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The spontaneous location recognition task for assessing spatial pattern separation and memory across a delay in rats and mice

Amy C. Reichelt[1,2,3]✉, Cecilia P. Kramar[4], Olivia R. Ghosh-Swaby[5], Paul A. S. Sheppard[1,2], Brianne A. Kent[6], Pedro Bekinschtein[7], Lisa M. Saksida[1,2,8] and Timothy J. Bussey[1,2,8]✉

Keeping similar memories distinct from one another is a critical cognitive process without which we would have difficulty functioning in everyday life. Memories are thought to be kept distinct through the computational mechanism of pattern separation, which reduces overlap between similar input patterns to amplify differences among stored representations. At the behavioral level, impaired pattern separation has been shown to contribute to memory deficits seen in neuropsychiatric and neurodegenerative diseases, including Alzheimer's disease, and in normal aging. This protocol describes the use of the spontaneous location recognition (SLR) task in mice and rats to behaviorally assess spatial pattern separation ability. This two-phase spontaneous memory task assesses the extent to which animals can discriminate and remember object locations presented during the encoding phase. Using three configurations of the task, the similarity of the to-be-remembered locations can be parametrically manipulated by altering the spatial positions of objects—dissimilar, similar or extra similar—to vary the load on pattern separation. Unlike other pattern separation tasks, SLR varies the load on pattern separation during encoding, when pattern separation is thought to occur. Furthermore, SLR can be used in standard rodent behavioral facilities with basic expertise in rodent handling. The entire protocol takes ~20 d from habituation to testing of the animals on all three task configurations. By incorporating breaks between testing, and varying the objects used as landmarks, animals can be tested repeatedly, increasing experimental power by allowing for within-subjects manipulations.

Introduction

Keeping memories distinct—for example, where you parked your car today, as opposed to where you parked yesterday—is a critical cognitive process. Without differentiation of memories for events, we would become confused and lost in the world. Memories of our everyday lives typically involve similar routines in familiar environments, causing episodic memory to be particularly vulnerable to interference[1]. When events that occur contain similar information, such as when identical landmarks are placed in similar locations, the inputs into the brain overlap. To distinguish between these similar events, a computational process—referred to as *pattern separation*—amplifies differences in the representations to render related memories more distinct[2,3]. So, by separating overlapping inputs during memory encoding, interference is reduced among stored memories, enabling the accurate retrieval of distinct memories. Aspects of the neural basis of pattern separation can be studied using spatial discrimination tests in humans and nonhuman animals. The hippocampus is one of the key brain regions where spatial discrimination occurs[4–8]. In the hippocampus—composed of the sub-regions of the dentate gyrus (DG), CA1 and CA3—plasticity-related mechanisms, including adult hippocampal neurogenesis, are involved in the process of pattern separation[9–15].

Insight into the neural processes that control pattern separation is critical for understanding how the hippocampus forms memories in both normal and pathological states. Translatable behavioral tests are essential to reliably measure pattern separation performance in rodents and have important

[1]Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada. [2]Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada. [3]Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia. [4]Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada. [5]Neuroscience Graduate Program, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada. [6]Department of Psychology, Simon Fraser University, Burnaby, British Columbia, Canada. [7]Instituto de Neurociencia Cognitiva y Traslacional (CONICET-INECO-Universidad Favaloro), Buenos Aires, Argentina. [8]Brain and Mind Institute, Western University, London, Ontario, Canada.
✉e-mail: amy.reichelt@adelaide.edu.au; tbussey@uwo.ca

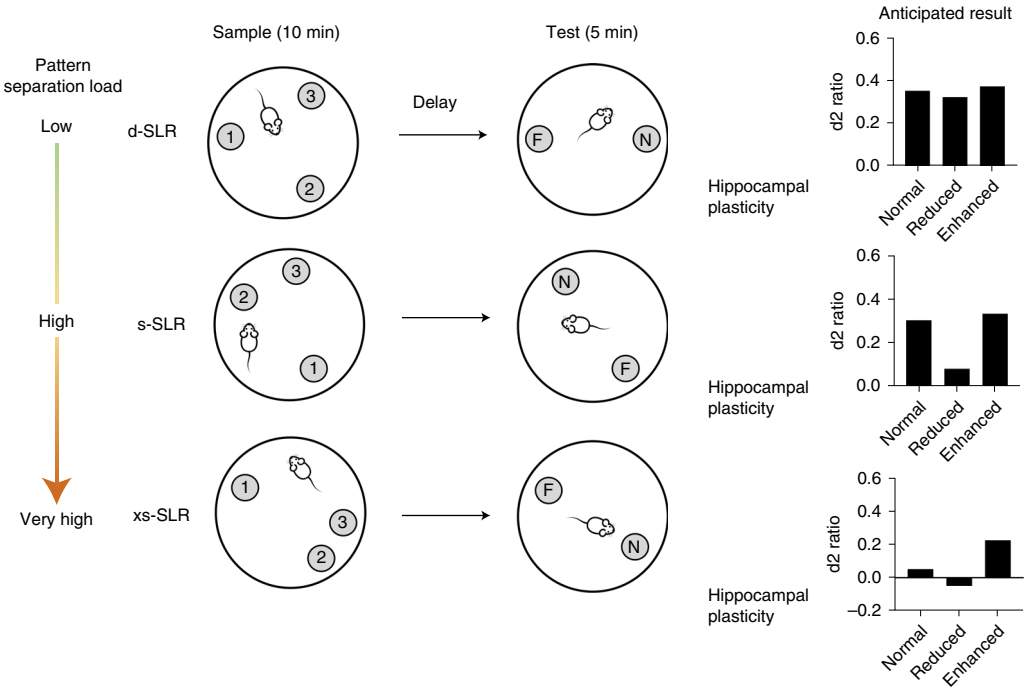

Fig. 1 | SLR task to assess pattern separation in rats or mice. During the sample phase, animals explore an arena containing identical landmarks in three locations arranged in a triangular formation. After a variable delay, memory is tested by assessing the extent to which mice can discriminate and remember the familiar location presented during sample (F) and preferentially explore the novel object location (N). Pattern separation load is varied across three configurations under which the similarity of the to-be-remembered locations are manipulated parametrically: dissimilar (d-SLR), similar (s-SLR) or extra similar (xs-SLR).

utility in the development of therapies for neuropsychiatric and neurodegenerative conditions (see refs. [16,17]), and understanding cognitive processes across the lifespan. More importantly, establishing behavioral tests that are specific for examining memory encoding processes is essential for the evaluation of pattern separation performance, rather than pattern completion, a cognitive process engaged during memory recall.

Development of the SLR task

In this protocol, we describe a spontaneous location recognition (SLR) memory test that can be used in both rats and mice. Rats or mice are exposed to two identical objects in an open field arena during the sample phase. Following a variable delay, one of the objects is moved to a novel location within the arena. Typically, rats and mice show a spontaneous preference for novelty and thus spend more time exploring the stimulus in the novel location[18]. Thus, the time spent exploring objects in the novel and familiar locations can be analyzed and used to infer memory, or lack thereof.

The SLR test can be implemented in a standard rodent facility, requires minimal specialist equipment, and is usable by personnel with basic animal behavioral training. SLR was first successfully implemented by researchers using a variety of rat strains including pigmented[9,19–21] and albino rats[22–25]. Most recently, we have successfully adapted this task for use in mice[26,27], opening up a wealth of transgenic manipulations available only in murine models, and allowing the testing of neuropathologies associated with mouse models of neuropsychiatric and neurodegenerative disease. The SLR task has been used to examine spatial memory performance in states of reduced plasticity[9,22,24,25] or in enhanced plasticity[19,20].

Prior to testing on the SLR task, the animals are habituated to the testing apparatus, which consists of a circular open field arena surrounded by easily distinguished spatial cues. Following repeated exposures to the arena during the habituation stage, the SLR task consists of two phases. First, during the sample phase, the rodent experiences the spatial arrangement of three identical objects (1, 2, 3) arranged in a triangle formation in a trial that consists of 10 min of free exploration time (Fig. 1). After a 24 h delay interval (rats), or 3 h delay interval (mice), the animal is returned to the arena for

the test phase. Here, one of the objects is in the same (F, familiar) location as the sample phase, and one object has been moved to a new location (N, novel), equidistant between two of the original objects in the sample phase configuration. Critically, with reduced distance between two of the object locations (objects 2 and 3) during the sample phase, pattern separation processes can be challenged, which later affects the recall of the memory during the test phase. This is measured when the animal re-explores the environment with an object in a familiar location and an object in a novel location. It is easier to discriminate or recognize the new position as novel when it can be more easily distinguished from the other locations in memory.

Pattern separation performance is indicated by the amount of time the animal spends exploring the object in the novel location. The relative time the animal takes to explore the two objects at test, one in a novel position and one in a familiar position, can be expressed by calculating a percentage preference to the novel position[28] or a discrimination index. Discrimination indices include the d1 ratio[29], which is the difference in time spent exploring the novel and familiar objects: $d1 = time(novel) - time(familiar)$; or the d2 ratio, which is the proportion of total exploration time spent exploring the novel object for each pair of objects $d2 = time(novel) - time(familiar)/time(novel + familiar)$. The d2 ratio takes into account individual differences in the total amount of exploration time and has been used to assess memory in the SLR test[19]. A d2 ratio of 0 indicates equal exploration of novel and familiar object locations, indicating that the memories for the locations could not be differentiated due to insufficient pattern separation. A d2 ratio of 0.33 indicates that the animal spends twice as long exploring the novel object in comparison with the familiar object, a demonstration of accurate spatial memory.

In the SLR task, we use three configurations: dissimilar (d-SLR), in which objects 2 and 3 are maximally separated; small/similar (s-SLR), in which objects 2 and 3 have a smaller separation; and extra-small/extra-similar (xs-SLR), in which objects 2 and 3 have the smallest spatial separation. These parametric shifts in distance during the encoding (sample) phase allow memory performance to be assessed under increasing cognitive load on pattern separation.

As shown in Fig. 2, manipulations that reduce plasticity within the hippocampus, such as the infusion of brain-derived neurotrophic factor (BDNF) antisense oligonucleotides (ASO) into the DG, result in memory impairments when the cognitive load on pattern separation is increased by reducing the distance between objects 2 and 3, as seen in the s-SLR configuration[19]. Manipulations that increase hippocampal plasticity during memory encoding, such as the infusion of BDNF into the DG, can facilitate memory performance under conditions where animals struggle to discriminate between the object in the familiar location and the object in the novel location, i.e., the xs-SLR configuration[19].

Comparison of the SLR test with other pattern separation tasks in rodents

A range of behavioral tasks described below have been used to study memory dependent on pattern separation in rodents. The assumption underlying these behavioral tasks is that memory representations formed after effective pattern separation is key for performing discriminations between similar contexts, locations or events.

One of the first behavioral paradigms to study spatial pattern separation in rodents was an appetitive delayed match-to-sample task developed by Gilbert and colleagues[5]. In this task, rats were trained to discriminate between the location of a food reward (correct choice) and an empty food well (incorrect choice) following a short delay (5–7 s). The locations were marked by identical objects within an open field arena, and the distance between them was systematically varied, ranging from 15 cm (small separation) to 105 cm (largest separation), with intermediate separation distances included to parametrically assess memory capabilities. Normal rats were able to perform the task accurately; however, hippocampal and DG lesions caused discrimination errors at small spatial separations[5,30].

Other pattern separation tasks that use positive reinforcement include automated touchscreen tests such as the location discrimination task[7,31], and trial-unique nonmatching-to-location (TUNL/cTUNL)[31–33]. These protocols vary the locations of to-be-remembered stimuli on a touchscreen array, with correct choices rewarded with food. Rats with hippocampal lesions were selectively impaired when discriminating between similar locations on the touchscreen[7,31–33]. These tests have been very successful; however, the requirement for positive reinforcement could present difficulties if motivational levels differ across experimental groups.

Contextual fear conditioning experiments examine whether a rodent can differentiate between 'unsafe' environments associated with a footshock and 'safe' environments associated with no shock, with features varying in similarity—including odors, shapes and lighting[13–15]. Rodents with reduced

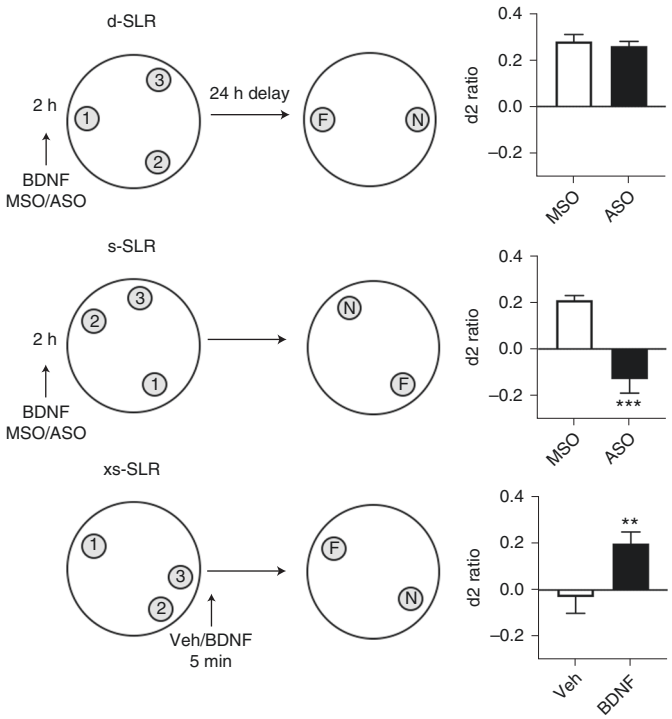

Fig. 2 | Experimental data showing the effect of manipulations that reduce or augment hippocampal plasticity on SLR performance. a,**b**, Effect of the microinfusion of BDNF antisense oligonucleotides (ASO) or BDNF missense oligonucleotides (MSO) into the DG of the hippocampus 2 h before the sample phase on discriminative memory performance in the choice phase 24 h later in the d-SLR (**a**) or the s-SLR (**b**) configuration of the task (male Sprague Dawley rats, $n = 7$). **c**, Recombinant human BDNF or saline injected into the DG 5 min after the xs-SLR sample phase enhanced memory performance during the choice phase 24 h later (male Sprague Dawley rats, $n = 7$). Discrimination is presented as the mean d2 ratio = *time(novel) − time(familiar)/time(novel + familiar)* with error bars showing ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$. A d2 ratio of 0 indicates equal exploration of novel and familiar located object. Graphs adapted with permission from ref. [19].

neurogenesis showed discrimination impairments when tested in similar contexts that shared many features, but not in dissimilar contexts[13–15]. A disadvantage of these tasks is the use of footshock, the stress of which can interact with manipulations such as drug treatments.

Naturalistic tasks that harness rodents' innate preference for novelty avoid caveats associated with altered motivation or aversive learning. A nonreinforced memory task was developed by Hunsaker, Rosenberg and Kesner[34]. Following habituation to an arena containing two objects, rats were exposed to either the same objects in a novel environment or were moved closer together. The detection of novelty was indicated by a spontaneous increase in exploration of the objects during the test phase. Rats with DG lesions exhibited lower levels of exploration to the objects when they were relocated to a new, round arena, or moved closer together, interpreted as a failure of pattern separation processes[34]. A modified spontaneous place recognition test was used by van Goethem and colleagues to explore pattern separation abilities in rats and mice[35–37]. This task uses two identical objects during the sample phase, and then moves one object to a novel location at test. Pattern separation abilities are inferred by the ability of the rodent to discriminate between spatial changes in one object's location that parametrically varies in distance from the original placement at test within a familiar environment, measured by the difference in exploration times at test, and was dependent on DG and hippocampal function. Under the protocol used by van Goethem and colleagues, the location of the objects is kept constant during the sample phases; as such, the conjunctive representations of each object's spatial location information encoded into memory during the sample is the same regardless of test trial object configuration. Thus, in this protocol, and in the delayed match to sample[5,30] and novel context recognition[34] paradigms, the location of objects is manipulated at retrieval only, rather than during encoding when pattern separation is thought to occur.

The SLR test similarly capitalizes on the ability of the animal to discriminate between familiar and novel locations; importantly, however, the similarity of spatial landmarked locations is varied during the sample phase, to systematically manipulate the load on pattern separation at the time it is thought

to happen, i.e., when memory is encoded. This design has the additional advantage that, during the choice phase, when memory ability is being assessed, testing conditions are identical across all three configurations of the task. This is a better design than one in which testing conditions vary across configurations, thus introducing potential confounds.

Experimental design

The SLR task measures location memory performance across a delay period, with behavior assessed in two phases: sample and test. To allow reliable and accurate measurement of SLR performance, we detail the important parameters that experimenters should consider before conducting a study, and considerations for the equipment setup. The Procedure itself starts with four preparatory sections: acclimatization to the housing and experimental handling (Steps 1–2); habituation to the arena (Step 3); testing in the SLR task (Steps 4–6); behavioral data analysis (Step 7). We also describe how animals can be tested repeatedly in this procedure, and the combination of pharmacological/neuromodulatory interventions.

Rodent species and strains

Laboratory-bred or commercially available rats (e.g., Long Evans, Lister Hooded, Sprague Dawley) and mice (on a C57Bl/6 background) have been behaviorally tested on the SLR task.

Because the SLR task is reliant on visual cues, the visual acuity of different mice or rat strains should be taken into consideration. Studies that have examined visual acuity in other tasks reliant on pattern discrimination and spatial cues have found that rodents with impaired visual acuity such as albino strains require either more time to learn a task or perform at chance levels when in bright light conditions[38–40]. However, studies have successfully used the albino Sprague Dawley rat strain in this SLR task[22–25], under slightly dimmed ambient lighting conditions (20–30 lux). We anticipate that other pigmented (e.g., dark agouti) and albino rat strains (e.g., Wistar) will successfully perform this task. If researchers are planning on using a strain that has not been previously tested on SLR, a pilot study should be run first to gauge performance of that strain on the task.

Ages of animals. So far, the youngest rats tested in SLR were mid/late adolescent postnatal day 40 (ref. [23]); all other rats tested have been adults (at least 8 weeks old). Adult mice that are up to 6 months old have been tested on the SLR.

Sex considerations. While biomedical and behavioral scientists have historically conducted the majority of experiments in male animals, a recent shift in many preclinical medical research policies encourages the consideration of both male and female animals in experimental designs. The use of only males is cited to avoid potential estrous-cycle-related performance variability in females, which has been shown to influence spatial memory performance in certain memory testing protocols[41]; however, male animals also show androgen and social hierarchy fluctuations that can influence behavior[42]. In the SLR task, female Sprague Dawley rats were shown to have reduced performance in the s-SLR task compared with male Sprague Dawley rats[22]; however, female Long Evans rats have been shown to perform at a similar level to males (P.B., unpublished results). Hormonal influences, such as estrous phase, pregnancy, puberty and mating status, have all been shown to affect hippocampal plasticity, and warrant further investigation using the SLR task. We encourage researchers to include male and female mice or rats when using this task and explore any sex differences if of interest.

When testing a cohort including both sexes on SLR, removing opposite sex odors from the testing arena is necessary to promote exploration of objects without the distraction of social odors. Ideally, SLR sessions can be conducted with females and males across separate days; however, if this is not possible, remove and replace at least half of the bedding from the arena floor (if used) and clean the arena walls with 50% ethanol solution when alternating rodent sexes. As per all testing sessions, the ethanol solution must be allowed to evaporate from the arena and objects as it can be an aversive irritant and decrease exploration.

Group size. In general, considerations such as group size should, ideally, be based on previous work using comparable animals (i.e., species, strain, age and background) and on the type of behavioral assay. Based on previous results where rats with reduced hippocampal plasticity showed impaired performance in the s-SLR condition[19,25], and in xs-SLR in groups with improved performance (e.g., those with increased hippocampal plasticity[19]), experimenters can expect large effect sizes

(Cohen's $d > 0.8$). Experimenters should take these effect sizes into account during the experimental planning stage to sufficiently power their experiments. The SLR task can be run within subjects with repeated testing; therefore, an experiment can be run with a relatively small sample size of 8–12 animals per experimental condition.

Animal handling

Rats and mice should be handled daily for at least 1 week prior to starting behavioral procedures to acclimate them to the experimental process and experimenters. Handling rats and mice prior to behavioral testing has been shown to facilitate exploratory behavior in novel environments and promotes activity[43,44]. Nonaversive handling to minimize stress during experiments can also impact exploration[45]. Anxiety-related reductions in exploratory behavior, caused by poor or limited handling, are likely to impair interaction with the stimulus and thus impact performance of the task[46].

Experimenters should not wear strong scents such as perfumes when handling animals for behavioral assessments; rodents have highly developed olfactory abilities, and strong scents or changing scents can be aversive or disruptive to testing. Additionally, experimenters should keep a personal laboratory coat that is not worn by someone else or change into clean clothes/scrubs upon arrival in the laboratory each day to prevent external odors (e.g., pet odors) being brought into the laboratory.

Timing of experiments

We advise that researchers consider light-phase conditions, as they can potentially interact with sex, strain, experimental manipulations and so on to influence performance. If the light cycle of the holding room is altered, experimenters should allow sufficient time for rats and mice to habituate before commencing behavioral testing. We generally allow 1 d per hour of shift.

Food restriction

Memory tasks driven by spontaneous exploration and novelty do not require food or water deprivation schedules to motivate behavior, and this task has been used successfully with animals undergoing diet manipulations[22,25]. However, food restriction to promote weight loss to 85–90% of free feeding weight can be used to encourage exploration and locomotion in rodents[47]. If restricting food, it is important to consider whether this has other effects on behavior as this could have differential effects in individuals.

Overview of the behavioral testing space

The behavioral testing space should contain a circular open field arena and video recording apparatus. The testing space containing the open field arena for rats should be contained behind a static room divider/curtain to allow the experimenter and rats not undergoing testing to remain away from the arena space, preventing distractions. Attach large black and white spatial cues to the walls of the testing room. These must remain in their locations throughout the whole testing procedure (Fig. 3b). For mice, a four-sided walled structure (61 cm width × 61 cm length × 122 cm height) can be constructed around the arena to reduce distractions and allow for animals to orient themselves in space, in which case black and white proximal spatial cues should be fixed to the walls of the structure (Fig. 3d, also see 'Visual cues').

Behavioral testing of animals should be conducted in a quiet room to minimize distractions. Animals should be familiarized with the testing room prior to undergoing behavioral assessments to reduce stress. Some groups suggest utilizing white noise or low-level background noise to reduce the impact of uncontrollable noises (e.g., external hallway doors closing).

Objects

Appropriate objects for the rodents to explore are very important (see Fig. 4 for examples of objects used with mice and rats.). A different set of three identical objects should be used for each test configuration to maintain the rodent's interest in the objects across multiple tests.

It is important that object sets are of equal interest to the rodents as this can lead to confounding results in the experiment, as reduced exploration during the sample phase is likely to impact memory encoding and subsequent performance. To make sure all used object sets are of equal interest to the rodents, pilot tests in which the exploration behavior toward all objects is assessed should be performed.

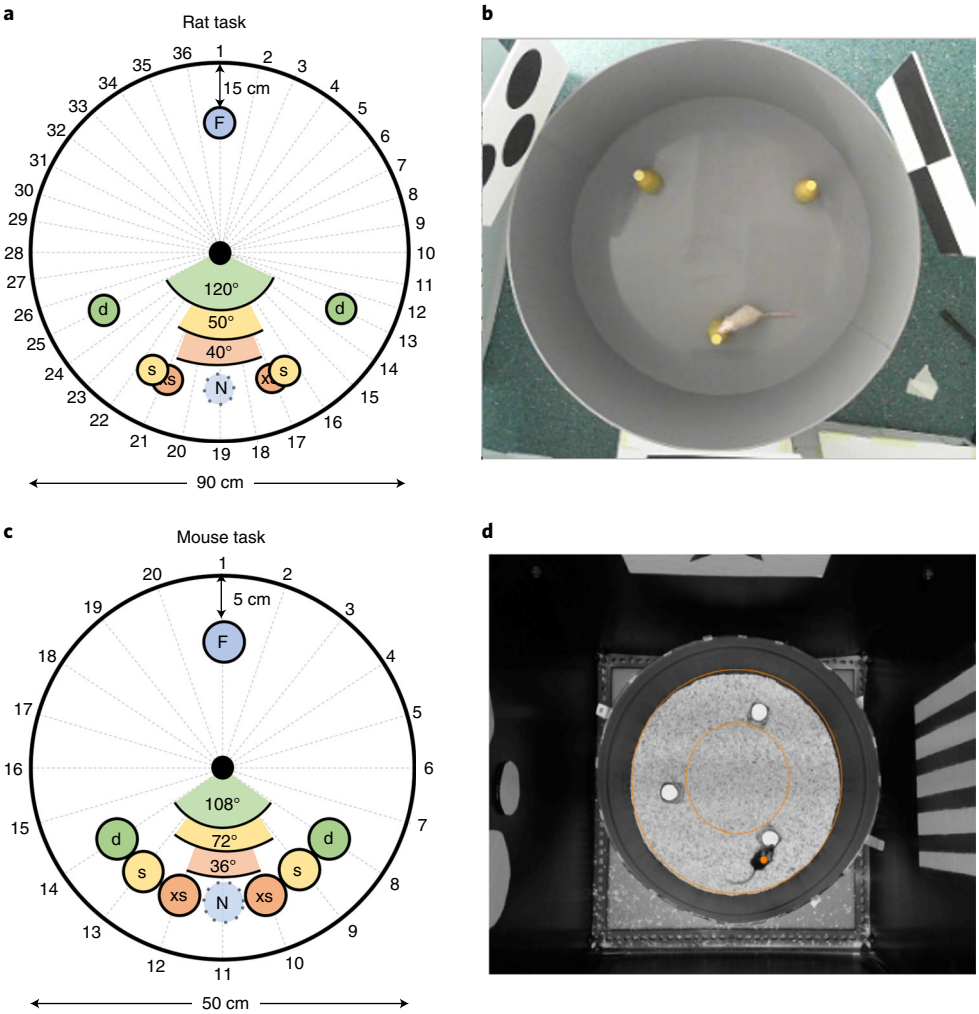

Fig. 3 | SLR apparatus setup for rats and mice. a, SLR apparatus setup for rats. Schematic of 36 marked segments from center at angles of 10°. SLR conditions (d-SLR, s-SLR, xs-SLR) vary in angle between objects to alter load on pattern separation capacity. **b**, Image of objects in the d-SLR configuration within a gray circular plastic arena as an example of the setup for rats showing two of three black and white arena spatial cues (bottom center cue out of frame). **c**, SLR apparatus setup for mice. Schematic of 20 marked segments from center at angles of 18°. SLR conditions (d-SLR, s-SLR, xs-SLR) vary in angle between objects to alter load on pattern separation capacity. **d**, Image of d-SLR as an example of an arena with surrounding four walls and three black and white cues. Each segment is marked along the outer edge of the walls of the arena.

Ideally, objects should be made from nonporous materials such as plastic, metal, glass or ceramic that are easy to clean to prevent animal odors being absorbed, as this can bias responding. Choosing objects that animals cannot climb on top of or chew can prevent this nonexploratory behavior and avoid potential arena escapes. Successfully used object choices include soda cans, glass or plastic bottles and ceramic ornaments with each set of objects differing in color, shape and material.

When selecting the objects, the size of the object in relation to the animal should be considered. For adult or adolescent rats, objects that are 15–20 cm high can be easily explored. Mice require smaller objects, and we have used objects with a height of 8–12 cm. Moreover, the width of the objects should also be taken into consideration; a narrower object allows a more precise location to be used, which is particularly important in the xs-SLR condition where two of the objects are in close proximity. These precise changes to object locations may be obscured when objects with a large base are used. Objects should be weighted at the base or fixed to the arena floor (using Blu-tak or similar) so they cannot be displaced from the location or knocked over while they are being explored; this can alter exploration, and the trial should be excluded.

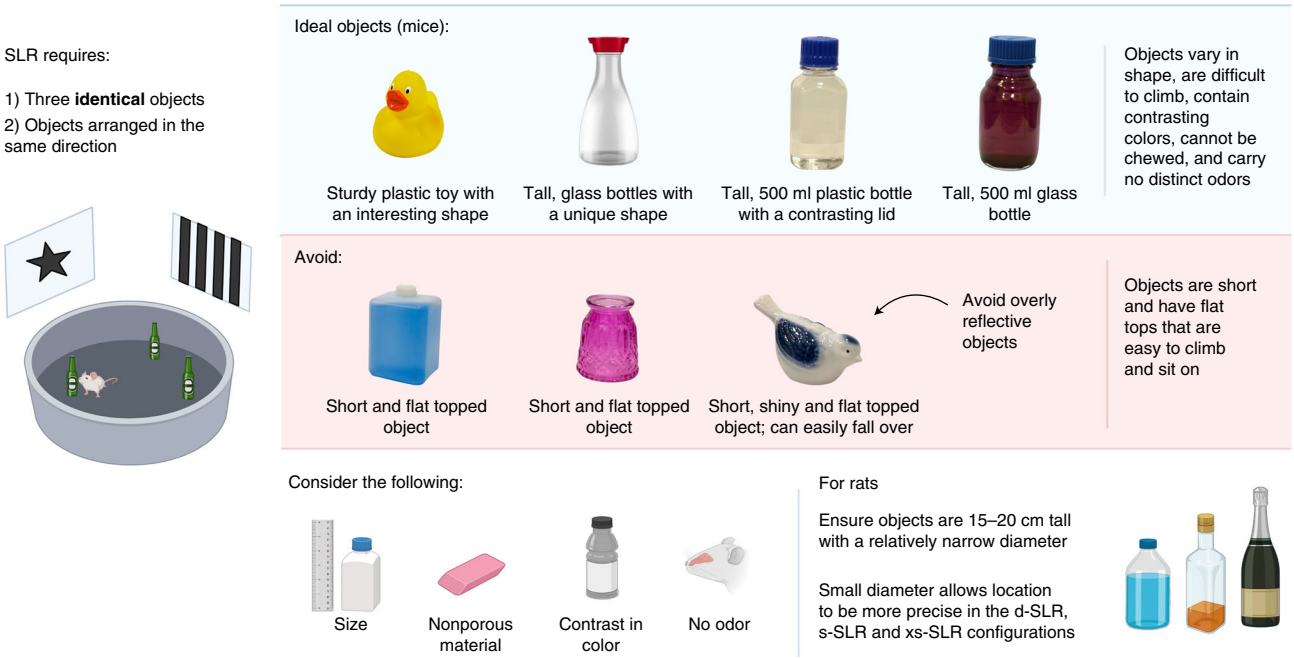

Fig. 4 | Objects used in SLR tests, showing examples of objects that we have previously used successfully with mice. Considerations that should be made when selecting objects include size, color and material. Figure created using BioRender.com.

Materials

Animals

- Rats or mice (the results shown in Fig. 5 are from adult male Sprague Dawley rats, adult male Lister Hooded rats and adult male transgenic mice on a C57BL/6 background aged 2–6 months) **! CAUTION** All experiments using live animals must be approved by and performed in accordance with all relevant governmental and institutional bodies ethical guidelines, codes and regulations involving the use of animals in research settings. We obtained permission from the National Animal Care and Use Committee of Favaloro University (CICUAL), the University of Cambridge, University of Western Ontario Animal Use Subcommittee and the Animal Ethics Committee at UNSW Sydney. **▲ CRITICAL STEP** Ensure reliable identification of each animal, making clear tail markings with permanent marker or ear notches.

Reagents

- Required for husbandry, such as rodent food pellets, low dust bedding materials (Bed-o'Cobs 1/8″ or similar)
- Cleaning materials to eliminate odors (e.g., 10% vol/vol ethanol solution, disposable paper towels)

Equipment setup

Animal housing

Rats and mice are social animals and should be housed in groups of two to four animals to prevent the negative effects of social isolation (for cage sizing suggestions[48,49]). Home cages should contain sawdust or corn cob bedding and (optional, although recommended) environmental enrichment items including tunnels, nesting materials and chew blocks, with cages cleaned regularly. The housing room should be maintained at a constant temperature (21 ± 2 °C) and humidity (55 ± 10%). Lighting is usually on a standard 12 h light–dark cycle, and we favor testing rats and mice in the active (dark) period of their circadian cycle, as this may enhance exploratory activity.

Arena to test rats

For testing rats, we use a circular open field arena 90 cm diameter with 45 cm high walls[9,19,20] or 100 cm diameter with 50 cm high walls[22,23,25] (Fig. 3b), constructed of black or dark-gray plastic, respectively. Divide the arena into 36 equal segments separated by a 10° angle (Fig. 3a). The floor of the arena should be covered with corncob or sawdust bedding to hide the location labels marked on

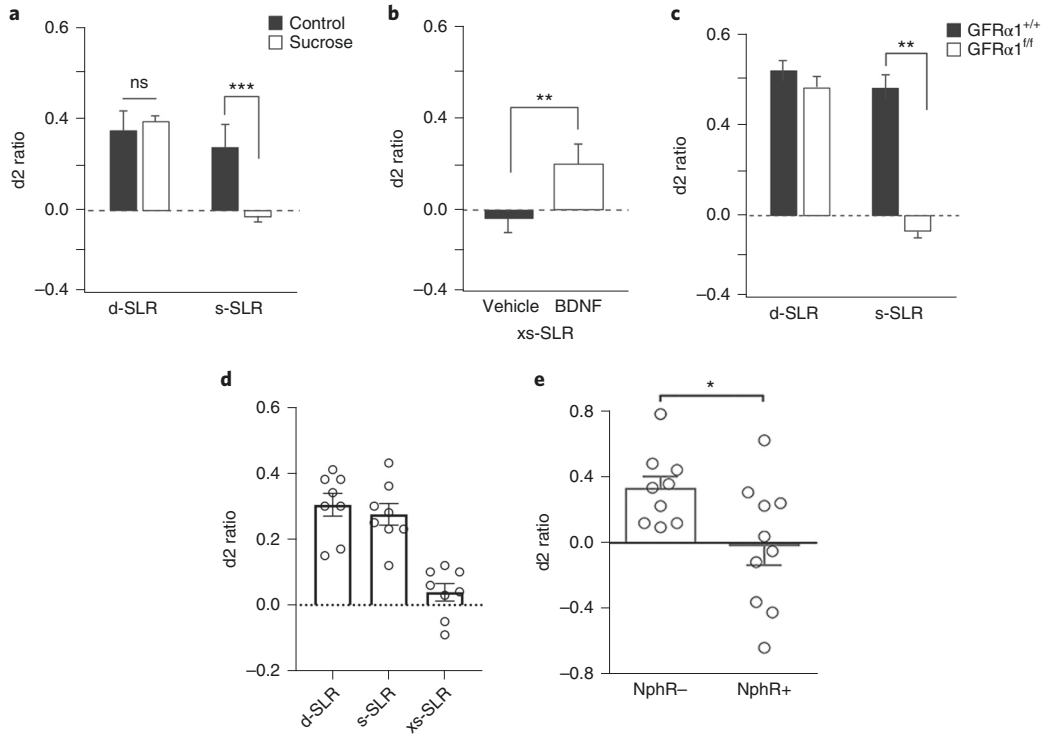

Fig. 5 | SLR test performance in rats and mice. a, Discrimination (d2) ratios of performance during the test phase in the d-SLR and s-SLR configurations showing that daily 2 h access to 10% sucrose solution for 28 d impaired s-SLR performance in 8-week-old adult male Sprague Dawley rats, $n = 6$ per group. **b**, d2 ratio of performance during the test phase of the xs-SLR configuration showing that intrahippocampal infusion of BDNF enhanced performance at the xs-SLR version of the task in adult male Lister Hooded rats (250–300 g). **c**, d2 ratios of performance during the test phase in adult male control ($Gfr\alpha1^{+/+}$) and Gfrα1 mutant ($Gfr\alpha1^{f/f}$) mice ($n = 8$–10 per genotype, aged 8+ weeks) in the d-SLR and s-SLR configurations of the SLR. **d**, d2 ratio of performance during the d-SLR, s-SLR and xs-SLR tests in 10-week-old male C57Bl/6 mice ($n = 8$) (A.C.R., L.M. S. & T.J.B., unpublished data; see source data). **e**, s-SLR version of the SLR test combined with optogenetic stimulation in adult (8–30-week-old) male and female double transgenic SST-IRES-Cre+/+ and Ai39+/− (NphR+, $n = 10$) and control mice (NphR−, $n = 9$). This example shows that optogenetic inhibition of somatostatin cells within the dorsoventral DG of the hippocampus using halorhodopsin (NphR-ON) disrupted performance of the s-SLR at test. Images adapted with permission from: **a**, ref. [25], **b**, ref. [19], **c**, ref. [26], **f**, ref. [27].

the arena floor[9,19–21]. Alternatively, the floor can be left clear of bedding and the locations of the objects determined by a standardized projection on the video monitor[22,23,25]. ▲ **CRITICAL** By numbering the segments, the locations used in each test can be counterbalanced for animals to avoid repetition and control for potential side biases (see 'Troubleshooting', Supplementary Fig. 1).

Arena to test mice

For testing mice, we use a circular open field arena 50 cm diameter with 30 cm high walls, made from black plastic. The floor of the arena should be covered with corncob bedding. Divide the arena into 20 equal segments separated by an 18° angle, and label these points with tape on the outside edges of the wall (Fig. 3c).

Setup of video recording

Videos of the behavioral tests should be made using a computer or external video recording device that is connected to a webcam or video camera. Videos should be recorded from directly overhead using a webcam (e.g., Logitech HD Pro Webcam C920) or video camera with sufficient resolution to allow the experimental observer to clearly determine when the objects are being explored.

Visual cues

Spatial cues are required that are distinct and easily visible to rodents while in the arena. For rats, black and white geometric cues, e.g., circle or square patterns, made of cardboard (40 cm wide × 30 cm high) can be mounted above the arena to provide discrete spatial cues (Fig. 3b). Incidental room cues can form additional geometric cues that are critical for encoding spatial memories[50]. For mice, white geometric cues, e.g., stars, stripes and circles, can be mounted on three of four black walls (each 61 cm wide by 122 cm high) housing the SLR arena (Fig. 3d). Lighting is also important. Mice and rats innately find brightly lit open spaces aversive[51]. Previously, we have used

20–30 lux white light using diffuse overhead lighting. This provides ambient lighting whereby spatial cues are visible but the light intensity is not aversive.

Objects to be explored

See Fig. 4 for examples of objects we have used successfully.

Procedure

Transportation and acclimatization ● Timing 7 d minimum

1 If animals are obtained from an outside source (i.e., different from the animal facility in which behavioral testing will occur), acclimatize them to the vivarium and housing after transport without any procedures, with food and water ad libitum, for a minimum of 7 d. Begin handling and weighing the animals (by proceeding to the next steps) after 3 d of acclimatization.

Habituation to the experimenter ● Timing 5–7 d; 2 min per rodent per day

2 Habituate animals to the experimenter by regular handling; each animal should be handled daily for at least 2 min for a 5 d period, or more if necessary.

▲ CRITICAL STEP Experimenters should keep everything consistent over the entire protocol. As rodents have a highly developed olfactory system, they rely heavily upon smell. Therefore, it is important that experimenters keep their smell as similar as possible across testing days and avoid the use of strong perfumes. Keep a personal laboratory coat that is not worn by someone else, or change into clean clothes/scrubs upon arrival in the laboratory each day.

▲ CRITICAL STEP We advise consulting with your institutional animal care regulatory body regarding matters such as food restriction, appropriate housing and other similar issues when you are planning and designing experiments.

Habituation to the arena ● Timing 2–5 d; 10 min per rodent per day

3 Once rats are habituated to the experimenter, habituate rats to the arena for 10 min for 2–5 d prior to testing. Habituate mice to the arena for 10 min for 3–5 d prior to testing. Video tracking software can be used to measure distance traveled and amount of time spent in the periphery versus the center of the arena as quantitative measures of habituation.

▲ CRITICAL STEP Pre-exposure to the testing apparatus is essential to familiarize rodents with the arena and facilitate learning about the environmental cues critical for encoding spatial memories. When placed into an unfamiliar open field arena, anxious mice show reduced movement around the arena, and are reluctant to enter or spend time in the open, unprotected central area[52]. Open-field behavior is subject to habituation; re-exposures to the apparatus typically result in reduced activity and a clear shift in behavior, with more time spent grooming or sitting still, and less time spent at the periphery of the arena (thigmotaxis[53]). Interestingly, habituation to the open field often differs between 'normal' animals in comparison with animals with lesions in the frontal cortex or hippocampus, in that lesions to these brain regions often result in increased baseline locomotion (hyperactivity) and no decrease in locomotion over habituation sessions[54–56]. This should be taken into consideration when testing rat/mouse models of neurodegeneration and/or lesions, and experimenters should consider increasing the number of habituation sessions or using an alternative metric (e.g., time spent in center of the arena versus periphery).

▲ CRITICAL STEP Time of day should be kept consistent for habituation and testing.

▲ CRITICAL STEP Following transport of animals to the testing room, allow 30 min for rodents to acclimatize. Rodents should be left undisturbed during this period.

▲ CRITICAL STEP If using bedding across the arena floor, between habituation sessions for each animal, remove one scoop of bedding from the arena and replenish with a scoop of clean bedding. Manually rustle the bedding in the arena around to disperse odors that may attract the animal to one particular area, and distract animals from exploring objects. At the end of testing for the day, remove at least half of the bedding, wipe down all walls with 50% ethanol and then replenish with fresh bedding for the next day of testing.

▲ CRITICAL STEP If not using bedding on the arena floor, the testing arena should be cleaned thoroughly between each animal using paper towels and a 10% ethanol solution to remove olfactory traces. At the end of testing for the day, wipe down all walls with 50% ethanol and allow to fully evaporate.

▲ CRITICAL STEP Make sure that the 10% ethanol solution has completely evaporated when the next animal is introduced into the testing arena, as ethanol can be aversive and a skin irritant.

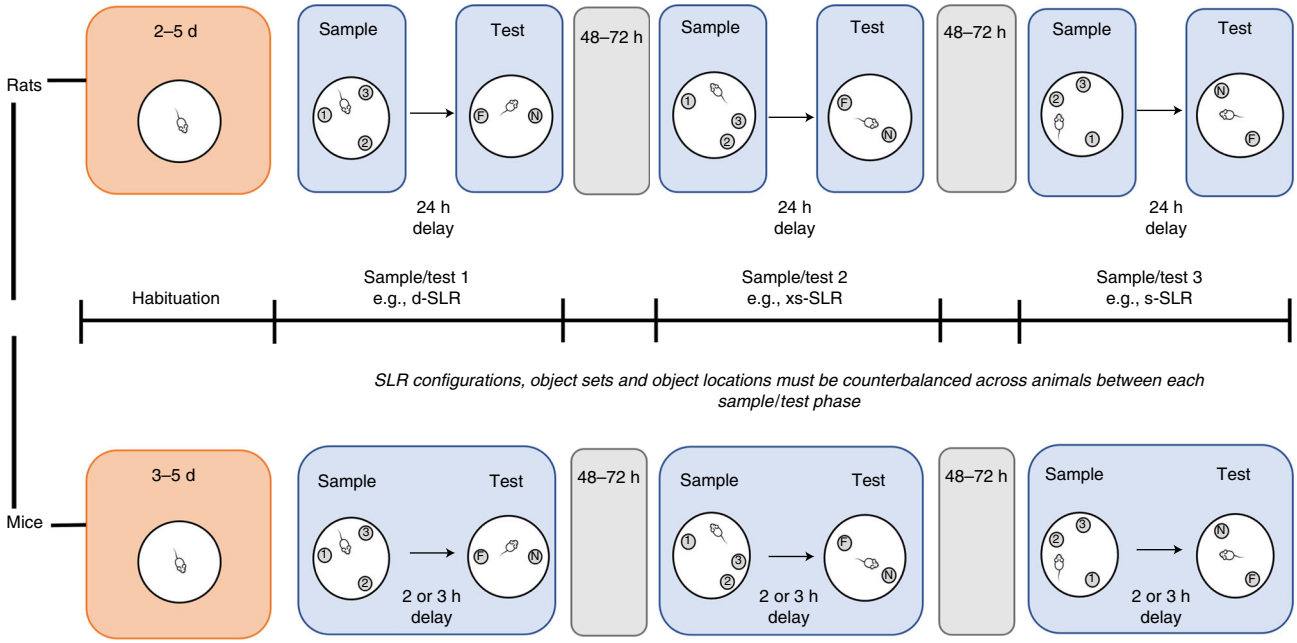

Fig. 6 | Flowchart of the experimental protocol for rats and mice. Following habituation, the test animals are exposed to different SLR configurations (d-SLR, s-SLR, xs-SLR) as shown. Rats have a 24 h delay between the sample and test phase, whereas mice have a 2 or 3 h delay. We advise a 48–72 h break (2–3 d) between testing on each configuration, particularly if animals are undergoing pharmacological manipulations or intracranial microinfusions to allow full washout of any drugs.

Flowchart of procedure

Sample phase ● Timing Variable; 10 min per rodent per day

4 For all SLR conditions, expose animals to three identical objects in a triangular formation for 10 min (Fig. 6). SLR configuration (d-SLR, s-SLR, xs-SLR) determines the distance between objects using the marked segments (angles of 18° from center). For *rats*, place objects 2 and 3 120° (d-SLR), 50° (s-SLR) or 40° (xs-SLR) apart from the center. For *mice*, the same angles for d-SLR and s-SLR have been used previously (120° and 50° (ref. [26])), or we have also used objects 2 and 3 placed 108° (d-SLR), 72° (s-SLR) or 36° (xs-SLR) apart from center (Fig. 3c). Place object 1 at an equal distance from the other two objects. Note that the location of object A1 landmarks the familiar location.

▲ **CRITICAL STEP** Following transport of animals to the testing room, allow 30 min for rodents to acclimatize prior to exposure to the objects. Rodents should be left undisturbed during this period.

▲ **CRITICAL STEP** Object choice, location quadrants of objects (i.e., placement of objects 2 and 3) and order of SLR conditions (d-SLR, s-SLR and xs-SLR) should be counterbalanced between animal groups (see Supplementary Fig. 1 for examples of counterbalanced arrangements of objects).

▲ **CRITICAL STEP** The sample and choice locations should be different for each animal, to avoid any potential location bias. Also, if repeated testing occurs, use the farthest distance to the ones used during the first and subsequent trials.

▲ **CRITICAL STEP** Time of day should be kept consistent for habituation and testing.

▲ **CRITICAL STEP** Between testing sessions for each animal, remove one scoop of bedding from the arena and replenish with a scoop of clean bedding. Manually redistribute the bedding in the arena around to disperse odors. At the end of testing for the day, remove at least half of the bedding, wipe down all walls with 50% ethanol and then replenish with fresh bedding for the next day of testing. If bedding is not used, the arena floor must be cleaned with 10% ethanol solution and dried thoroughly between testing sessions for each animal. Note that we have run the SLR with and without bedding and did not see differences in SLR performance

▲ **CRITICAL STEP** Clean objects and arena between animals. The testing arena and the objects must be cleaned thoroughly after each animal with paper towels and a 10% ethanol solution to remove all olfactory traces that could attract or repel an animal toward or away from an object or location.

▲ CRITICAL STEP Note that we recommend times of 10 min and 5 min for sample phase and test phase, respectively. Novelty preference tends to be strongest in the initial 2–3 min of the testing phase[28,29,57]. A time of 5 min in the SLR test phase maintains location novelty without the object location becoming more familiar over a course of time[57,58]. Additionally, the sample phase duration requires more time and opportunity for familiarization as there are three objects located in the arena.

Delay ● Timing 24 h for each rat, 2–3 h for each mouse

5 Following the sample phase, allow a 24 h delay (rats) or a 2 or 3 h delay (mice) before testing (Fig. 6). Prior studies have shown that these delays are appropriate for memory in rats or mice without extreme difficulty. If experimenters are interested in time-dependent memory or are looking to troubleshoot, the delay phase duration can be manipulated. Future studies could examine how variation in delays impact SLR performance, for example extending the delay to 48 h in rats or 6 h in mice to place additional load on memory processes. Critically, normal animals should be able to perform the d-SLR condition following the chosen delay duration, as this provides a baseline of performance to compare against.

Test phase and behavioral analysis ● Timing Varies depending on numbers of animals tested; 5 min per rodent per day

6 After the delay phase, give rats or mice a 5 min testing phase using two of the identical objects experienced during the sample phase (Fig. 6). To prevent olfactory cues, the test objects in the novel and familiar locations should have been thoroughly cleaned from the sample phase and always cleaned between animals. Place one object in a familiar location, and another in a novel location. The familiar location is defined as the same position as object 1 in the sample phase. The novel location is defined as a position exactly in between the ones in which objects 2 and 3 were located (i.e., 180° from A1) during the sample phase (see configurations in Fig. 3a,c).

? TROUBLESHOOTING

▲ CRITICAL STEP Time of day should be kept consistent for testing each animal.

7 Manually score animal exploration or use video tracking software. Commercial video tracking behavioral software (e.g., AnyMaze, Ethovision) or open-source software[59–61] can be used to measure distance traveled and amount of time spent in the periphery versus the center of the arena. However, we recommend manually scoring animal exploration as many video tracking software programs are less able to distinguish animal exploration from the animal rearing, standing on the object, or when in proximity of the object.

▲ CRITICAL STEP If manually scoring object exploration, we recommend defining exploration as active investigation, i.e., rodent's snout facing towards the object and vibrissae moving around the object at a distance >2 cm to the object, or, if rearing, contact with and active investigation (e.g., vibrissae moving) of the object without the head overlooking the object[58]. As the central features of exploratory behaviors are preserved between rats and mice[62], we do not suggest different criteria for exploration in rats and mice.

▲ CRITICAL STEP The experimenter scoring exploration behavior during the test phase should be blinded to the identity of the location of objects (i.e., novel, familiar). This is achieved by coding the objects as 'left' and 'right', and the identity along with the video information is recorded in a detailed spreadsheet maintained by the experimenter (see source data for Fig. 5d).

8 After an appropriate delay phase, repeat Steps 6 and 7 using a different SLR condition. There should be a 48–72 h break period before animals are tested in a different SLR condition. If all conditions are to be repeated again with the same animals, we recommend at least a 48 h break period before retesting. Additionally, new identical objects should be used to maintain novelty and interest.

▲ CRITICAL STEP Between testing sessions for each animal, remove one scoop of bedding from the arena and replenish with a scoop of clean bedding. Manually redistribute the bedding in the arena around to disperse odors. At the end of testing for the day, remove at least half of the bedding, wipe down all walls with 50% ethanol and then replenish with fresh bedding for the next day of testing. If bedding is not used, the arena floor must be cleaned with 10% ethanol solution and dried thoroughly between testing sessions for each animal.

▲ CRITICAL STEP The testing arena and the objects must be cleaned thoroughly after each animal with a 10% ethanol solution and dried with paper towel to remove all olfactory traces that could attract or repel an animal toward or away from an object or location.

Troubleshooting

Consider excluding animals that do not reach the criterion for exploring objects (see 'Statistical analysis' for further details of the criterion). Although generally we have found that <5% of animals do not explore the objects to criterion, pharmacological manipulations or transgenic strains may show differences in exploration that should be taken into consideration. General troubleshooting advice can be found in Table 1. It is generally good practice to have assessed the object sets being used in the study for exploration or preferences prior to starting an experiment.

Table 1 | Troubleshooting table

Problem	Possible reason	Solution
The rodent does not investigate objects sufficiently (i.e., does not meet set investigation criteria)	Rodent is stressed/anxious	Check for environmental stressors (e.g., noisy equipment, temperature, humidity, air flow, scents, bright lights, etc.), and remove or reduce where possible
		Increase the acclimation period following animal transport to testing room
		Ensure home cage changes do not occur on the same day of testing; a minimum of 24 h should elapse between cage change and testing
		Increase handling sessions to familiarize animals to the experimenter
		Increase the number of arena habituation sessions
	Rodent motivation/arousal is too low	Restricting food to reduce animals to 85–90% free feeding weight can invigorate exploration
		If food restricted, feeding should occur after the SLR session
	Experimental treatment (e.g., drugs) or condition (e.g., genotype/strain) has altered rodent motivation, locomotor abilities and/or arousal	Consider giving treatment at an earlier time point (if possible), and check to see if deficits are still observed
		Consider dividing the sample phase into two sessions of 5 min with a short delay (~10 min)
	Rodent is disinterested in objects	Ensure the objects used are interesting to rodents; increase attractiveness of objects by changing the tactile (e.g., adding holes/contours) and visual (e.g., color or contrast) properties
		The concentration of ethanol used to clean objects is too high, which is aversive to rats and mice
		Consider dividing the sample phase into two sessions of 5 min with a short delay (~10 min)
Rodents are avoiding object in novel location (neophobia)	Objects retain scents from previous animals or from ethanol cleaning	Ensure that objects are thoroughly wiped down with 10% ethanol solution between rodents/trials and that the ethanol has completely evaporated prior to starting the trial
Rodents are climbing onto objects or jumping up onto arena walls	Objects or arena walls are too short	Use taller objects or make the objects taller (e.g., attaching objects to the top or bottom); use an arena with taller walls that still allow observation of the distal spatial cues
Rodent not exploring objects and remaining at the edges of the arena	Rodent still anxious in SLR arena	More handling of rodent or habituation trials are required prior to testing
		Ensure the testing room is free from potential stressors
		Increase the acclimation period following animal transport to testing room
d2 ratio is 1 or −1	Side preference	Use different locations of objects across animals
		Check for stressors (e.g., noisy equipment, temperature, humidity, air flow, scents, bright lights, etc.), and remove or reduce where possible; ensure SLR arena is equally lit; move home cages farther away from SLR arena, or distribute equally around the SLR arena
	Novel object location is still too novel (leading to higher performance)	Increase delay duration
		Shorten sample duration
		Ensure objects are identical
	Scents left on objects by previous rodent leading to aversion or preference	Ensure objects are thoroughly wiped down with 50% ethanol solution between rodents. Allow ethanol to fully evaporate prior to the start of the next trial/rodent

Table continued

Table 1 (continued)

Problem	Possible reason	Solution
Unequal exploration of objects during the sample phase	Arena is located within a distracting environment	Enclose arena in separate testing room or enclosure
	Stressors (e.g., noises) inside the testing room	Check for stressors (e.g., noisy equipment, temperature, humidity, air flow, scents, bright lights, etc.), and remove or reduce where possible
	Unequal lighting in the SLR arena	Ensure the SLR arena is equally lit
d2 values in the xs-SLR configuration in control rodents are greater than chance	Object location is easily discriminable	Increase delay duration
		Ensure objects are identical
	Object placed in incorrect locations during sample (decrease in location similarity) or test phases	Ensure objects are placed in the correct locations during sample and test phases
	Objects have not been cleaned sufficiently between trials	Clean objects thoroughly with 10% ethanol, and allow to fully dry between trials
d2 values in d-SLR and/or s-SLR in control rodents are not greater than chance	Rodents are not investigating objects sufficiently during sample phase	See suggestions elsewhere in this table for increasing object investigation
	Object placed in incorrect locations during sample (decrease in location similarity) or test phases	Ensure objects are placed in the correct locations during sample and test phases
	Objects have not been cleaned sufficiently between trials	Clean objects thoroughly with 10% ethanol, and allow to fully dry between trials
Performance varies within a testing condition across testing days for similar groups and conditions	Testing conditions are not consistent across days	Ensure that animals are fully habituated to the testing environment
		Ensure that testing occurs at the same time on each testing day to minimize circadian influence on results
		Make sure you use different sets of objects for different trials
		Make sure home cage changes do not occur before animals undergo behavioral assessment; a minimum of 12 h should elapse between cage change and behavior testing
		Increase the acclimation period following animal transport to testing room
		Ensure testing apparatus is placed in the same location across testing days
Total investigation times during sample and test vary between object sets	Rodents prefer one or more object sets over others	Pilot testing with multiple different sets of objects is necessary before starting the experiment to ensure that exploration times do not differ
		Counterbalance object sets between animals for different trials

Timing

Step 1, transportation and acclimatization (if animals are arriving from a different facility/location): days 1–7
Step 2, habituation to the experimenter (including weighing and any food restriction): days 3–9
Step 3, habituation to the SLR arena: days 10–15 (rats), days 10–15 (mice); 10 min/rodent
Step 4, SLR task—sample: day 16 (rats), day 16 (mice); 10 min/rodent
Step 5, SLR task—delay: 24 h delay for rats, 2 or 3 h delay for mice
Step 6, SLR task—test: day 17 (rats), day 16 (mice); 5 min/rodent
Steps 7 and 8, SLR task—test for other SLR configurations, 5 min/rodent, allowing 48–72 h between testing runs

Anticipated results

The typical results presented in this section are based on the performance of male Sprague Dawley rats ($n = 24$)[25], male Lister Hooded rats ($n = 24$)[19] and male transgenic mice on a C57BL/6 background ($n = 8$ and 10 per group)[26,27]. All data shown in Fig. 5 are presented as means ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Statistical analysis

The SLR task provides measures for both the exploration times (time) and discrimination performance (d2 ratio). The outcome measures of the task are the times spent by rodents exploring each object location during the sample phase and the test phase.

During the sample phase, the times spent exploring the three objects are represented by '1', '2' and '3', respectively. The times spent exploring the object in the familiar location and the moved object in the novel location in the test phase are represented by 'familiar' and 'novel', respectively. From these exploration times, the following variables are calculated: total sample exploration (S), total test exploration (T) and d2 ratio. Total exploration times (i.e., sum of the exploration time of all objects) in both sample and test should be sufficient (i.e., sample ≥15 s and test ≥5 s) to reliably assess discrimination performance. Moreover, experimenters should set a criterion for exploration of each object[63]. In our previous work, we have set a criterion of ≥5 s per object in the sample phase and ≥2.5 s per object in the test phase. On average, we have observed typical sample exploration times of 25–43 s/object during the sample phase and 12–39 s/object during the test phase in rats[19] and mean exploration times of 13–16 s/object during the sample and 8–14s/object during the test in mice[26,27] (see Table 1 for further guidance regarding optimizing object exploration).

During the sample phase, animals should show equal exploration of each of the objects. This can be represented as a percentage of total time spent exploring the objects, with equal exploration equal to 33%. Statistical analysis by ANOVA can be conducted on exploration times between experimental groups to ensure that equal exploration of the objects occurs during the sample phase.

The d2 index is a relative measure of discrimination across the novel and familiar located objects at test and is corrected for total exploratory activity in the test trial. The d2 index can range from −1 to 1, with −1 indicating complete preference for the familiar object location, 1 indicating complete preference for the novel object location and 0 signifying no preference for either object location (chance performance). In general, a d2 score of 0.33 indicates that an animal spent twice as much time exploring the novel location compared with the familiar location. Statistical analysis by ANOVA can be conducted on d2 ratios between experimental groups in each configuration of the SLR task to determine group differences. If one group of animals is being used and tested in a within-subjects design, a repeated measures ANOVA can be conducted using the performance measures across each configuration (d-SLR, s-SLR and xs-SLR). One-way comparisons of group means against zero (chance) can also be performed.

A d2 index should never be 1 or −1, as this means an animal did not explore one of the objects. Zero, or very low, exploration of one object biases data, and the animal should be excluded from the dataset; this can emerge when an animal is anxious or distracted during the test session (see Table 1 for further guidance). As per the criteria described above, animals should explore the objects for a minimum amount of time and animals should always explore both objects to draw reliable conclusions about memory performance.

Additional measures, such as total distance (locomotion) during trials, can serve as an assessment of locomotor activity, which can be a valuable readout in, for example, pharmacological studies where drugs may have an impact on activity and movement, or when using cognitive deficit models (e.g., transgenic, diet manipulation or pharmacological models). Total distance in the arena can be recorded and analyzed when using an automated animal tracking program, such as AnyMaze (Stoelting) or Ethovision (Noldus). Furthermore, the integration of machine learning driven analysis software such as DeepLabCut[60] could further automate the accurate measurement of exploration behaviors during the sample and test. Moreover, future research using this protocol could be used to address questions regarding the neural correlates and circuitry of memory when conducted in tandem with cutting-edge neurotechnologies such as head-mounted miniscopes for calcium imaging, providing insights to questions that have never before been answerable.

Data availability

The authors declare that the main data supporting the findings of this protocol are available within the article and and/or are already published and included with permission. Extra data are available from the corresponding author upon request. Source data are provided with this paper.

References

1. Tulving, E. Episodic and semantic memory. in *Organization of Memory* (eds Tulving, E. & Donaldson, W.) 381–403 (Academic Press, 1972).
2. Rolls, E. The mechanisms for pattern completion and pattern separation in the hippocampus. *Front. Syst. Neurosci.* **7**, 74 (2013).
3. Marr, D. & Thach, W. T. A theory of cerebellar cortex. in *From the Retina to the Neocortex* (ed. Vaina, L.) 11–50 (Springer, 1991).
4. Frankland, P. W., Cestari, V., Filipkowski, R. K., McDonald, R. J. & Silva, A. J. The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. *Behav. Neurosci.* **112**, 863 (1998).
5. Gilbert, P. E., Kesner, R. P. & DeCoteau, W. E. Memory for spatial location: role of the hippocampus in mediating spatial pattern separation. *J. Neurosci.* **18**, 804–810 (1998).
6. McDonald, R. J. & White, N. M. Hippocampal and nonhippocampal contributions to place learning in rats. *Behav. Neurosci.* **109**, 579 (1995).
7. McTighe, S. M., Mar, A. C., Romberg, C., Bussey, T. J. & Saksida, L. M. A new touchscreen test of pattern separation: effect of hippocampal lesions. *Neuroreport* **20**, 881–885 (2009).
8. Kent, B. A., Hvoslef-Eide, M., Saksida, L. M. & Bussey, T. J. The representational–hierarchical view of pattern separation: not just hippocampus, not just space, not just memory? *Neurobiol. Learn. Mem.* **129**, 99–106 (2016).
9. Bekinschtein, P. et al. Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories. *Hippocampus* **24**, 905–911 (2014).
10. Clelland, C. et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **325**, 210–213 (2009).
11. Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H. & Bussey, T. J. Running enhances spatial pattern separation in mice. *Proc. Natl Acad. Sci. USA* **107**, 2367–2372 (2010).
12. Kheirbek, M. A., Klemenhagen, K. C., Sahay, A. & Hen, R. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat. Neurosci.* **15**, 1613–1620 (2012).
13. Nakashiba, T. et al. Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell* **149**, 188–201 (2012).
14. Sahay, A. et al. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466–470 (2011).
15. Tronel, S. et al. Adult-born neurons are necessary for extended contextual discrimination. *Hippocampus* **22**, 292–298 (2012).
16. Quian Quiroga, R. No pattern separation in the human hippocampus. *Trends Cogn. Sci.* **24**, 994–1007 (2020).
17. Suthana, N., Ekstrom, A. D., Yassa, M. A. & Stark, C. Pattern separation in the human hippocampus: response to Quiroga. *Trends Cogn. Sci.* **25**, 423–424 (2021).
18. Berlyne, D. E. Novelty and curiosity as determinants of exploratory behaviour. *Br. J. Psychol.* **41**, 68 (1950).
19. Bekinschtein, P. et al. BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories. *Cell Rep.* **5**, 759–768 (2013).
20. Kent, B. A. et al. The orexigenic hormone acyl-ghrelin increases adult hippocampal neurogenesis and enhances pattern separation. *Psychoneuroendocrinology* **51**, 431–439 (2015).
21. Miranda, M. et al. NMDA receptors and BDNF are necessary for discrimination of overlapping spatial and non-spatial memories in perirhinal cortex and hippocampus. *Neurobiol. Learn. Mem.* **155**, 337–343 (2018).
22. Buyukata, C., Vukalo, M., Xu, T. J., Khore, M. A. & Reichelt, A. C. Impact of high sucrose diets on the discrimination of spatial and object memories with overlapping features. *Physiol. Behav.* **192**, 127–133 (2018).
23. Gilchrist, C. P. et al. Hippocampal neurogenesis and memory in adolescence following intrauterine growth restriction. *Hippocampus* **31**, 321–334 (2021).
24. Hueston, C. M. et al. Chronic interleukin-1β in the dorsal hippocampus impairs behavioural pattern separation. *Brain Behav. Immun.* **74**, 252–264 (2018).
25. Reichelt, A. C., Morris, M. J. & Westbrook, R. F. Daily access to sucrose impairs aspects of spatial memory tasks reliant on pattern separation and neural proliferation in rats. *Learn. Mem.* **23**, 386–390 (2016).
26. Bonafina, A. et al. GDNF and GFRα1 are required for proper integration of adult-born hippocampal neurons. *Cell Rep.* **29**, 4308–4319. e4304 (2019).
27. Morales, C. et al. Dentate gyrus somatostatin cells are required for contextual discrimination during episodic memory encoding. *Cereb. Cortex* **31**, 1046–1059 (2021).
28. Clark, R. E., Zola, S. M. & Squire, L. R. Impaired recognition memory in rats after damage to the hippocampus. *J. Neurosci.* **20**, 8853–8860 (2000).
29. Dix, S. L. & Aggleton, J. P. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav. Brain Res.* **99**, 191–200 (1999).
30. Gilbert, P. E., Kesner, R. P. & Lee, I. Dissociating hippocampal subregions: a double dissociation between dentate gyrus and CA1. *Hippocampus* **11**, 626–636 (2001).
31. Oomen, C. A. et al. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. *Nat. Protoc.* **8**, 2006–2021 (2013).
32. Kim, C. H. et al. Trial-unique, delayed nonmatching-to-location (TUNL) touchscreen testing for mice: sensitivity to dorsal hippocampal dysfunction. *Psychopharmacology* **232**, 3935–3945 (2015).

33. Talpos, J., McTighe, S., Dias, R., Saksida, L. & Bussey, T. Trial-unique, delayed nonmatching-to-location (TUNL): a novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiol. Learn. Mem.* **94**, 341–352 (2010).
34. Hunsaker, M. R., Rosenberg, J. S. & Kesner, R. P. The role of the dentate gyrus, CA3a, b, and CA3c for detecting spatial and environmental novelty. *Hippocampus* **18**, 1064–1073 (2008).
35. Van Goethem, N., Schreiber, R., Newman-Tancredi, A., Varney, M. & Prickaerts, J. Divergent effects of the 'biased'5-HT 1 A receptor agonists F15599 and F13714 in a novel object pattern separation task. *Br. J. Pharmacol.* **172**, 2532–2543 (2015).
36. van Goethem, N. P., van Hagen, B. T. & Prickaerts, J. Assessing spatial pattern separation in rodents using the object pattern separation task. *Nat. Protoc.* **13**, 1763–1792 (2018).
37. Van Hagen, B., Van Goethem, N., Lagatta, D. & Prickaerts, J. The object pattern separation (OPS) task: a behavioral paradigm derived from the object recognition task. *Behav. Brain Res.* **285**, 44–52 (2015).
38. Prusky, G. T., Harker, K. T., Douglas, R. M. & Whishaw, I. Q. Variation in visual acuity within pigmented, and between pigmented and albino rat strains. *Behav. Brain Res.* **136**, 339–348 (2002).
39. Brown, R. E. & Wong, A. A. The influence of visual ability on learning and memory performance in 13 strains of mice. *Learn. Mem.* **14**, 134–144 (2007).
40. Wong, A. A. & Brown, R. E. Visual detection, pattern discrimination and visual acuity in 14 strains of mice. *Genes Brain Behav.* **5**, 389–403 (2006).
41. Abbott, K. N., Morris, M. J., Westbrook, R. F. & Reichelt, A. C. Sex-specific effects of daily exposure to sucrose on spatial memory performance in male and female rats, and implications for estrous cycle stage. *Physiol. Behav.* **162**, 52–60 (2016).
42. Shansky, R. M. Are hormones a "female problem" for animal research? *Science* **364**, 825–826 (2019).
43. Costa, R., Tamascia, M. L., Nogueira, M. D., Casarini, D. E. & Marcondes, F. K. Handling of adolescent rats improves learning and memory and decreases anxiety. *J. Am. Assoc. Lab. Anim. Sci.* **51**, 548–553 (2012).
44. Rebouças, R. C. & Schmidek, W. R. Handling and isolation in three strains of rats affect open field, exploration, hoarding and predation. *Physiol. Behav.* **62**, 1159–1164 (1997).
45. Gouveia, K. & Hurst, J. L. Optimising reliability of mouse performance in behavioural testing: the major role of non-aversive handling. *Sci. Rep.* **7**, 44999 (2017).
46. Deacon, R. M. Housing, husbandry and handling of rodents for behavioral experiments. *Nat. Protoc.* **1**, 936 (2006).
47. Heiderstadt, K., McLaughlin, R., Wrighe, D., Walker, S. & Gomez-Sanchez, C. The effect of chronic food and water restriction on open-field behaviour and serum corticosterone levels in rats. *Lab. Anim.* **34**, 20–28 (2000).
48. Brown, K. J. & Grunberg, N. E. Effects of housing on male and female rats: crowding stresses males but calms females. *Physiol. Behav.* **58**, 1085–1089 (1995).
49. Kappel, S., Hawkins, P. & Mendl, M. T. To group or not to group? Good practice for housing male laboratory mice. *Animals* **7**, 88 (2017).
50. Gallistel, C. R. *The Organization of Learning* (MIT Press, 1990).
51. Sousa, N., Almeida, O. & Wotjak, C. A hitchhiker's guide to behavioral analysis in laboratory rodents. *Genes Brain Behav.* **5**, 5–24 (2006).
52. Walsh, R. N. & Cummins, R. A. The open-field test: a critical review. *Psychol. Bull.* **83**, 482 (1976).
53. Simon, P., Dupuis, R. & Costentin, J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav. Brain Res.* **61**, 59–64 (1994).
54. Kolb, B. Some tests of response habituation in rats with discrete lesions to the orbital or medial frontal cortex. *Can. J. Psychol.* **28**, 260 (1974).
55. Klein, J. et al. Lesion of the medial prefrontal cortex and the subthalamic nucleus selectively affect depression-like behavior in rats. *Behav. Brain Res.* **213**, 73–81 (2010).
56. Godsil, B. P., Stefanacci, L. & Fanselow, M. S. Bright light suppresses hyperactivity induced by excitotoxic dorsal hippocampus lesions in the rat. *Behav. Neurosci.* **119**, 1339–1352 (2005).
57. Broadbent, N. J., Gaskin, S., Squire, L. R. & Clark, R. E. Object recognition memory and the rodent hippocampus. *Learn. Mem.* **17**, 5–11 (2010).
58. Antunes, M. & Biala, G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn. Process.* **13**, 93–110 (2012).
59. Pennington, Z. T. et al. ezTrack: an open-source video analysis pipeline for the investigation of animal behavior. *Sci. Rep.* **9**, 19979 (2019).
60. Mathis, A. et al. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).
61. Sridhar, V. H., Roche, D. G. & Gingins, S. Tracktor: image-based automated tracking of animal movement and behaviour. *Methods Ecol. Evol.* **10**, 815–820 (2019).
62. Drai, D., Kafkafi, N., Benjamini, Y., Elmer, G. & Golani, I. Rats and mice share common ethologically relevant parameters of exploratory behavior. *Behav. Brain Res.* **125**, 133–140 (2001).
63. Akkerman, S. et al. Object recognition testing: methodological considerations on exploration and discrimination measures. *Behav. Brain Res.* **232**, 335–347 (2012).

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Author contributions

All authors contributed to the writing of the manuscript. A.C.R. coordinated this effort.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Amy C. Reichelt or Timothy J. Bussey.

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Bekinschtein, P. et al. *Cell Rep.* **5**, 759–768 (2013): https://doi.org/10.1016/j.celrep.2013.09.027
Bonafina, A. et al. *Cell Rep.* **29**, 4308–4319 (2019): https://doi.org/10.1016/j.celrep.2019.11.100
Reichelt, A.C. et al. *Learn. Mem.* **23**, 386–390 (2016): https://doi.org/10.1101/lm.042416.116