tse and Richard Morris

Response letter:

JOVE 63512
Manuscript title: A behavioural task modelling 'everyday memory' in an event arena designed to foster allocentric representations for rodents.

We appreciate the comments from the editor and reviewers. Our responses are described below in a point-to-point fashion below. Corrected or added portions in the revised manuscript text are in red.

Editorial comments:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

This is a very helpful comment. We have thoroughly proofread the manuscript and made sure that there are no spelling or grammar issues.

2. For in-text formatting, corresponding references should appear as numbered superscripts after the appropriate statement(s) and before the punctuation.

We have changed all the corresponding references to the numbered superscripts (text in red).

3. Please submit each figure individually as a vector image file to ensure high resolution throughout production: (.psd, ai, .eps.).

We have submitted each figure individually in AI format.

4. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example, OBS studio, Mulititimer, etc.

We have removed all commercial language from the manuscript and have now used generic terms instead. For example: Step 2.4.2 This will provide a live feed to the adjacent control room for both custom video capture and custom computer software used control the arena startbox doors and manual timers for the task (developed by P.A. Spooner, University of Edinburgh).

5. The Protocol should be made up almost entirely of discrete steps that direct the reader to do something without large paragraphs of text between sections. Please move the discussion about the protocol to the Discussion.

We have shortened those paragraphs and moved the discussion about the protocol to the Discussion.

6. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

We thank the editor for pointing this out. We have simplified some individual steps in the protocol.

7. Avoid usage of phrase “should be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

Thank you for pointing this out. We have changed relevant parts in the manuscript to reflect this. For example: Step 2.3.1 A spherical, perforated plastic bowl “should be” inserted 4 cm from the top… and now we changed to “Insert a spherical, perforated plastic bowl 4 cm from the top...”

8. There is no important section in the JoVE manuscript format. Please provide details either as a Note or Caution statement. If any information given is an actionable item, please convert it as a protocol step.

We have now amended this in the manuscript.

9. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed?

We have now addressed this in the Protocol steps.

Step 1.1: What is the strain of rats used for this experiment? Please mention.

We used Lister-hooded male rats in this experiment. We have added this information in the manuscript “The experimental subject of the protocol outlined below is for Lister-hooded rats, but it can be adapted for other rodent strains” and “Allow one week for Lister-hooded male rats to settle after arrival”.

Step 4.2.1: there is no table 1 provided in the manuscript. Please correct this.

Thank you for pointing that out. We have changed table 1 to Figure 9.

Step 4.3.3, 4.3.11, 4.4.6: Please provide details as to how this is done such as “click this”, “select that”, “observe this”, etc. Please mention all the steps that are necessary to execute the action item. Please provide details so a reader may replicate your analysis including buttons clicked, inputs, screenshots, etc. Please keep in mind that software steps without a graphical user interface (GUI) cannot be filmed.

We have now clarified all these steps in our manuscript. For example:
Step 4.3.3 Press the on-screen start button to record the trial on the in-house video capture system.
Step 4.3.11 Press the on-screen stop button on the custom video capture software. Then, click the stop button on the on-screen timer on the custom computer software.
Step 4.4.6 is now Step 4.4.7 Click and hold the on-screen sandwell icons for the duration the rat digs to record the time they spend digging in each sandwell. Continue to record the rat’s dig time in each sandwell visited until the end of the recall choice trial.

Step 4.4.4: What is the timer setting? How can it be assessed using the sandwell map? Please mention.

We have now revised 4.4.5 “On the custom computer software, select the timers matching the sandwells (sandwell timers) to be used on this particular session (figure 4B).”

Step 7.2: What does the choice in the equation indicate? Is it the sandwell which the rat visited and was incorrect? Please specify this.

‘Choice’ is defined as the number of sandwells that rats dig in, up to and including the correct sandwell, during the recall choice and recall probe trials. The maximum possible value of the ‘choice’ is 5, as there are 5 sandwells in total. I have now repeated this definition of “choice” in step 7.2 (it is also defined in 7.1, as before)

10. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.

We have now changed the figure legends to accommodate this.

11. Figure 7B, 8B, 8D: What do the error bar in the figures indicate: standard error or standard deviation. Which test was used to calculate significance? Please mention in the figure legend.

We have now included this information in the figure legends 7B, 8B and 8D.

12. Figure 8B, 8D: What does the * stand for? Please mention in the figure legend.

We have now added this information in the figure legends 8B & 8D.

13. Please also include in the discussion the critical steps in the protocol along with appropriate citations.

We have now included this in the discussion. “To ensure that the integrity of this home-base measure and the effective encouragement of an allocentric spatial strategy was achieved and maintained, several mandatory control measures and critical steps were incorporated into this protocol. First…”

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We have obtained the explicit copyright permission from the European Journal of Neuroscience.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
This is a fine and thorough protocol for a very complex memory testing procedure.

We appreciate the constructive comments from Reviewer #1. Our responses are described in a point-to-point fashion below. Corrected or added portions in the text are in red text.

I have some questions and remarks:
House the rats in a 12 hr (light on)/12 hr (light off) light cycle and conduct all experiments during the light phase (7 am - 7 pm): why test during the light phase?

In a previous study (Tse et al., 2007) using a similar task in the event arena, we conducted the experiment in the rats' night/dark phase, partly because the study likely involved overnight consolidation. However, since this time, we have conducted multiple event arena tasks during the light phase, including the task from our 2007 paper, and there were no differences in behaviour found between light and dark phases. The light phase is more practical, in many respects, and training during the animal's night phase nonetheless involves having lights on in the training room in order that the animals can see cues relevant for allocentric coding. This creates an ambiguity which is avoided by simply training in the light phase.

2.1.1 Using transparent plexiglass: capitalize Plexiglass

We have changed this in the manuscript.

As an alternative to reducing the animal's body weight, why not use a highly prederred food the incentive for which does not require a "need" for the animal?

Studies have shown that rats under food restriction are healthier and live longer than those with free access to food (Weindruch and Segal, New England Journal of Medicine, 1997). In addition, the food pellets we used contain essential nutrients for the rats in a balanced diet format. We have been successfully using these food pellets since 2003 (Day et al., 2003; Bast et al., 2005, Tse et al., 2007, 2011; Wang et al., 2010, 2012; Bethus et al., 2010; Takeuchi et al., 2016; Nonako et al., 2017; Broadbent et al., 2021).

5.7 After the 120-s probe trial has elapsed, put 3 pellets in the correct sandwell (i.e. the location of the rewarded sandwell in the encoding trial) to avoid "extinction" of the rats' memory: Extinction of memory? This is a very mentalistic expression: behavior undergoes extinction.

We have now changed the text. "After the 120-s probe trial has elapsed, put 3 pellets in the correct sandwell (i.e., the location of the rewarded sandwell in the encoding trial) to prevent memory decline."

To conclude, our stable home-base protocol provides a powerful rodent model for episodic like everyday memory:

There is a lot of confusion about animal modelling of episodic memory. Episodic memory is considered to be an integrated memory for an event in time and place. The most convincing episodic memory models are based on the use of object-recognition tasks. I would like to have the authors add a section providing an argument for why they believe their procedure can model episodic memory.

Thanks for raising this point. We have now alluded to this in the discussion. Specifically, we have added the following paragraph.

"Episodic memory is considered to be an integrated memory for an event in time and place. Following introduction of spontaneous novel object recognition as a method for studying recognition memory[9], an important sophistication was added in the work of Dix and Aggleton[23] which added in location and context as additional associative attributes. There have thence been further developments, including the Langston and Wood[24] studies of object-place-context as a triple association. These are important approaches but they all rely on recognition memory. The event arena represents a conceptually distinct development as it is a recall task rather than one reliant only on recognition memory. In remembering where an event (digging up food) recently occurred in a specific context, different from where it happened the day before, the animal must approach today's location from its starting position at the edge of the arena without there being any local cues which mark out that sandwell from any other – they all look alike. Consolidated long-term memory is of no value, only a rapidly shifting recency memory. We judge this protocol to be much more analogous to episodic recall such as remembering where one has recently put down one's glasses than would be to choose between a set of objects or images depicting where the glasses might have been placed. We recognise, however, that there are limitations, for example the lack of any test of context-specificity in the manner of the Dix and Aggleton[23] innovation. However, in still unpublished work, we have shown that rats can perform the event arena task in two separate arenas with distinct extra-maze cues, and will successfully search for the sandwell in the correct position in each context."

Reviewer #2:
Manuscript Summary:
This is a well written manuscript about a previously published, well regarded, but poorly used (outside the primary lab) rodent behavioural task. The manuscript describes the task well and I can see publication in JoVE to be very useful in helping other labs use and adapt this apparatus for their own behavioural tasks. There are no changes required from this extremely well presented manuscript and I thank the authors for being so thorough in their discussion.

We really appreciate the positive comments from Reviewer #2.

Reviewer #3:
Manuscript Summary:
The manuscript entitled "A behavioural task modelling "everyday memory" in an event arena designed to foster allocentric representations for rodents" written by Tse D, Norton AC, Spooner PA and Morris RGM presents a detailed description of how to set, conduct and analyze the "everyday memory" paradigm. The "everyday memory" paradigm is a spatial processing task that asks rats to find a specific location with the allocentric, but not allocentric, strategy. The authors carefully provides step-by-step illustrations and practical data of the "everyday memory" paradigm, which make the manuscript is invaluable and suitable to be published in JoVE. I just have some minor points that do not compromise the manuscript:

We appreciate the constructive comments from Reviewer #3, but wish to note that by "everyday", we also imply that the information being processed changes from day to day and not just that it complies with an allocentric strategy. Our responses are described in a point-to-point fashion below. Corrected or added portions in the text are in red text.

Major Concerns:
N.A.

Minor Concerns:
1. In the introduction (page 2), line 64, "…have provided valuable insights into recognition memory, they do not involve recall." This is overly-simplicity. The spontaneous object exploration paradigms depend on animals' natural tendency towards novel stimuli, which indicates that the information of "familiar" stimuli is encoded. During test trial of spontaneous object exploration paradigms, animals likely "retrieve" the stored memory, and thus, tend to explore preferentially novel stimuli. Although spontaneous object exploration paradigms cannot clearly reflect recollection per se, as they involves the processes of familiarity (knowing) and/or recollection (remembering), spontaneous object exploration paradigms are not un-related to recall. Details of spontaneous object exploration paradigms can be found: Chao et al., 2020 Neurosci Biobehav Rev 113:373-407.

Thanks for pointing this out. We have now changed the text to "And, although novel object recognition[9] and permutations of this spontaneous memory task, such as object-place memory[10], have provided valuable insights into recognition memory, they do not test explicit recall of events".

2. In page 3, line 126-127, "…in which the experimenter is at the mercy of what the experimental animals choose to do.." It is not clear. Neither spontaneous object exploration paradigms nor the "everyday memory" task forces animals to choose, and both need "the mercy" of animals to behave. Please use scientific language to describe the sentence.

We have now removed this language from the paragraph.

3. In page 5, 2.1.2., "…clean them regularly." Use what substance to clean the objects? And what exactly is the frequency of "regularly"?

Thanks for pointing this out. We have added "clean them daily with 70% ethanol".

4. In page 7, line 278, please add the institute of P.A. Spooner.

We have added this in the text "developed by P.A. Spooner, University of Edinburgh".

5. In page 14, point 7.2, what if a rat re-visits incorrect sandwells?

When a rat re-visits the incorrect sandwell (i.e., for a second time), we do not count this as another error as the maximum number of errors is 4 due to there being 5 sandwells. We have included this information in the text.

6. In page 14, point 7.3., again, what if a rat re-visits incorrect sandwells?

When a rat re-visits the incorrect sandwell, we do not count this as another error as the maximum number of errors is 4 due to there being 5 sandwells. We have included this information in the text. See 5. Above.

7. About the figures, what software do the authors use to create the figures, e.g., figure 1?

We used Adobe Illustrator to create the figures.

8. Figure 6, it is a little bit confusing to understand the counterbalance table. It looks like the "1A, 1B, 2C" used in Figure 6B matches Figure 6C. If so, please clearly indicate or otherwise describe.

We have clarified this matter in figure legend 6. We have now changed to “C) Table outlining the sandwell sets counterbalanced within and across sessions. There are 15 sandwells in total and 3 sets (set 1-3) of sandwells, each containing 5 wells (A-E). Each rat uses different wells in each encoding and recall choice trials. For example, as mentioned in Figure 6B, Rat 1 will use Sandwell 1A in encoding trial 1, Sandwell 1B in encoding trial 2 and Sandwell 2C in the recall choice trial.”

Experiment _____ – Training Session ____ Date ____/____/____

Experimenters: _____ **LUX:** _____

Lat. 1: latency to dig in correct well; Lat. 2: latency to retrieve food; Ent. Arena: latency to enter the arena from startbox (1st pellet) or homebase (2nd pellet).

- -

Rat-No.: _____

Encoding Trials

	Location	SB	Ent. Arena	Lat1	Lat 2	Eats@	Time
E1-1st			0				
E1-2nd							
E2-1st			0				
E2-2nd							

Recall Choice/ Probe Trial **Time:**

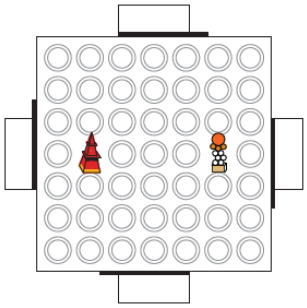
	Location	SB	Ent. Arena	Lat1	Lat 2	Eats@	Order	Choice
C1-1			0					
C1-2								

Additional **Time:**

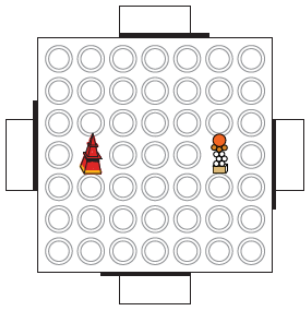
	Location	SB		Lat1	Lat 2	Eats@	Order	Choice
			0					

Encoding 1

Pellet 1

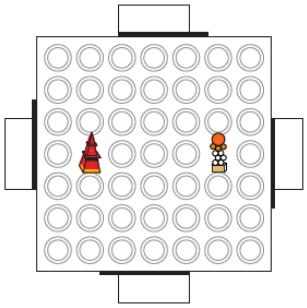


Pellet 2



Encoding 2

Pellet 1



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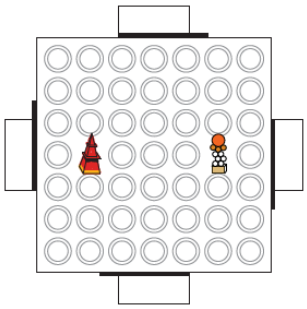
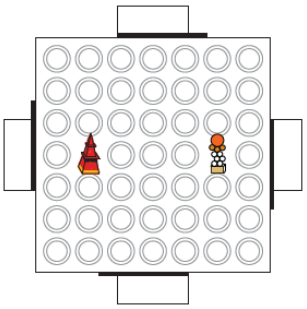
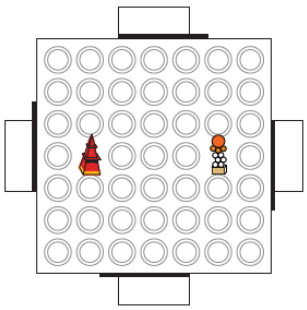


Recall Choice

Pellet 1



Pellet 2



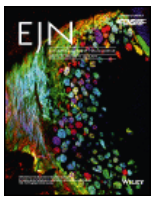
For Probe Trial Use Only

Trial Duration: 60s

Location	Dig Time	Latency
L1		
L2		
L3		
L4		
L5		

Trial Duration: 120s

Location	Dig Time	Latency
L1		
L2		
L3		
L4		
L5		



A stable home-base promotes allocentric memory representations of episodic-like everyday spatial memory

Author: Dorothy Tse, Richard G. M. Morris, Marco Peters, et al

Publication: European Journal of Neuroscience

Publisher: John Wiley and Sons

Date: Mar 20, 2020